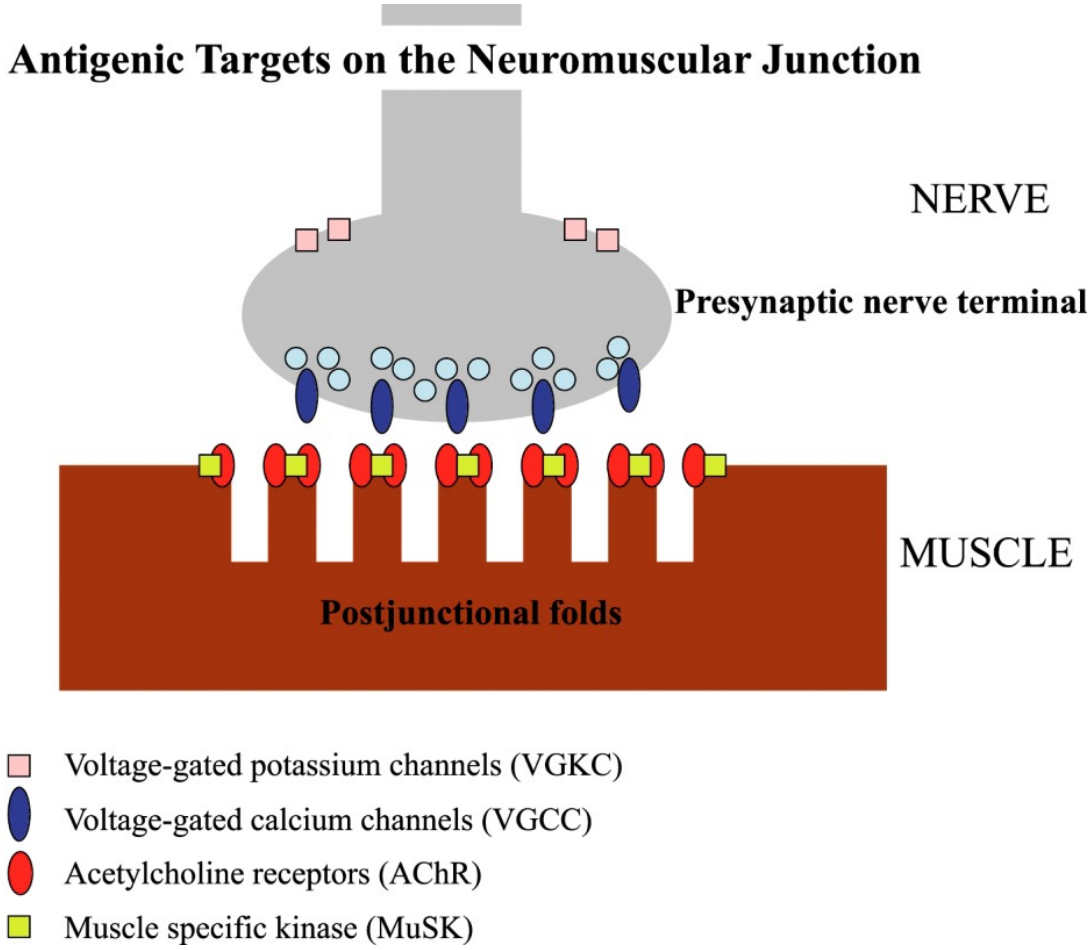
Synaptic Transmission in PNS: the **Neuromuscular Junction**

1. The Synaptogenesis
2. The Synaptic Transmission at NMJ
3. The Safety Factor
4. **Myasthenic Syndromes**
5. The Tripartite Synapse



Antigenic targets on the mammalian neuromuscular junction; AChR (red) and muscle-specific kinase (MuSK, yellow) situated on the postsynaptic membrane and voltage-gated potassium (VGKC, pink) and calcium channels (VGCC, blue) present on the presynaptic nerve terminal. The presynaptic nerve terminal (grey), contains the synaptic vesicles (light blue). These vesicles contain the neurotransmitter, acetylcholine (ACh). When the nerve is depolarised, the vesicles fuse with the presynaptic terminal and discharge the transmitter into the synaptic cleft. ACh binds to the AChR and causes an influx of sodium ions, which finally results in the muscle action potential.

B. Lang, A. Vincent / Autoimmunity Reviews 2 (2003) 94–100

Table 1
Autoimmune disorders of the peripheral nervous system and neurotransmission

Disorder	Antibody specificity	Associated conditions (% patients with condition)	Associated CNS disorders
MG	Anti-AChR (muscle)	Thymoma (15%)	
'Seronegative' MG	Anti-MuSK	No tumours	
LEMS	Anti-VGCC (P/Q-type VGCC)	SCLC (50%)	Cerebellar ataxia
NMT (Isaac's syndrome)	Anti-VGKC	Thymoma (20%) MG (10–20%)	Morvan's syndrome Limbic encephalitis/epilepsy

Isaac's Syndrome: is a neuromyotonia, not a MS

4. Myasthenic Syndromes

1. Autoimmune diseases

2. Congenital diseases

Autoimmune diseases

(auto) Antibody

+

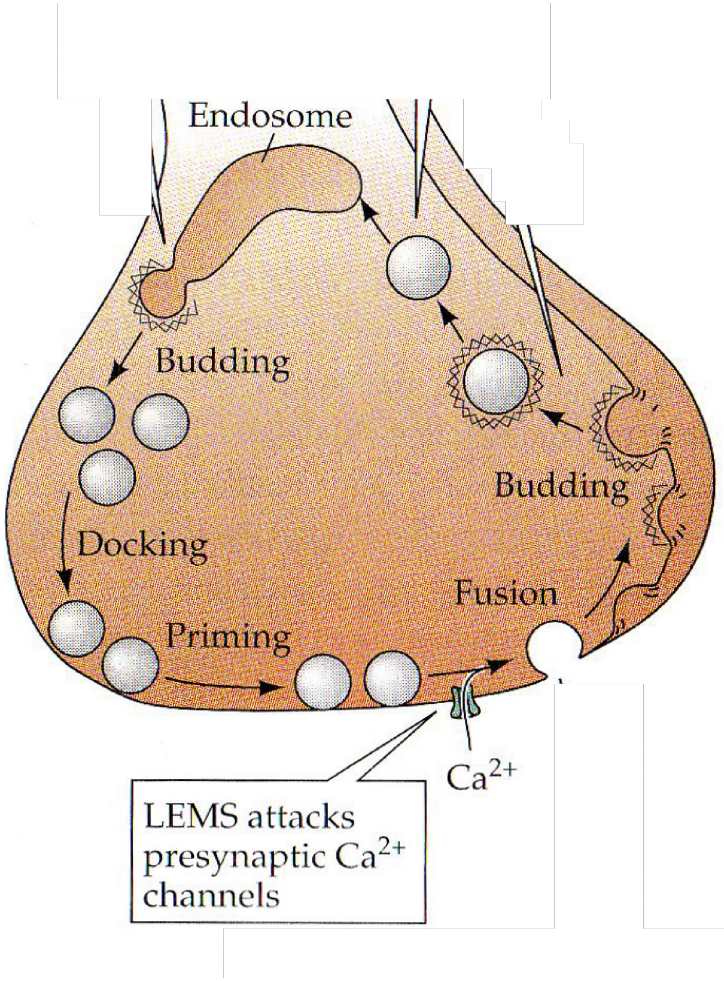
Muscle weakness

Lamber-Eaton Myasthenic Syndrome (LEMS)

Reduction in release of ACh

* Normal miniature endplate potential amplitude, demonstrating normal postsynaptic sensitivity to acetylcholine (ACh).

* Markedly reduced evoked endplate potential amplitude, suggesting a significant reduction in ACh release.



MG: Myasthenia Gravis

Myasthenia gravis

“grave muscle weakness”

• Asymmetrical ptosis (a drooping of one or both eyelids).

• Diplopia (double vision) due to weakness of the muscles that control eye movements.

Distribution of MG subtypes

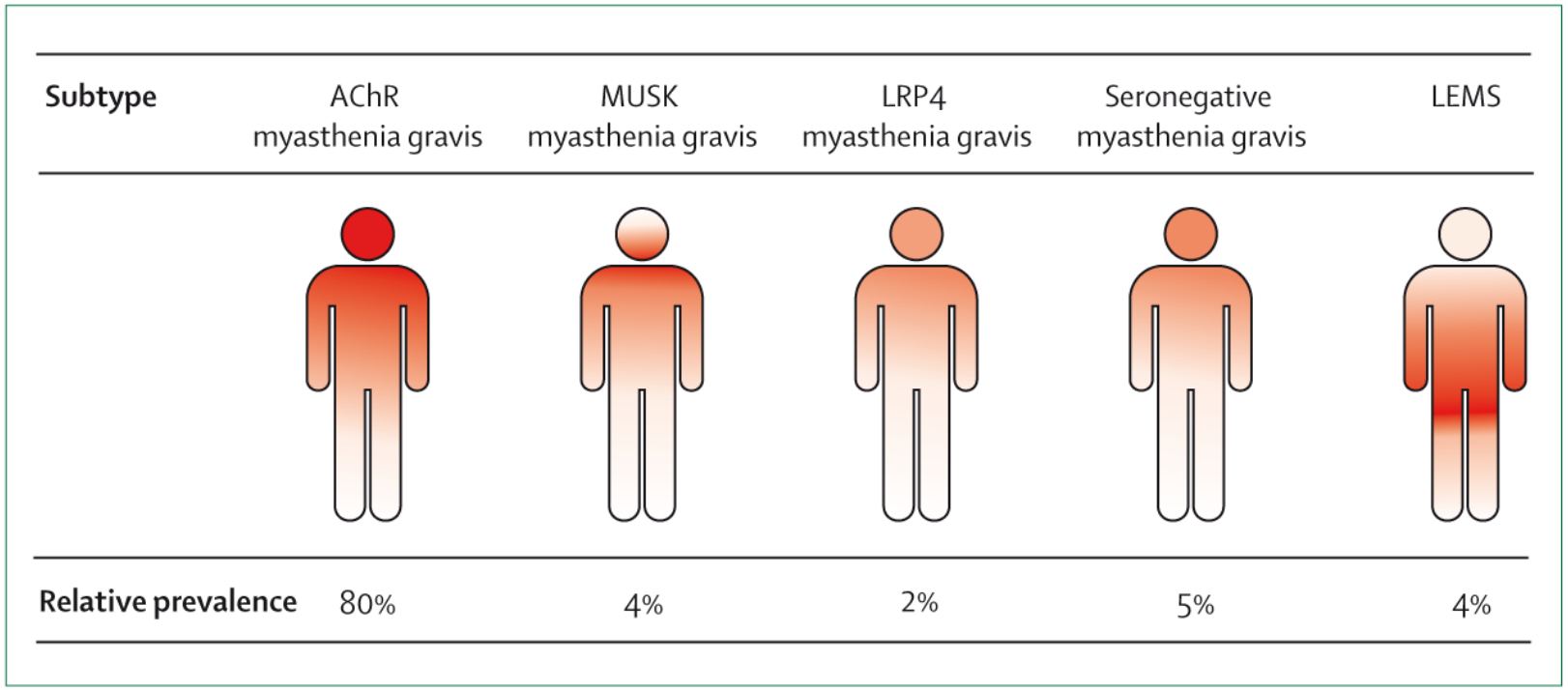
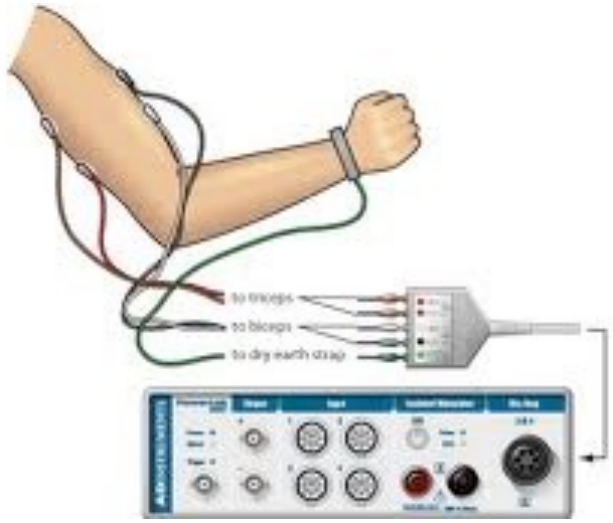
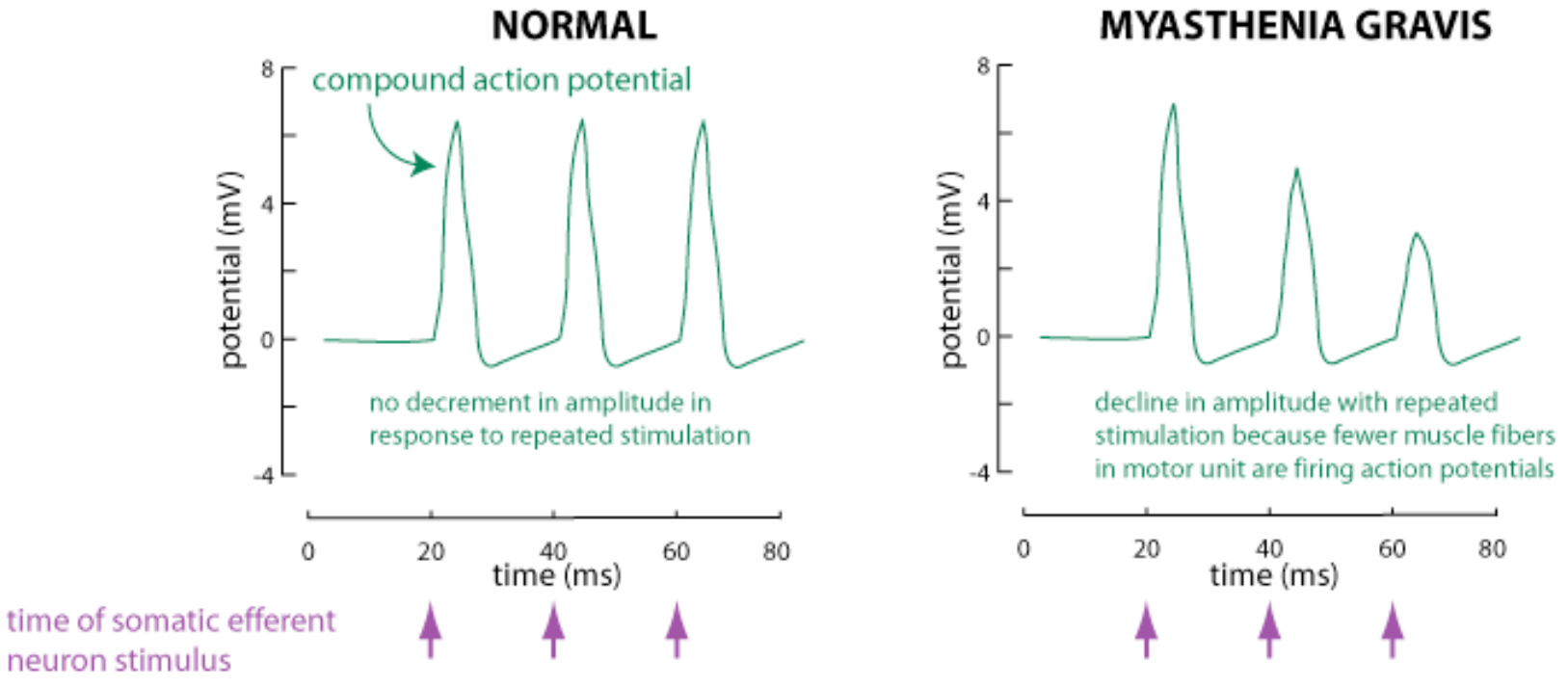


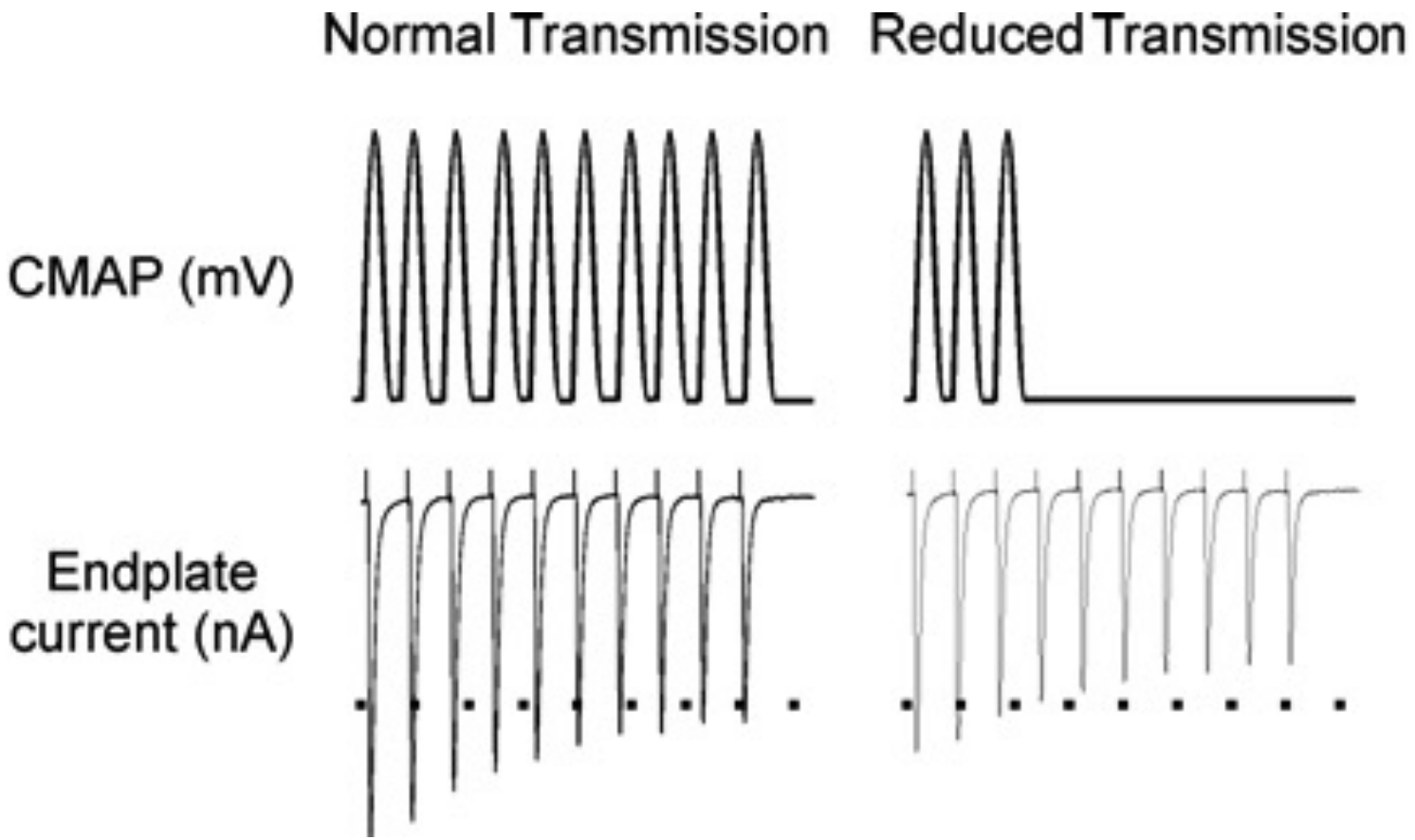
Figure 2: **Distribution of weakness and relative prevalence of subtypes of myasthenia gravis**
AChR=acetylcholine receptor. MUSK=muscle-specific kinase. LRP4=lipoprotein-related protein 4. LEMS=Lambert–Eaton myasthenic syndrome.

MG: Diagnostic test



Electromyography:
The electromyogram is an extracellular recording of the electrical activity of motor units. A **motor unit** consists of a somatic efferent neuron and all the muscle fibers that it innervates. In the electromyogram, one records a potential known as the **compound action potential**, which is the summed action potentials of all the muscle fibers in the motor unit.





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), wherea 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.

MG Activities of Daily Living (MG-ADL) Scale

	Score = 0	Score = 1	Score = 2	Score = 3	Your Score
Talking	Normal	Intermittent slurring or nasal speech	Constant slurring or nasal speech, but can be understood	Difficult to understand speech	
Chewing	Normal	Fatigue with solid food	Fatigue with soft food	Gastric tube	
Swallowing	Normal	Rare episode of choking	Frequent choking necessitating changes in diet	Gastric tube	
Breathing	Normal	Shortness of breath with exertion	Shortness of breath at rest	Ventilator dependence	
Brushing teeth or hair	Normal	Extra effort, but no rest periods needed	Rest periods needed	Cannot do one of these functions	
Arising from chair	Normal	Mild, sometimes uses arms	Moderate, always uses arms	Severe, requires assistance	
Double vision	Normal	Occurs, but not daily	Daily, but not constant	Constant	
Eyelid droop	Normal	Occurs, but not daily	Daily, but not constant	Constant	
				Your Total Score =	

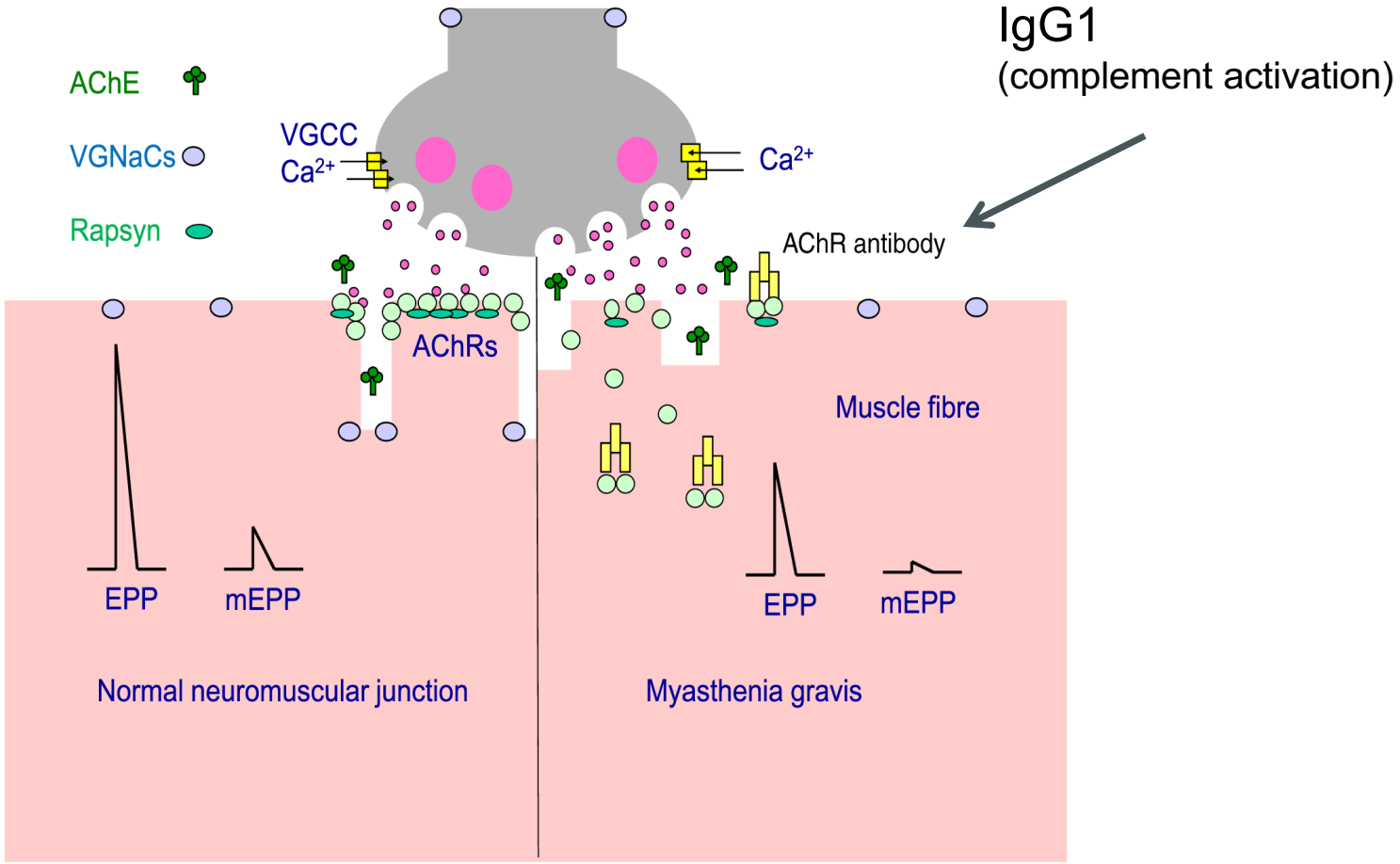


Figure 1. Assessing neuromuscular transmission. (**A**) Healthy neuromuscular transmission. The nerve terminal can release the contents of each vesicle (quanta) of acetylcholine by exocytosis. Spontaneous release of single quanta of acetylcholine activates the intrinsic cation channels of acetylcholine receptors (AChRs) in the postsynaptic membrane to produce a small, transient depolarisation called a miniature endplate potential (mEPP). The nerve action potential opens voltage-gated calcium channels (VGCCs) and triggers exocytosis of many quanta of acetylcholine, simultaneously producing the (much larger) EPP. In healthy individuals, the amplitude of the EPP is more than enough to reach the threshold required to activate the postsynaptic voltage-gated sodium channels (VGNaCs) and generate a muscle action potential. (**B**) The myasthenia gravis neuromuscular junction. AChR antibodies (mainly immunoglobulin [Ig]G1) activate complement, resulting in membrane attack complex-mediated damage to the post-junctional membrane architecture. The postsynaptic AChR numbers are depleted by divalent antibodies inducing AChR internalisation. The loss of AChRs results in smaller mEPP and EPP amplitudes. The EPP may not reach threshold, especially when the nerve is repetitively activated. Abbreviations: AChE, acetylcholinesterase

IgG4
(NO activation of complement)

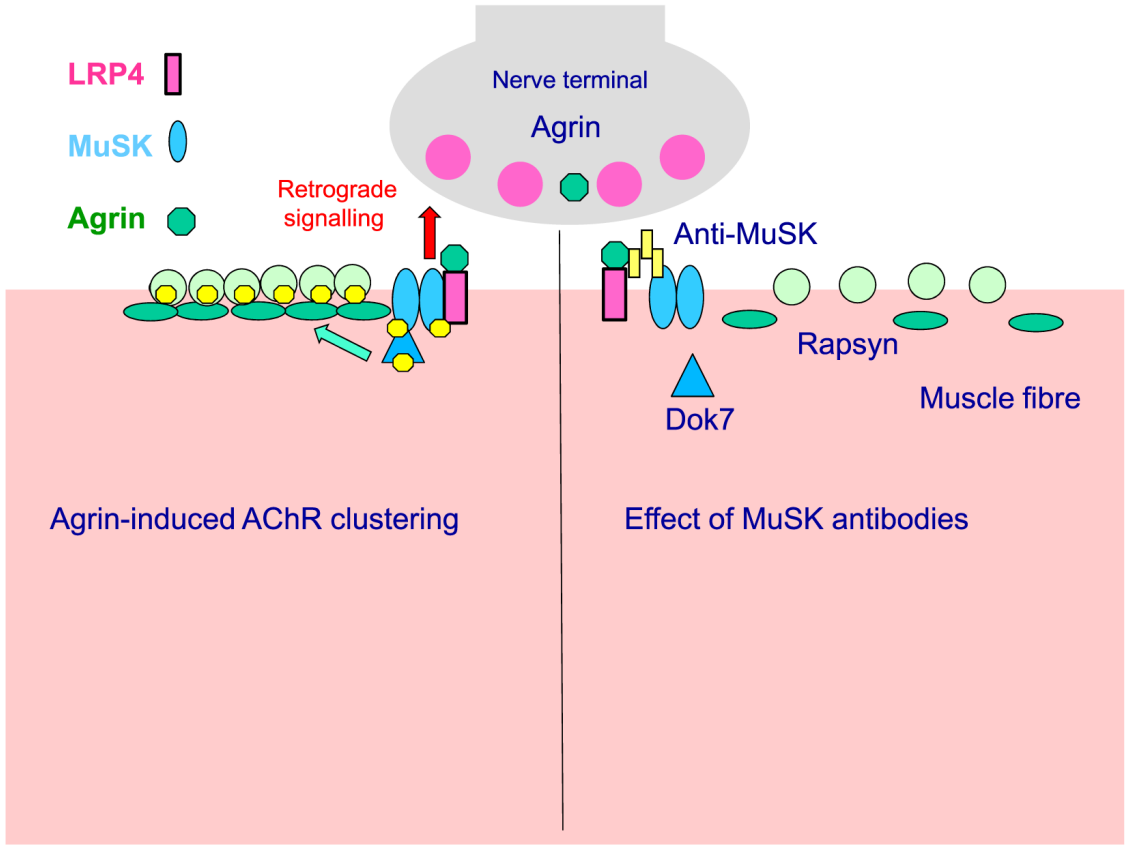


Figure 2. Disruption of postsynaptic differentiation pathway by muscle-specific kinase (MuSK) autoantibodies. (**A**) Healthy MuSK-mediated postsynaptic differentiation pathway at the neuromuscular junction (NMJ). Neural agrin secreted by the motor nerve terminal binds to LRP4, low-density lipoprotein receptor-related protein 4 (LRP4), which causes the dimerisation of MuSK. MuSK dimerisation causes phosphorylation of MuSK and associated proteins of the MuSK pathway, including Dok7 and the acetylcholine receptor (AChR) β-subunit. Rapsyn is recruited to the phosphorylated AChRs, stabilising postsynaptic clusters of AChRs. (**B**) Impaired postsynaptic differentiation in animal models of MuSK myasthenia gravis. MuSK autoantibodies are mainly of the immunoglobulin (Ig)G4 subclass. They block the assembly of the agrin-LRP4-MuSK complex. Interruption of MuSK kinase signalling leads to slow disassembly of the postsynaptic AChR clusters. A resultant decline in miniature endplate potential (mEPP) and EPP amplitude (not shown) results in failure of the muscle action potential and fatiguing weakness. Co-existing IgG1-3 antibodies, although lower concentration, may contribute but their pathogenic roles are not yet well defined. The compensatory presynaptic upregulation of quantal release found in AChR MG does not occur in MuSK MG.

2 major groups of antibodies:

- Those that recognized extracellular/ transmembrane autoantigens (nAChR, MuSK, Lrp4, Agrin and AChE).

- Those that recognized intracellular autoantigens (RyR, titin and cortactin).

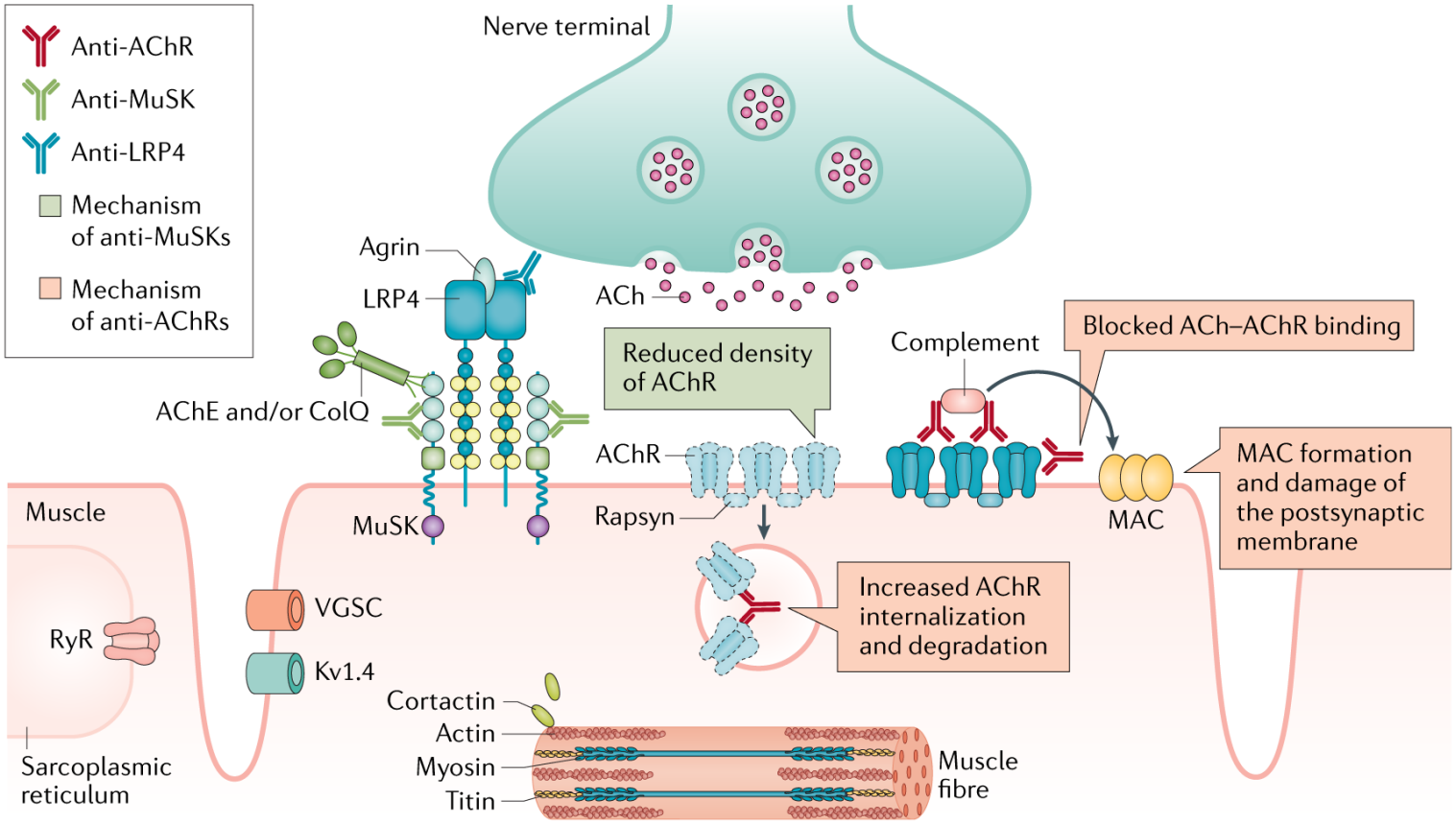
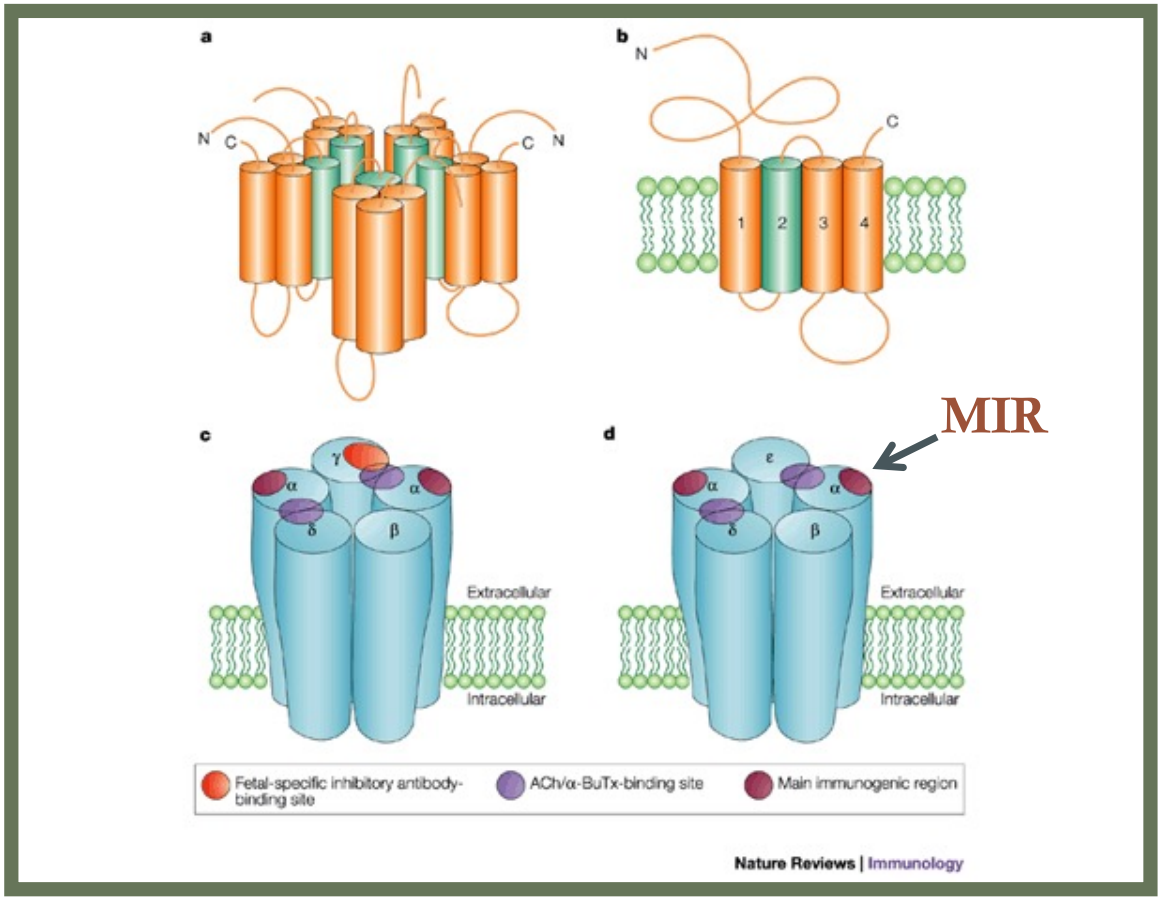
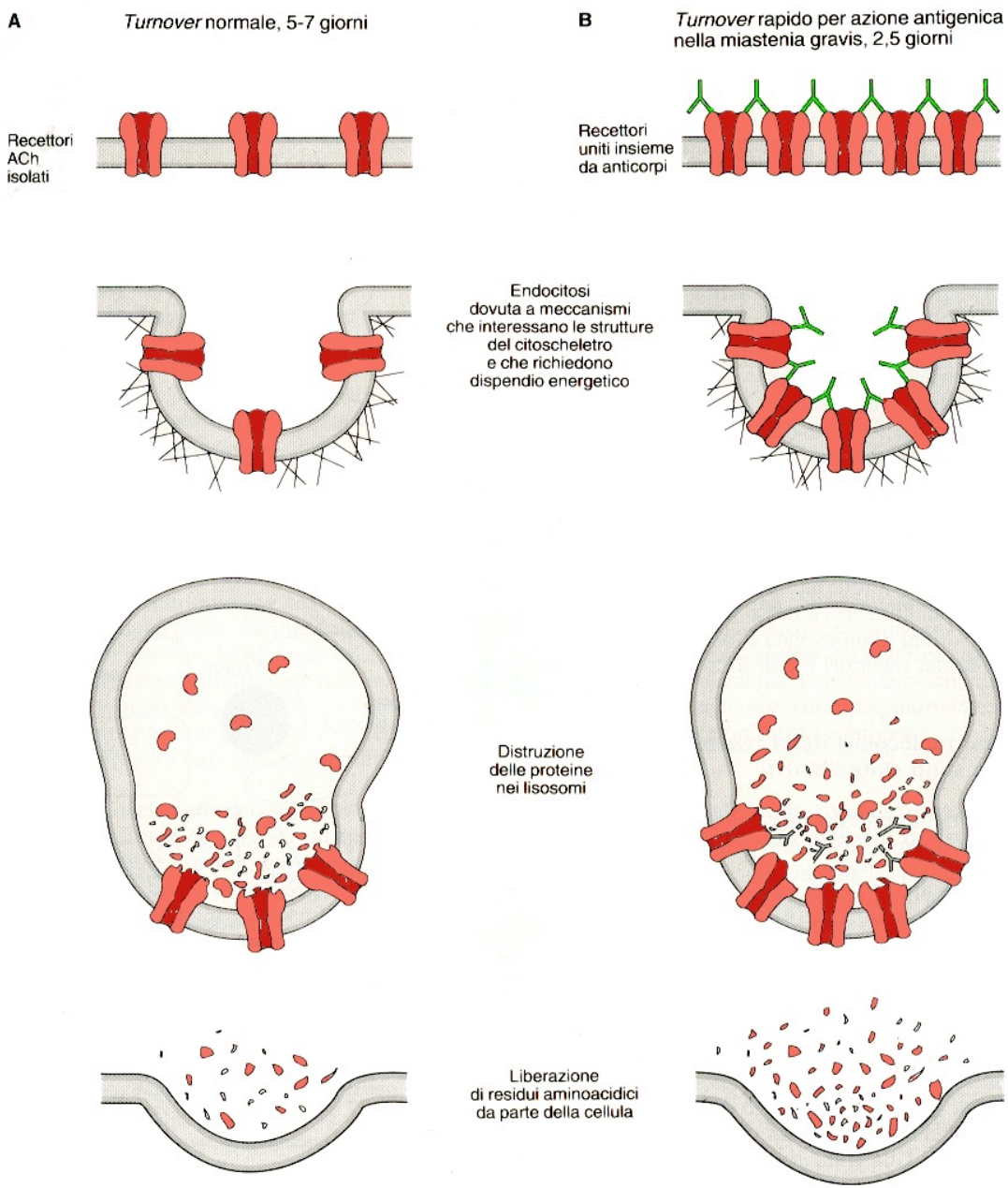


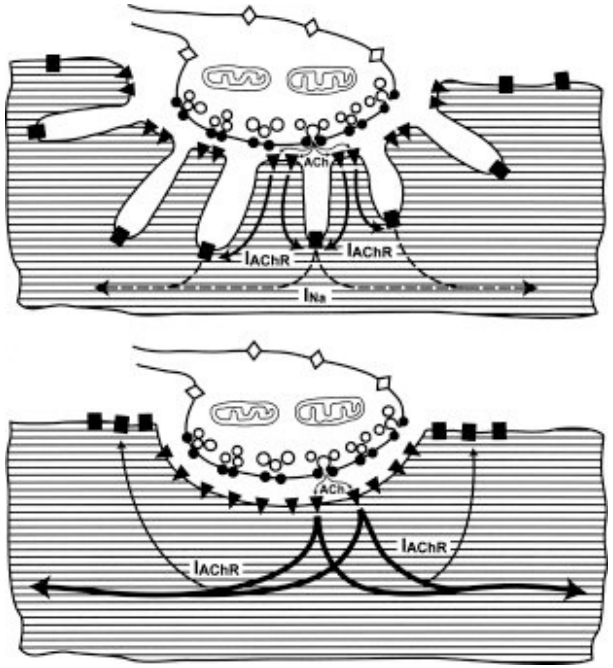
Fig. 3 | **Pathophysiology of MG at the neuromuscular junction.** Anti-acetylcholine (ACh) receptor (AChR) antibodies activate complement, leading to damage of the postsynaptic membrane at the neuromuscular junction through production of the membrane attack complex (MAC). Anti-AChR antibodies can also crosslink AChRs, leading to their accelerated internalization and degradation rate. Some antibodies can directly block the ACh binding site. Anti-muscle-specific kinase (MuSK) antibodies do not activate complement and typically prevent the interaction of MuSK and lipoprotein-receptor-related protein 4 (LRP4), among other proteins, leading to reduced AChR clustering on the postsynaptic membrane. The pathogenicity of anti-LRP4 antibodies in myasthenia gravis (MG) remains to be established. Additional antibodies, such as anti-collagen Q (ColQ), anti-titin, anti-ryanodine receptor (RyR), anti-cortactin and anti-voltage-gated potassium channel (Kv1.4) have been demonstrated in patients with MG, although any pathogenetic significance remains unknown. AChE, acetylcholinesterase; VGSC, voltage-gated sodium channel.

85% of patients affected by MG are positive for anti-AChR antibodies and 50% of them bind **MIR**

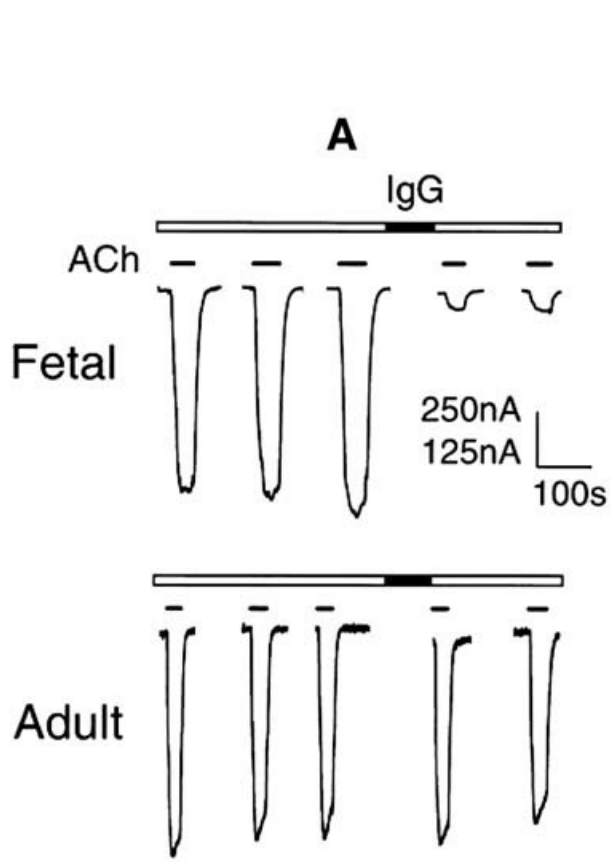


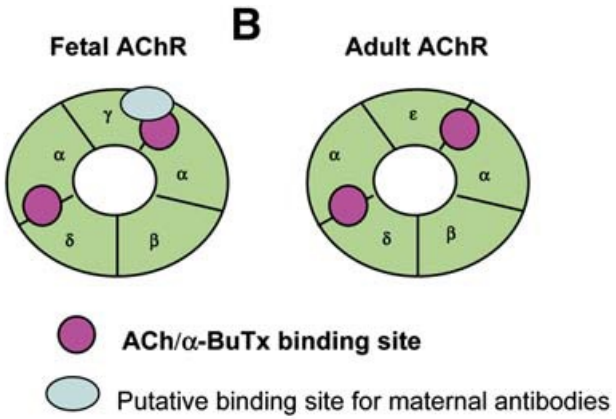
a | The acetylcholine receptor (AChR) is a pentameric membrane protein. b | Each of the subunits consists of an extracellular domain, four transmembrane regions and a cytoplasmic domain. The receptor consists of (alpha)2, beta, gamma and delta subunits in the fetal form (c), and (alpha)2, beta, delta and alt epsilon subunits in the adult form (d). A large proportion of the antibodies in the sera of myasthenia gravis patients bind to the **main immunogenic regions** that are on both of the alpha subunits, to which the sequence alpha64–76 makes an important contribution. In addition, many patients' antibodies bind to the fetal-specific gamma subunit. In some cases, these antibodies inhibit the function of the AChR in vitro, and in pregnant mothers, they can cross the placenta, causing fetal muscle paralysis and severe, and often fatal, deformities. alpha-BuTx, alpha-bungarotoxin.

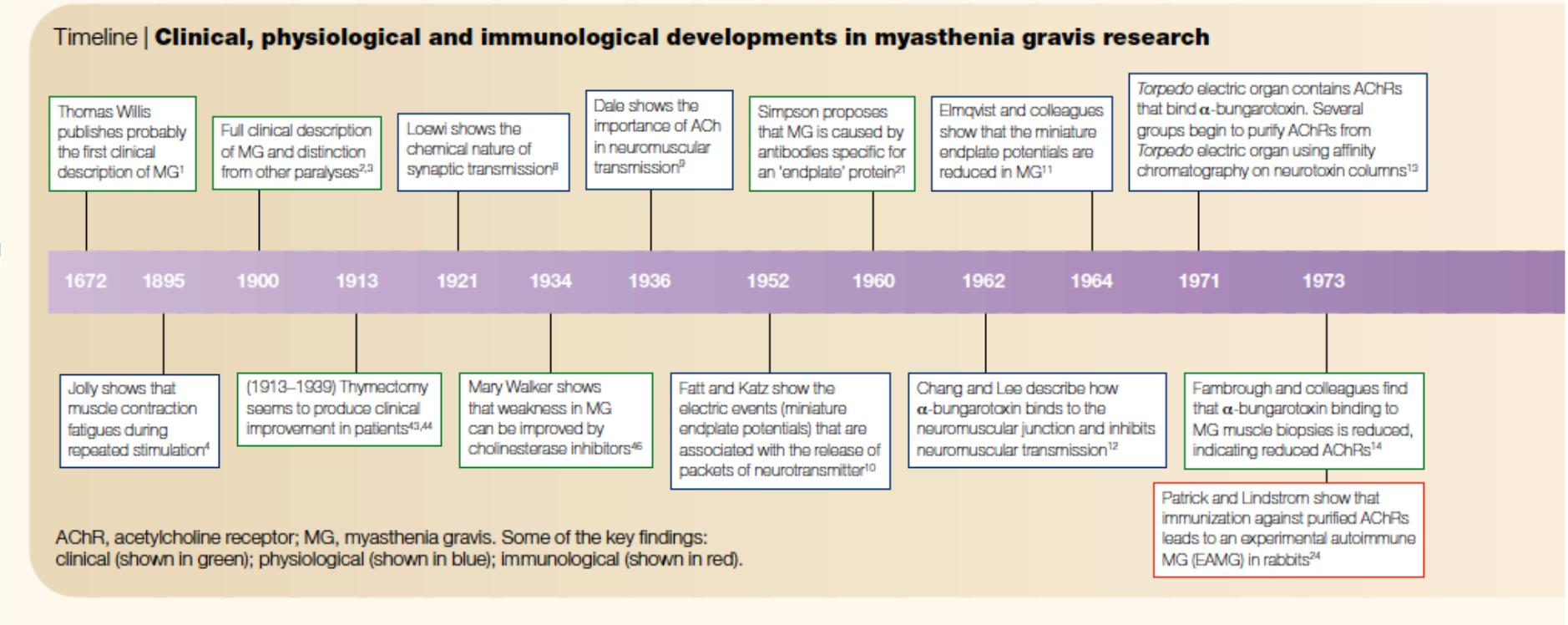

A
Turnover normale, 5-7 giorni
B
Turnover rapido per azione antigenica nella miastenia gravis, 2,5 giorni
Recettori ACh isolati
Recettori uniti insieme da anticorpi
Endocitosi dovuta a meccanismi che interessano le strutture del citoscheletro e che richiedono dispendio energetico
Distruzione delle proteine nei lisosomi
Liberazione di residui aminoacidici da parte della cellula


ACh
I_{AChR}
I_{AChR}
I_{Na}
ACh
I_{AChR}
I_{AChR}

Maternal antibodies to fetal nAChR cause developmental disorders







-repetitive stimulation of the nerve that innervates a MG muscle produces a decreasing muscle contraction

-mepp are reduced

- the number of AChR at the NMJ is reduced

The discovery of MG….

Thomas Willis
(circa 1667)

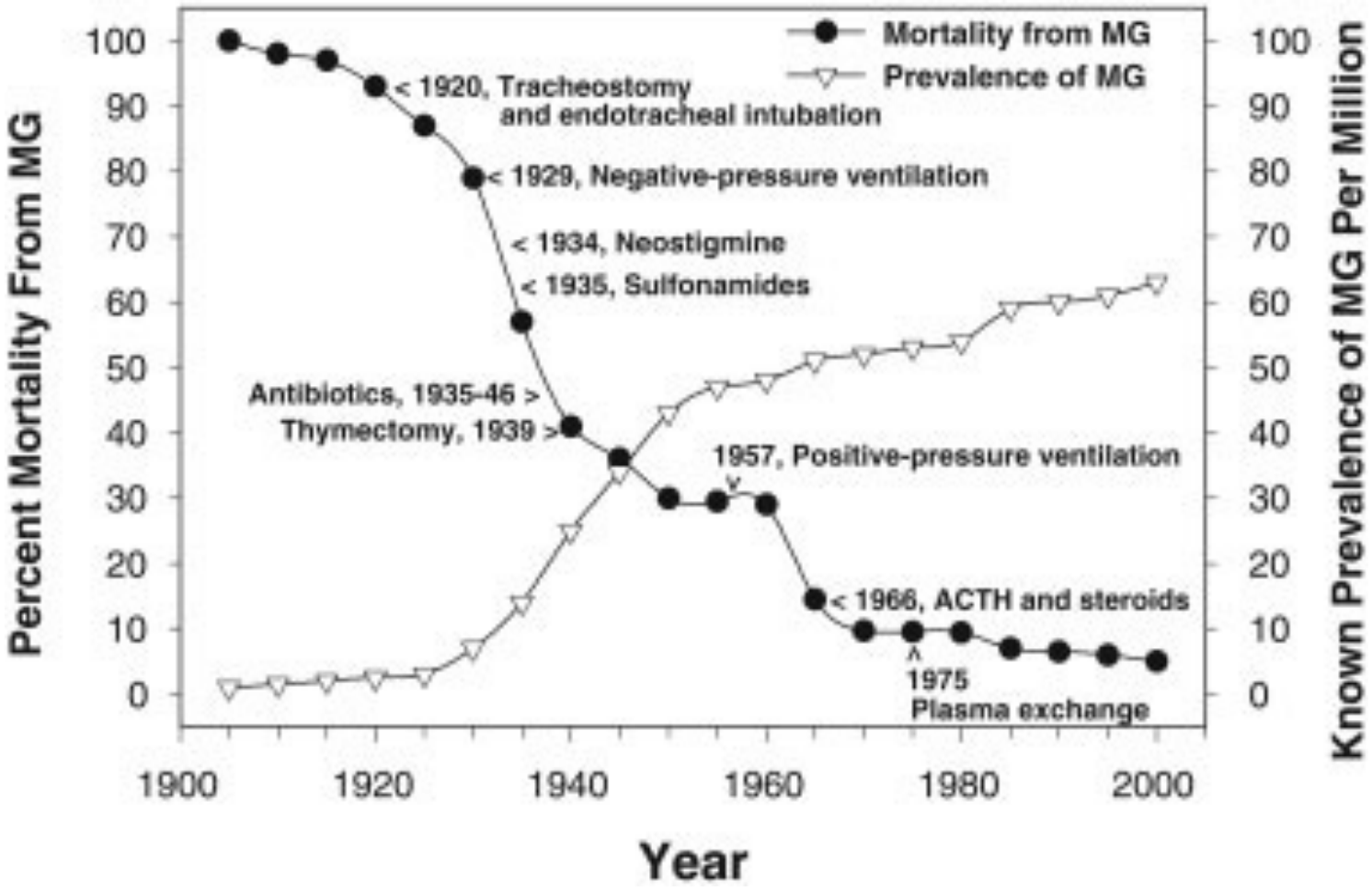
A London physician who made pertinent observations of many neurological diseases, including arguably the first description of myasthenia gravis.

Mary Walker
(circa 1935)

Nature Reviews | Immunology

She was the first doctor to try **acetylcholinesterase inhibitors** for the treatment of myasthenia gravis.

Prevalence & Mortality during 1900-2000



At the moment there are not therapies that prevent or cure the disease.

Unfortunately, the therapies do not target the specific antibody.

Table 1 | **Subgroups of MG patients with AChR-specific antibodies***

Subtype of MG	Age at onset	Sex M:F	Typical thymic pathology	MHC association	Associated autoantibodies
Early onset	<41 years	1:3	Hyperplastic	B8, DR3	Might have other tissue-specific antibodies (such as thyroid specific)
Thymoma-associated	Mainly 40–60 years	1:1	Epithelial tumour containing many lymphocytes	No clear association	Antibodies specific for titin and ryanodine receptor are very common[51,52]. Also, anti-cytokine antibodies[53].
Late onset	>40 years	1.5:1	Normal or atrophied	B7, DR2 in males	Antibodies specific for titin and ryanodine receptor, particularly after 60 years[51–53].

*These subdivisions relate particularly to Caucasians who have generalized myasthenia gravis (MG) and are positive for acetylcholine receptor (AChR)-specific antibodies. These subgroups are not appropriate in patients who have purely ocular MG or who lack AChR-specific antibodies, or possibly in other ethnic populations. F, female; M, male.

Animal (rodent) models

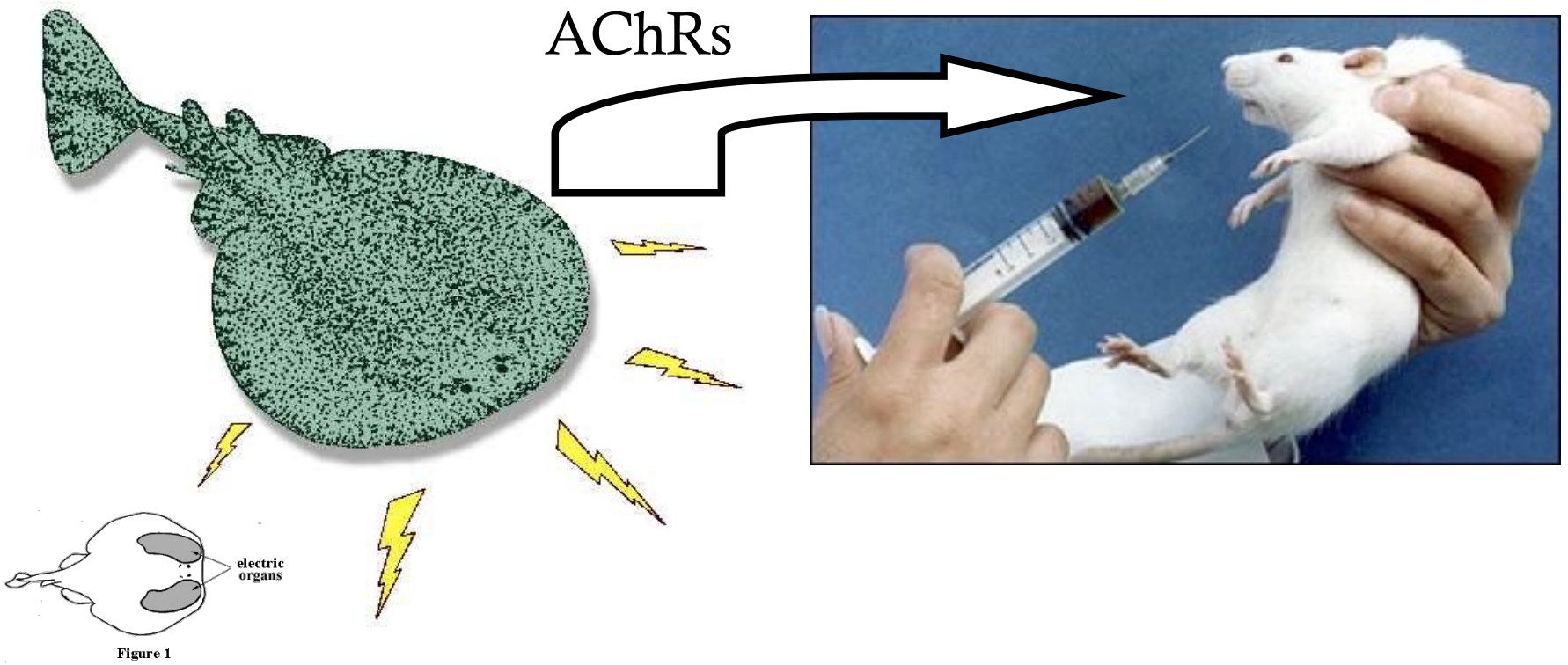


Table 1
Comparison of mouse models of myasthenia gravis.

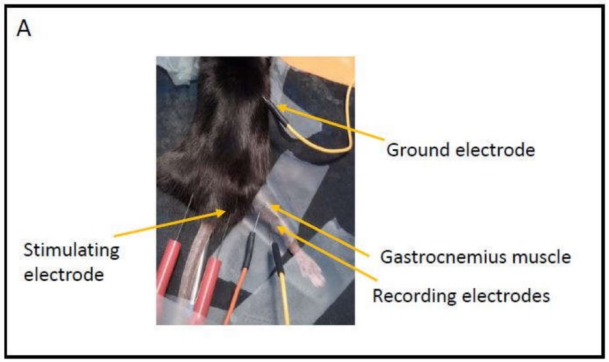
Type of MG model	Advantages	Disadvantages	Laboriousness	Relative costs	Suitability for electrophysiological NMJ analysis	Suitability for drug studies
Passive transfer of patient IgG	– High human relevance: tests pathogenicity of MG patient IgG – Muscle weakness reasonably well titratable (in case of MuSK MG)	– Need of large amounts (g) of patient IgG, purified from plasmapheresis material – Sudden, stepwise elevation of autoantibody titre, unlike gradual rise in patients – Not all strains equally suitable (e.g. variability in complement activity)	High (multiple IgG purifications, daily injections over several weeks)	High	High	– Very high, due to possibility of creating stable disease – Suitable for drugs targeting the NMJ, the pathogenic antibodies or complement – Not suitable for drugs aimed at autoimmunity mechanisms
Passive transfer of monoclonal antibodies against AChR	– Robust weakness after single injection – Reproducibility	– Need to produce large amounts of monoclonal antibodies – Monoclonals are non-human	Low to moderate	Moderate	High	Suitable for drugs targeting the NMJ or the pathogenic antibodies or complement
Active immunization	– Involvement of the animal's own immune system – Gradual increase of autoantibody titre, like in patients	– Muscle weakness not titratable, uncontrollable disease can lead to death – Sometimes non-responders – Mouse IgG subclass characteristics differ from human	Low to moderate (requires regular monitoring over several months)	Moderate	High	– High, particularly suitable for drugs aimed at autoimmunity mechanisms as well as at NMJ and complement – Intrinsic variability may require larger number of mice
α-Bungarotoxin-induced	– 'Clean' NMJ effects, i.e. no involvement of immune system – Muscle weakness easily titratable	Less clinical relevance for autoimmune-mediated MG (e.g. no complement activation component)	Moderate (requires regular injections over several weeks)	Low	High	Moderate, no immunological drug targets, only for drugs targeting the NMJ
Genetic (natural and transgenic mutations)	– Human congenital MG with known mutation can be exactly mimicked (knock-in)	Breeding of strain needs to be maintained	– Initially high (in case of transgenesis) – Moderate once established (ongoing genotyping and breeding)	Moderate to high	High	High, for drugs targeting the NMJ (e.g. the mutated protein) or drugs acting on DNA/RNA

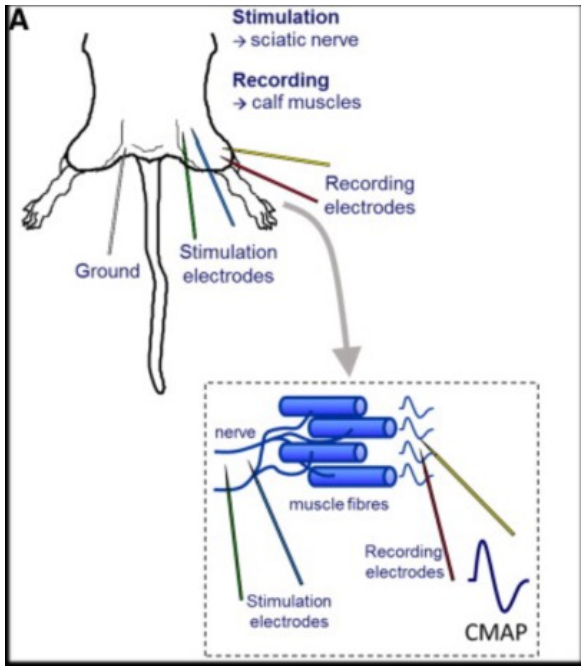
The animal models for MG are useful to:

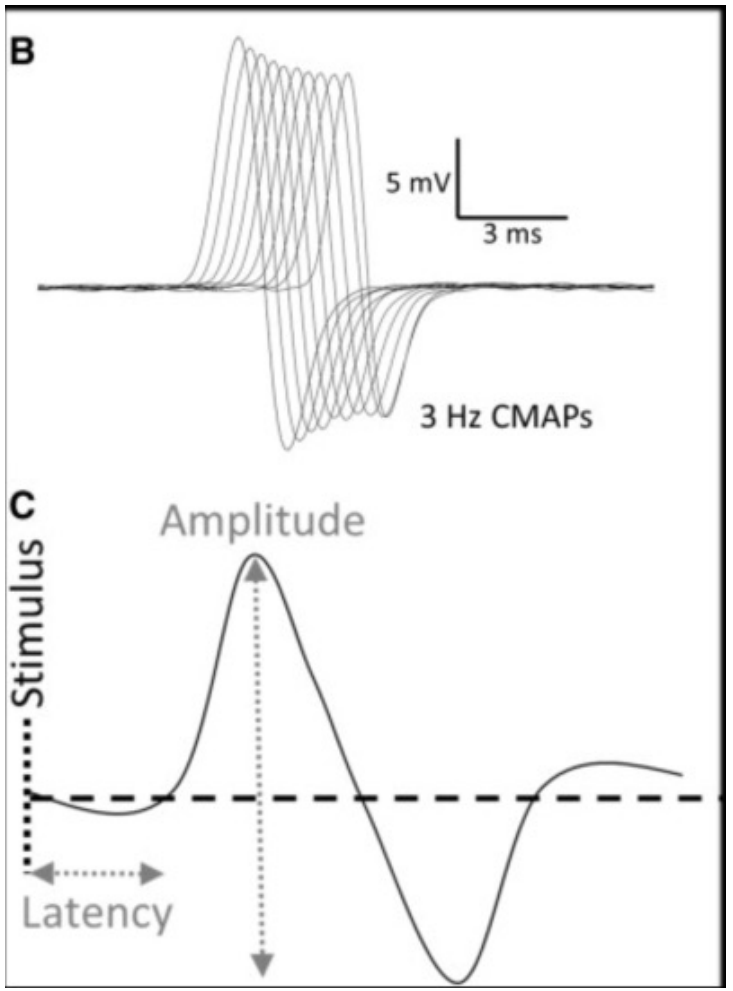
- Prove the pathogenicity of patient antibody
- Study pathophysiology
- Test potential therapeutic effect of new or existing drugs

IN VIVO STUDIES

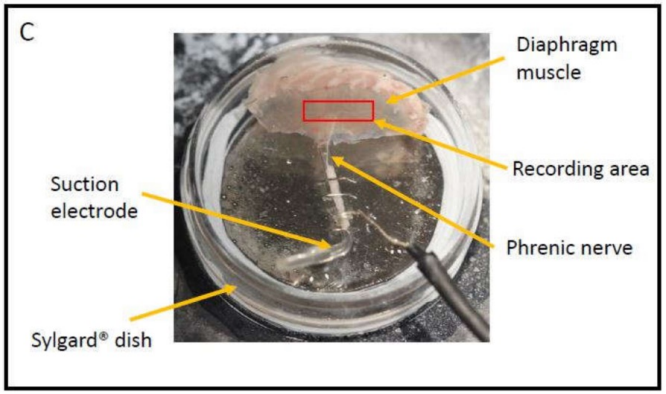
Repetitive nerve stimulation electromyography in the anaesthetized mouse.
(A) Cartoon showing the electrode configuration used to record compound muscle action potentials (CMAPs). (B) Example of CMAP recording from the gastrocnemius muscle of a myasthenic mouse, demonstrating a decrement in amplitude during 3 Hz repetitive nerve stimulation. Superimposed CMAPs are spaced somewhat for easier visualization of the decrement. (C) Schematic drawing of a single CMAP on an expanded timescale.

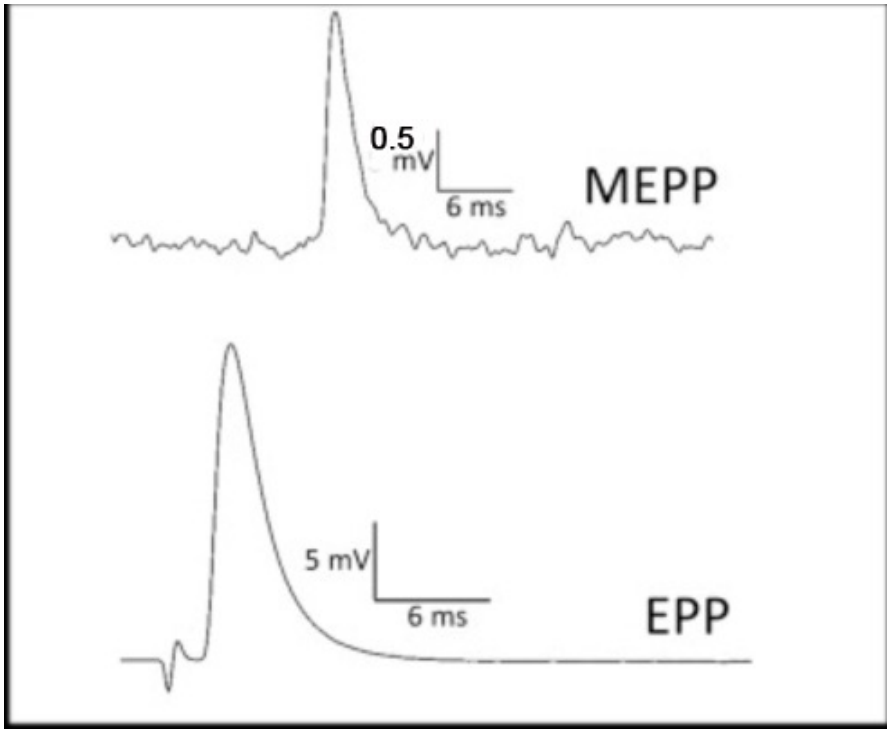


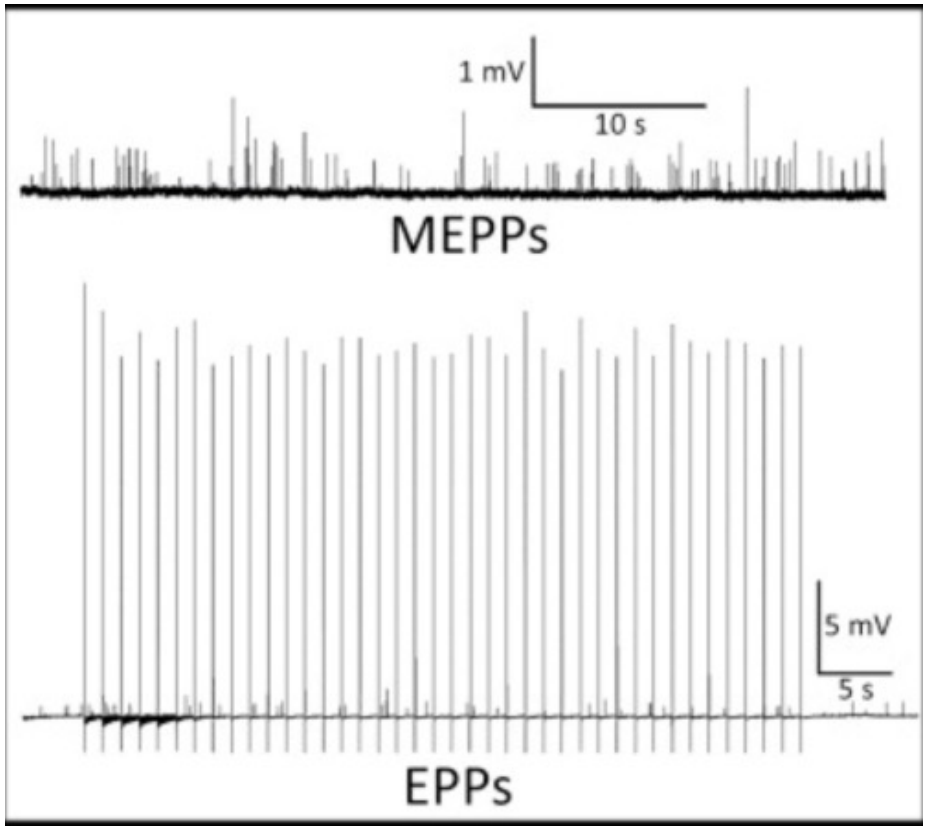


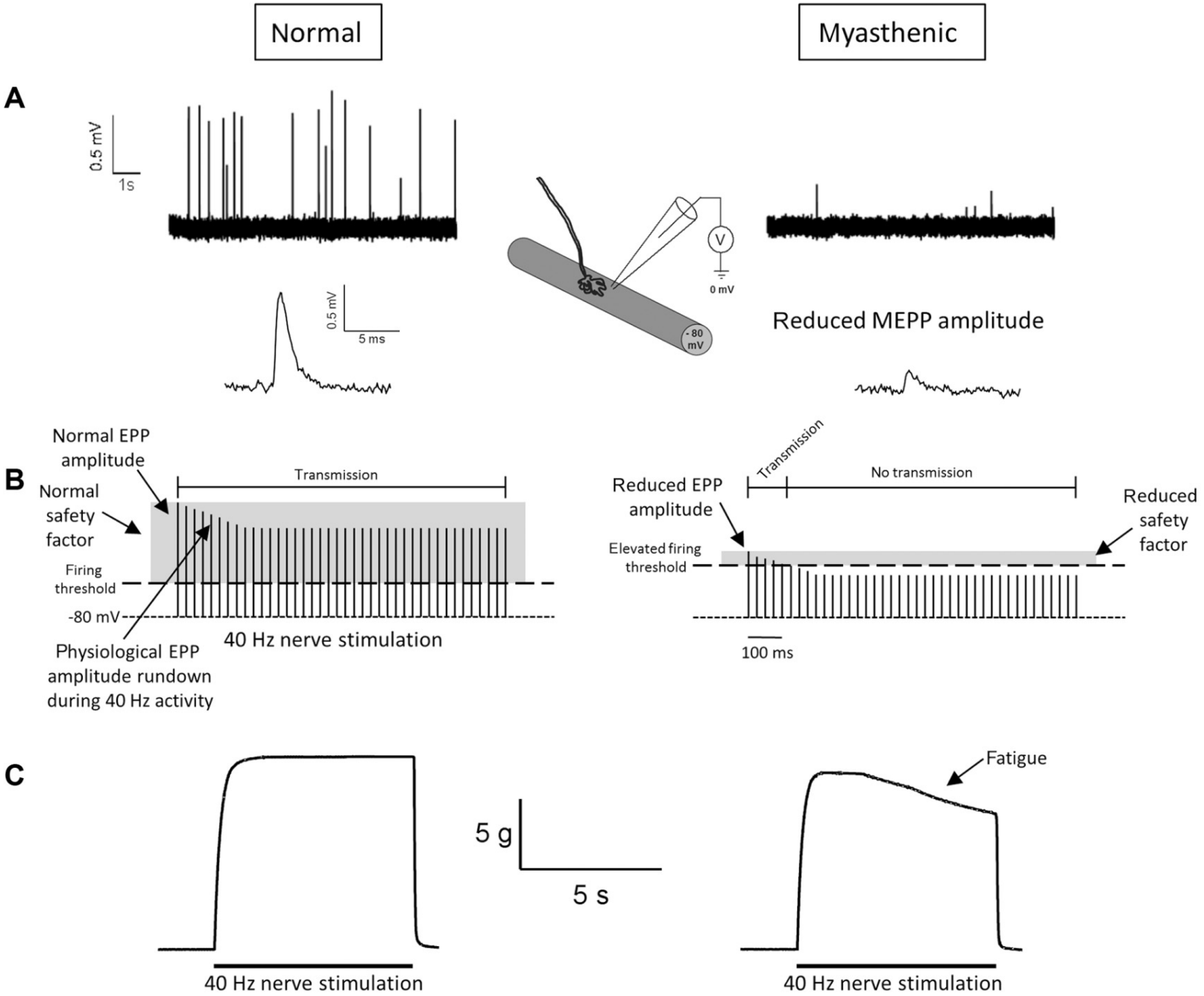


IN VITRO STUDIES








Normal
Myasthenic
A
0.5 mV
1s
0.5 mV
5 ms
V
0 mV
- 80 mV
Reduced MEPP amplitude
B
Normal EPP amplitude
Normal safety factor
Firing threshold
-80 mV
Transmission
40 Hz nerve stimulation
Physiological EPP amplitude rundown during 40 Hz activity
Transmission
No transmission
Reduced EPP amplitude
Elevated firing threshold
Reduced safety factor
100 ms
C
5 g
5 s
40 Hz nerve stimulation
Fatigue
40 Hz nerve stimulation

Some biophysical properties of MG human skeletal muscle

Table 1
Resting membrane potential (RP) and action potential (AP) properties on the endplate border compared to extrajunctional membrane of type IIb intercostal muscle fibers from 7 control subjects and 5 patients with MG

	RP	AP Threshold	AP dV/dt	E_{AP}
	(mV)	(mV)	(Vs^{-1})	(mV)
Endplate border				
Control	−85.4	−71.9	617	13.5
(n=38 fibers)	±1.1	±2.2	±23	±2.4
MG	−83.9	−62.3	412	21.6
(n=34 fibers)	±1.5	±2.7	±18	±2.9
		$p<0.001$	$p<0.001$	$p<0.01$
Extrajunctional membrane				
Control	−85.6	−60.1	364	25.5
(n=38 fibers)	±1.2	±2.3	±19	±3.1
MG	−85.1	−59.7	351	25.4
(n=34 fibers)	±1.3	±2.5	±17	±3.0

AP properties are threshold, maximum rate of rise (AP dV/dt) and membrane depolarization required to reach threshold (E_{AP}).

Table 2
EPP and MEPP size recorded from type IIb external intercostal muscles of 5 patients with MG and 7 control patients

	MEPP	EPP
	(mV)	(mV)
Control	0.91	40.2
(n=38 fibers)	±0.03	±1.3
MG	0.48	23.5
(n=34 fibers)	±0.02	±1.7
	$p<0.001$	$p<0.001$

Table 3
The safety factor (SF) for neuromuscular transmission for type IIb external intercostal muscles of 5 patients with MG and 7 control patients

	SF
Control	2.98
MG	1.09

SF=EPP/E_{AP} using data from Tables 1 and 2.

MG treatments:

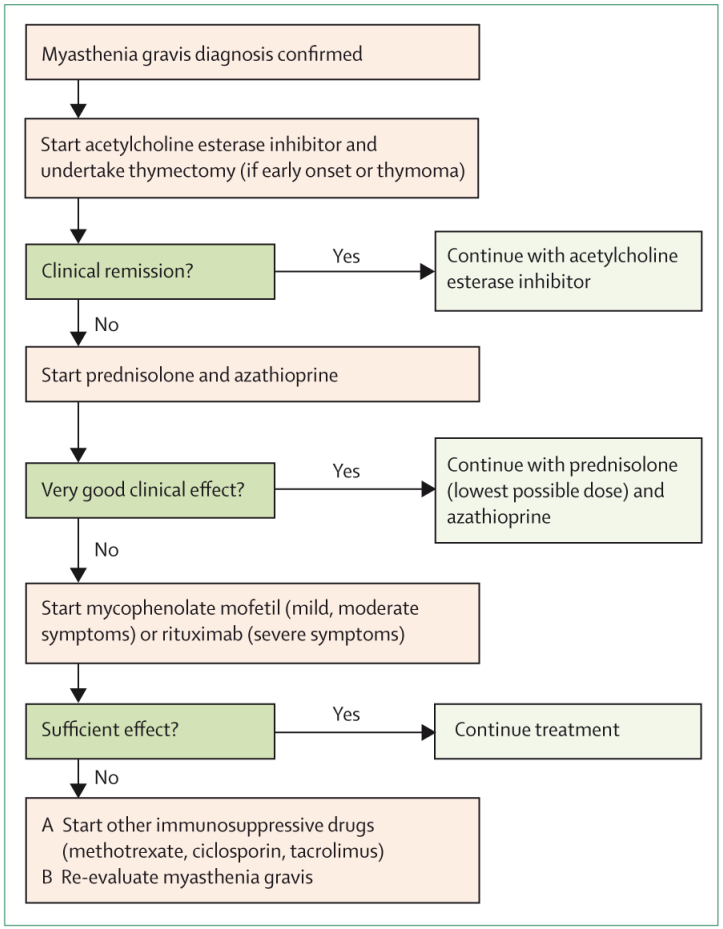


Figure 4: **Treatment of generalised myasthenia gravis**

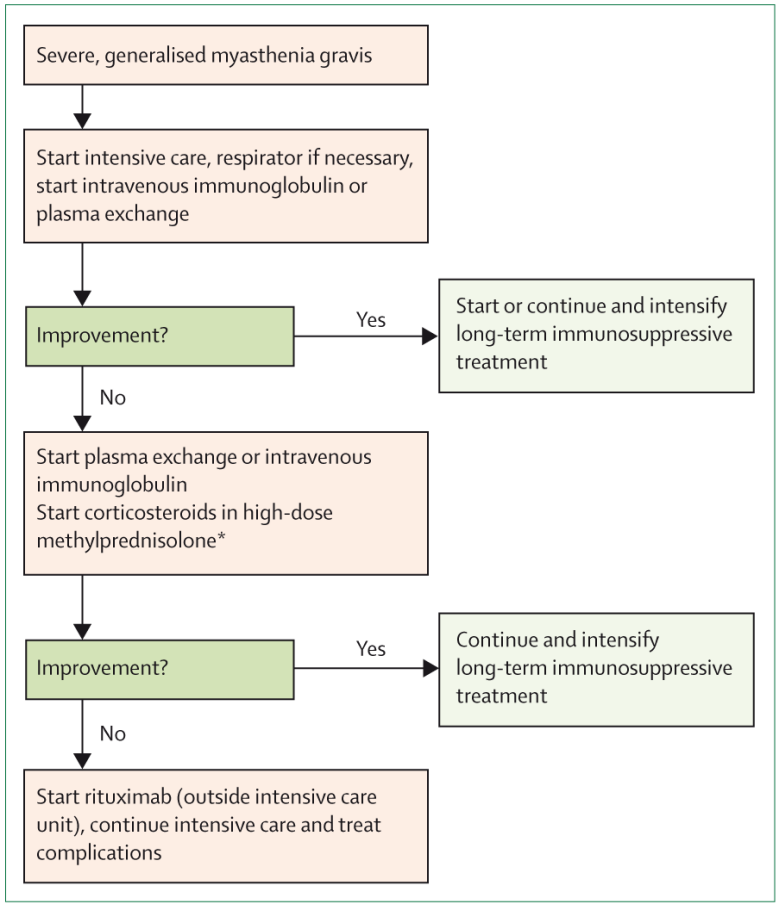


Figure 5: **Treatment of severe myasthenia gravis exacerbations**
*1000 mg a day for 3 days.

Elevation in the threshold for muscle fiber action potential initiation

Autoimmune response directed against the endplate AChR in MG may cause loss of sodium channels from the postsynaptic region of the endplate. The loss of sodium channel significantly increase the amount of current needed to initiate an action potential.

Congenital Myasthenic Syndromes

Fast-channel syndrome

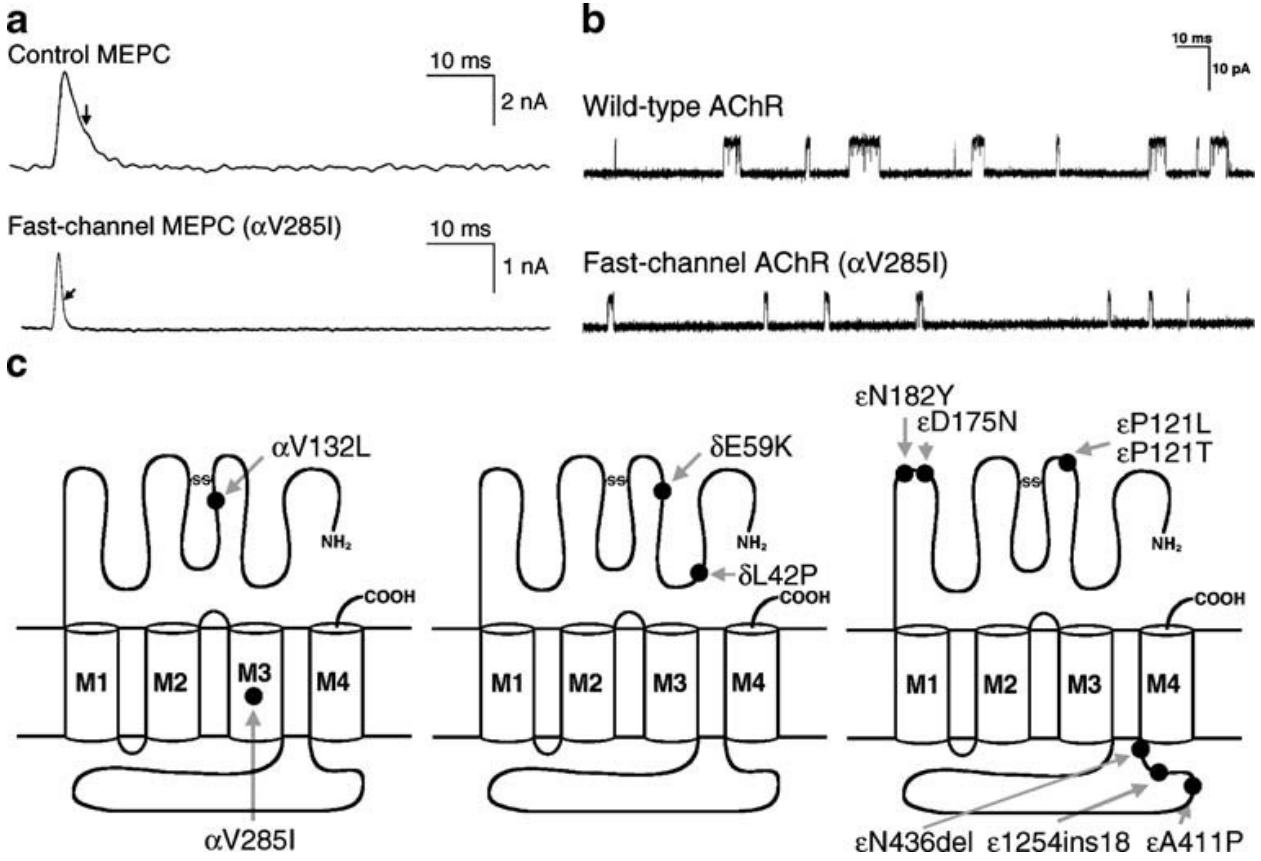


Figure 4 Fast-channel syndromes. a MEPC recorded from EPs of a control subject and a patient harboring the αV285I fast-channel mutation. Arrows indicate decay time constants. b Single-channel currents from wild-type and fast-channel (αV285I) AChRs expressed in HEK cells. c Schematic diagram of fastchannel mutations in the AChR α, β, and δ subunits. (From Engel 2004, by permission)

Slow-channel syndrome

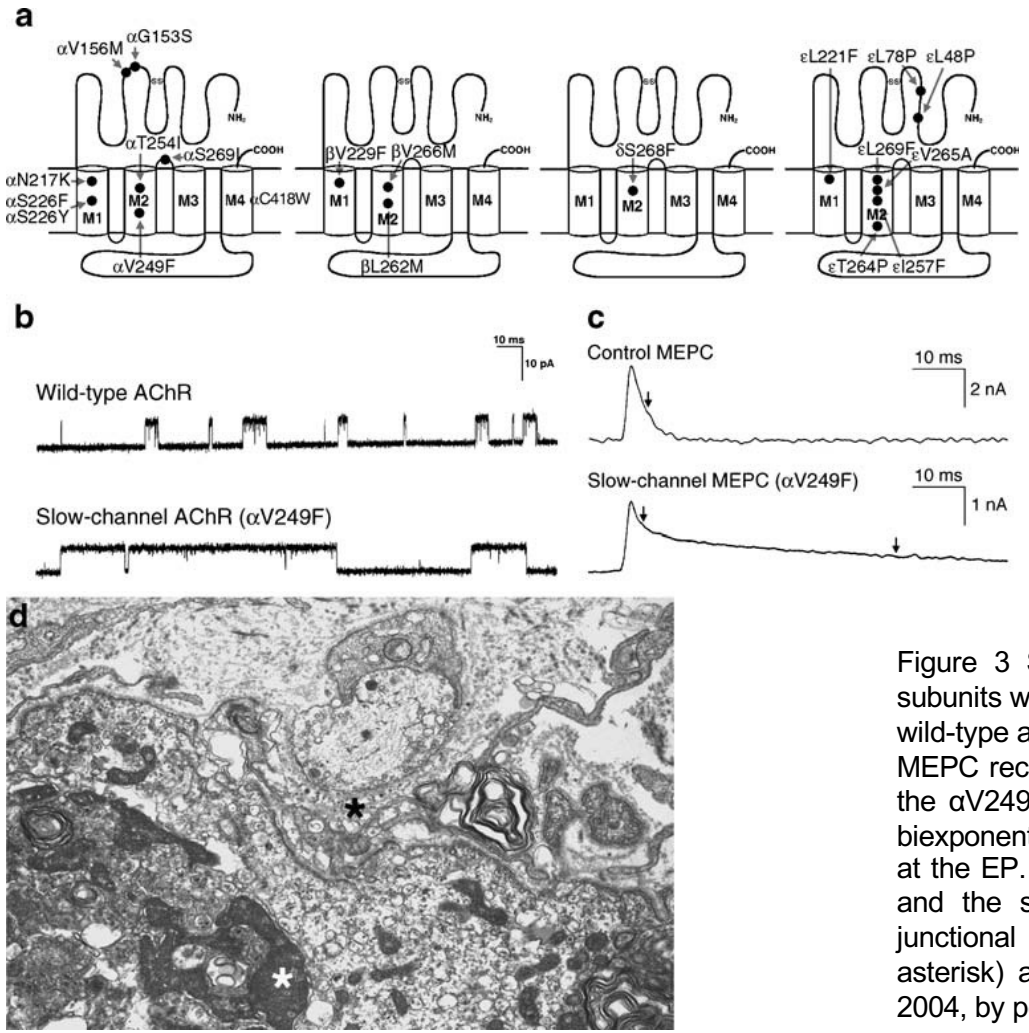


Figure 3 Slow-channel syndromes. a Schematic diagram of AChR subunits with slow-channel mutation. b Single-channel currents from wild-type and slow-channel (αV249F) AChRs expressed in HEK cells. c MEPC recorded from EPs of a control subject and a patient harboring the αV249F slow-channel mutation. The slow-channel MEPC decays biexponentially due to expression of both wild-type and mutant AChRs at the EP. d Slow-channel EP. The junctional folds have disintegrated and the synaptic space is filled with debris (black asterisk). The junctional sarcoplasm displays fragmented apoptotic nuclei (white asterisk) and myeloid structures. Bar=1 μm. (a to c are from Engel 2004, by permission)