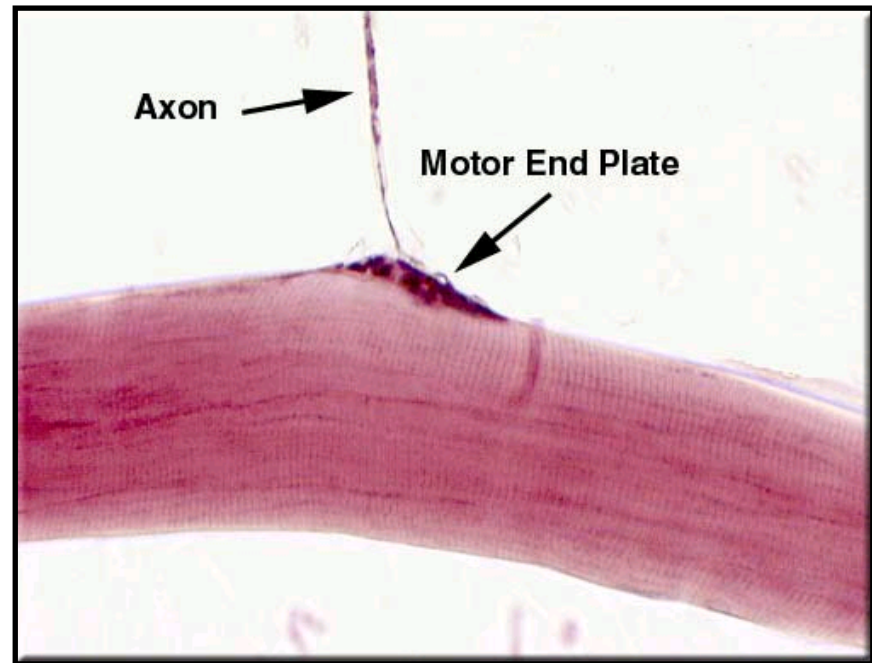


SINAPSI CHIMICHE

- Sono localizzate prevalentemente nel SNC e SNP
- I citoplasmi della cellula pre e della cellula post-sinaptica non sono in continuità
- La comunicazione avviene attraverso la liberazione di un neurotrasmettitore
- La comunicazione avviene in una sola direzione (dalla cellula pre alla cellula post-sinaptica)

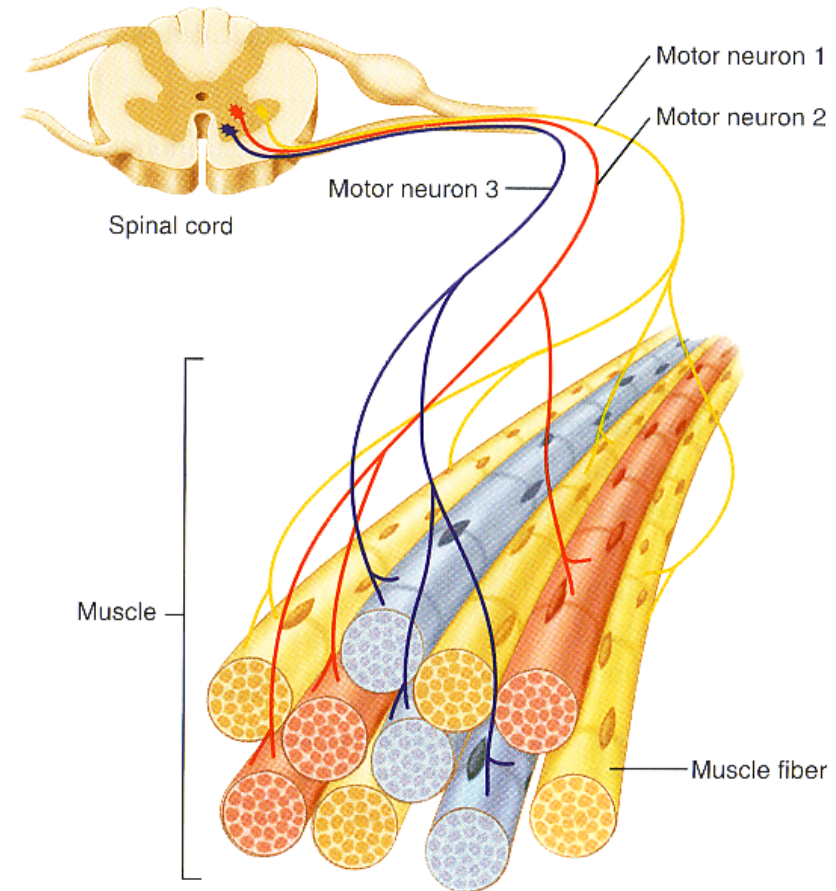
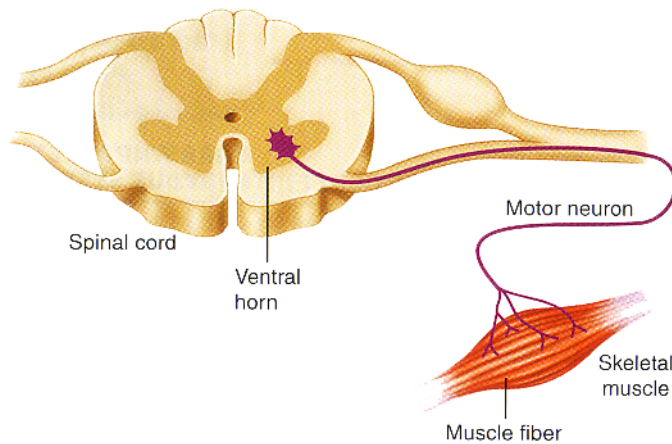
Esempio di sinapsi chimica:

La giunzione neuromuscolare



Gli elementi della sinapsi neuromuscolare

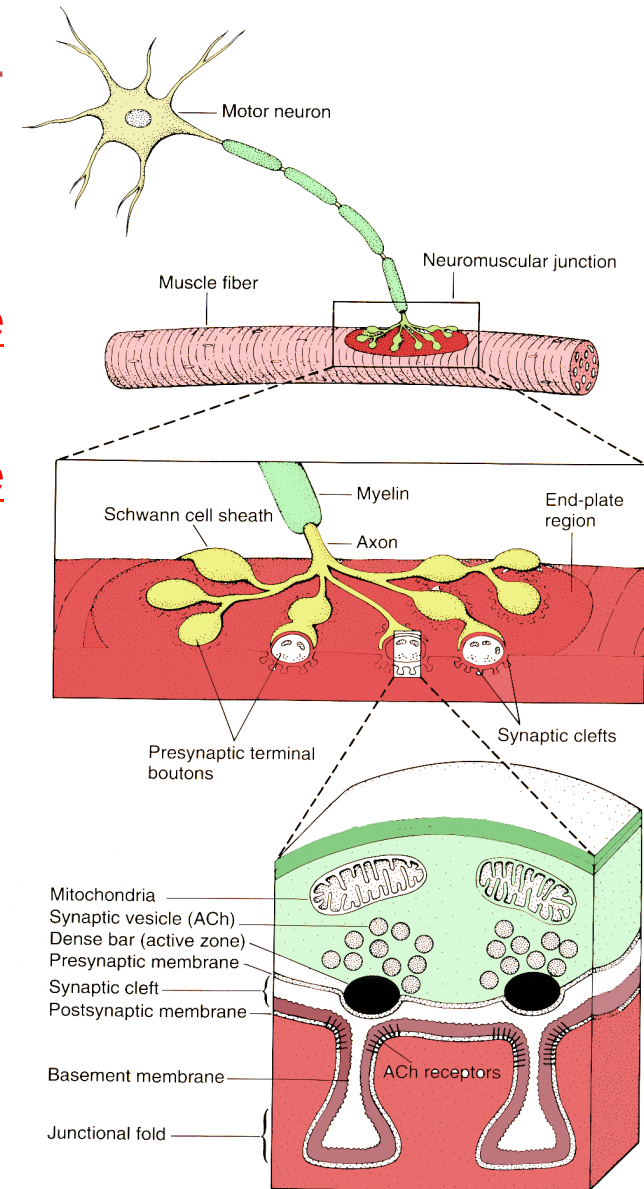
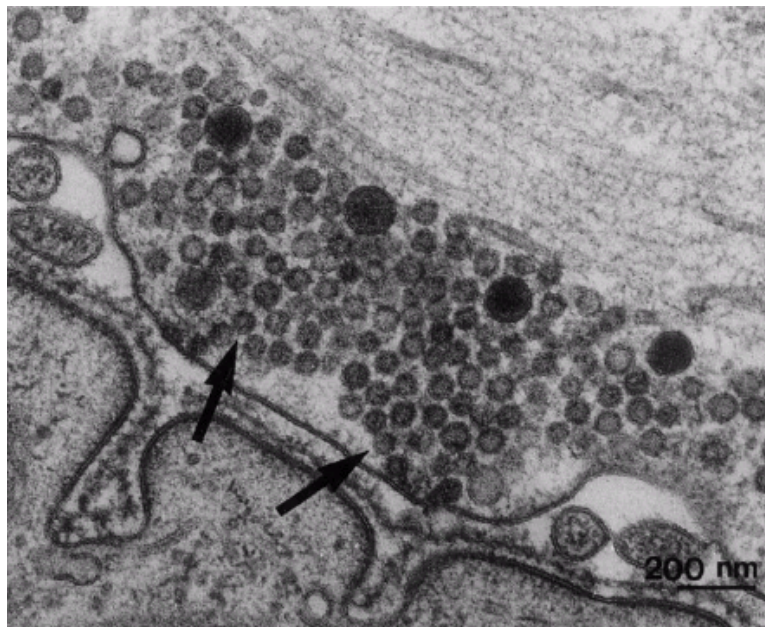
- **cellula presinaptica**
il motoneurone (α - motoneurone)
- **cellula postsinaptica**
la fibra muscolare scheletrica
- **il mediatore chimico**
(o neurotrasmettitore)
l'acetilcolina

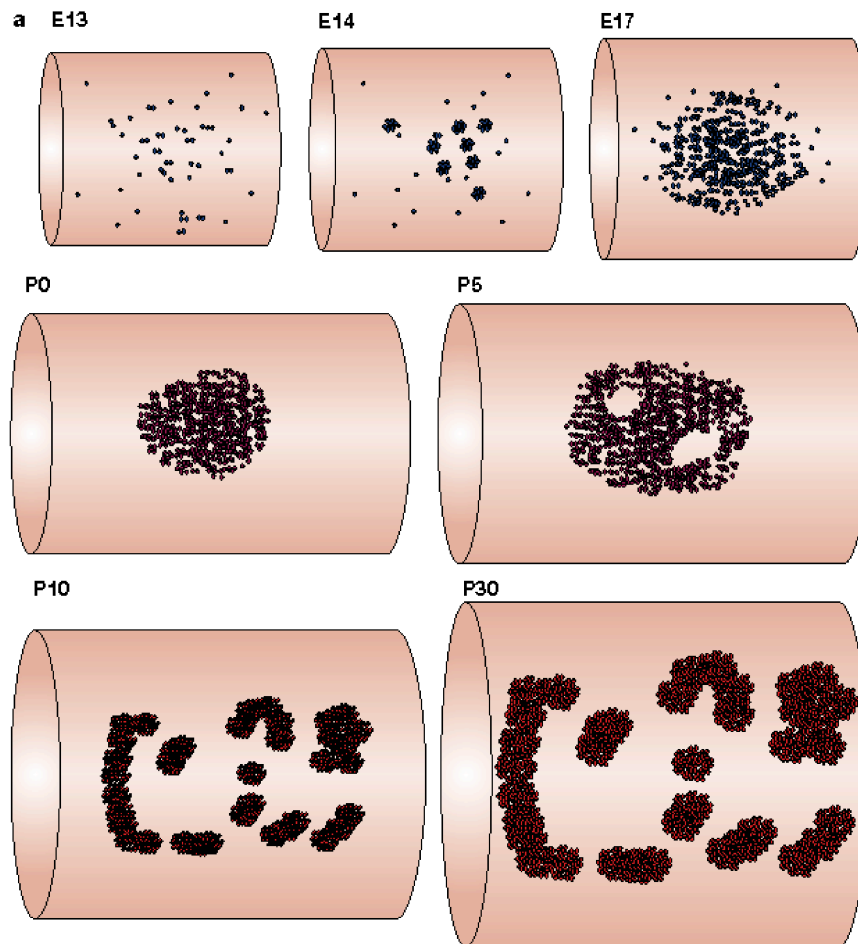


Gli elementi caratterizzanti le cellule sinaptiche:

- le vescicole sinaptiche

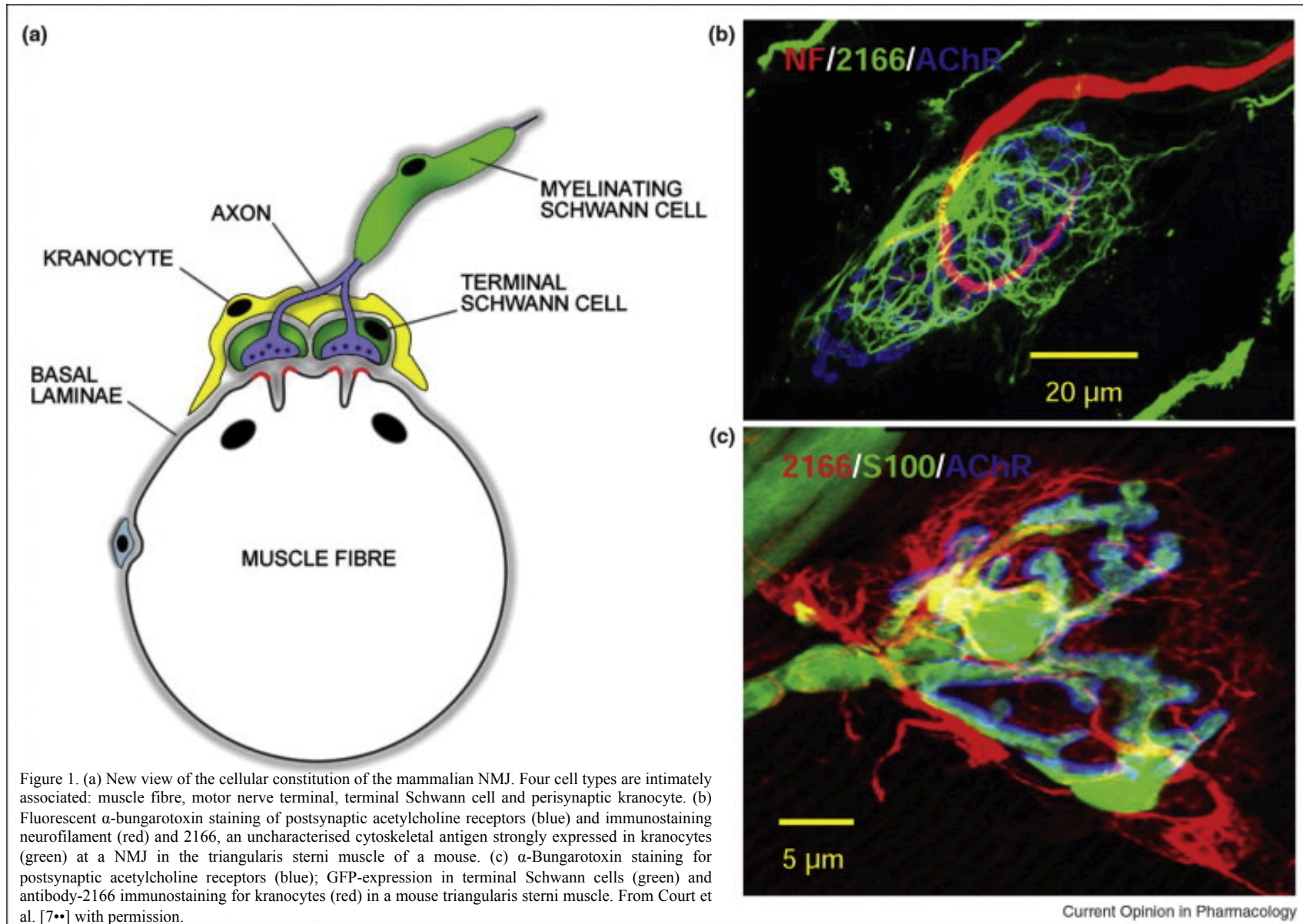
- i recettori per il neurotrasmettitore





Maturation of the postsynaptic apparatus. a | Acetylcholine receptor (AChR) microclusters coalesce to form a loose aggregate. Late in embryogenesis, the aggregate consolidates to form a plaque: its borders sharpen, its length decreases, and AChR density increases. Postnatally, the plaque becomes perforated to eventually form a pretzel-like array of branches. The branches then expand in an intercalary fashion as the muscle grows. Change in AChR colour denotes the switch from γ - to ϵ -containing AChRs.

A modern view of the NMJ



AChRs on the move

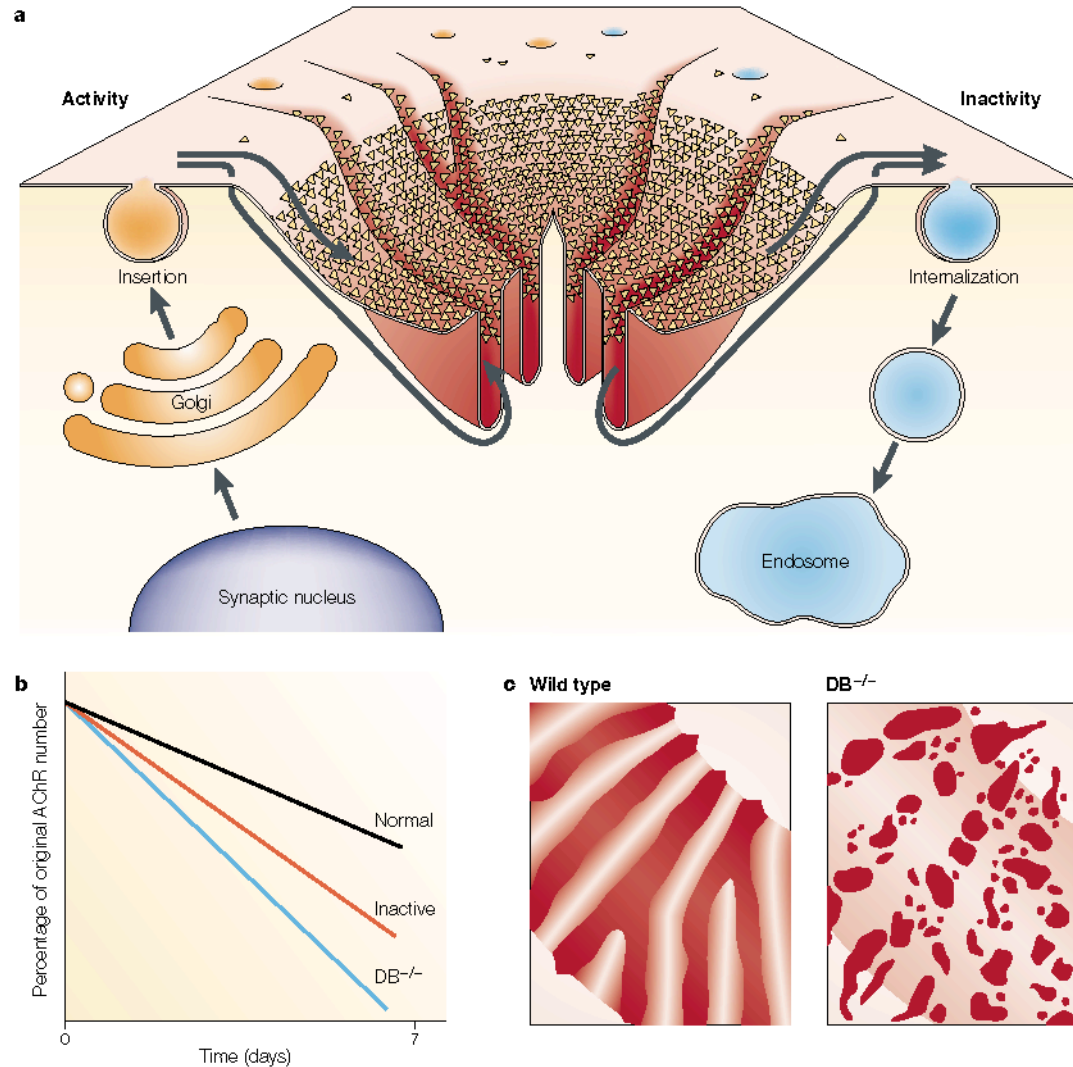
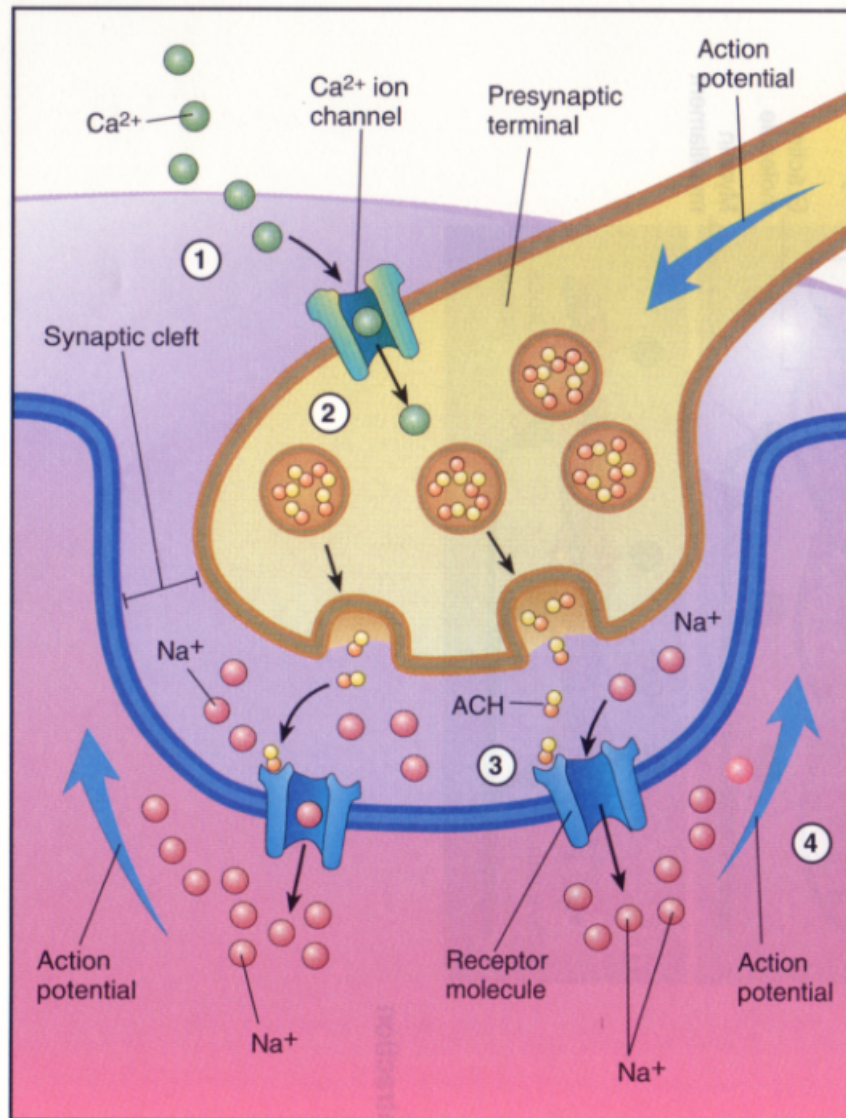


Figure 6 | **Turnover of AChRs in active, inactive and mutant muscles.** **a** | Three-dimensional view of a bouton, showing acetylcholine receptors (AChRs) at the crests of folds. In one model, new AChRs are added by exocytosis and old AChRs are removed by endocytosis, both in perisynaptic areas. The folds provide a route by which AChRs could enter or leave the dense patch. Arrows show processes that are believed to be affected by activity in adult muscle. **b** | AChR turnover (loss) is slow from normal, active NMJs, but accelerated by paralysis and by knocking out dystrobrevin (DB) (M. Akaaboune, R. M. Grady, J. R. S. and J. W. L., unpublished observations). **c** | *En face* views of a bouton in control and α -dystrobrevin mutant mice. Striations in the control are in folds. In the mutant, AChRs break up into granular microclusters.

Le fasi della trasmissione in una sinapsi chimica



1. An action potential arrives at the presynaptic terminal causing voltage-gated Ca^{2+} ion channels to open, increasing the Ca^{2+} ion permeability of the presynaptic terminal.
2. Ca^{2+} ions enter the presynaptic terminal and initiate the release of a neurotransmitter, acetylcholine (ACH), from synaptic vesicles in the presynaptic terminal.
3. Diffusion of ACH across the synaptic cleft and binding of ACH to ACH receptors on the postsynaptic muscle fiber membrane causes an increase in the permeability of ligand-gated Na^+ ion channels.
4. The increase in Na^+ ion permeability results in depolarization of the postsynaptic membrane; once threshold has been reached a postsynaptic action potential results.

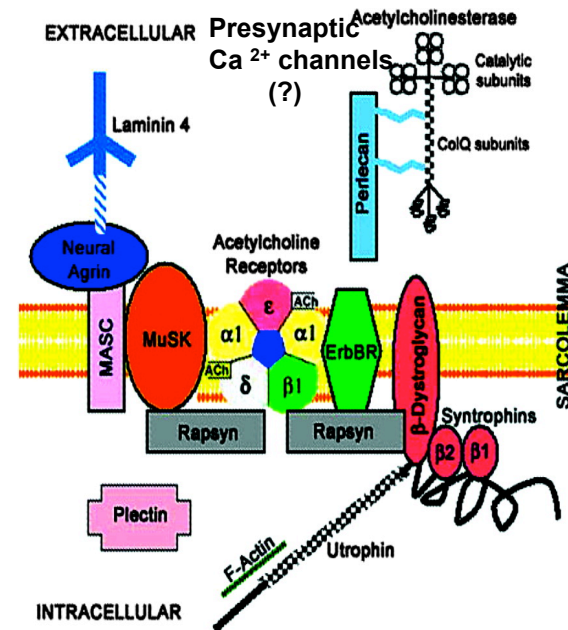
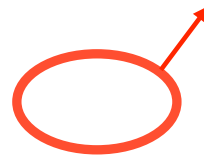
(a)

Synaptic cleft

Space of about 50 nm that separated the nerve and the muscle membrane and is comprised of basal lamina.

Basal lamina is made by:

- collagen IV, laminin, fibronectin, entactin, perlecan
- Agrin
- AChE



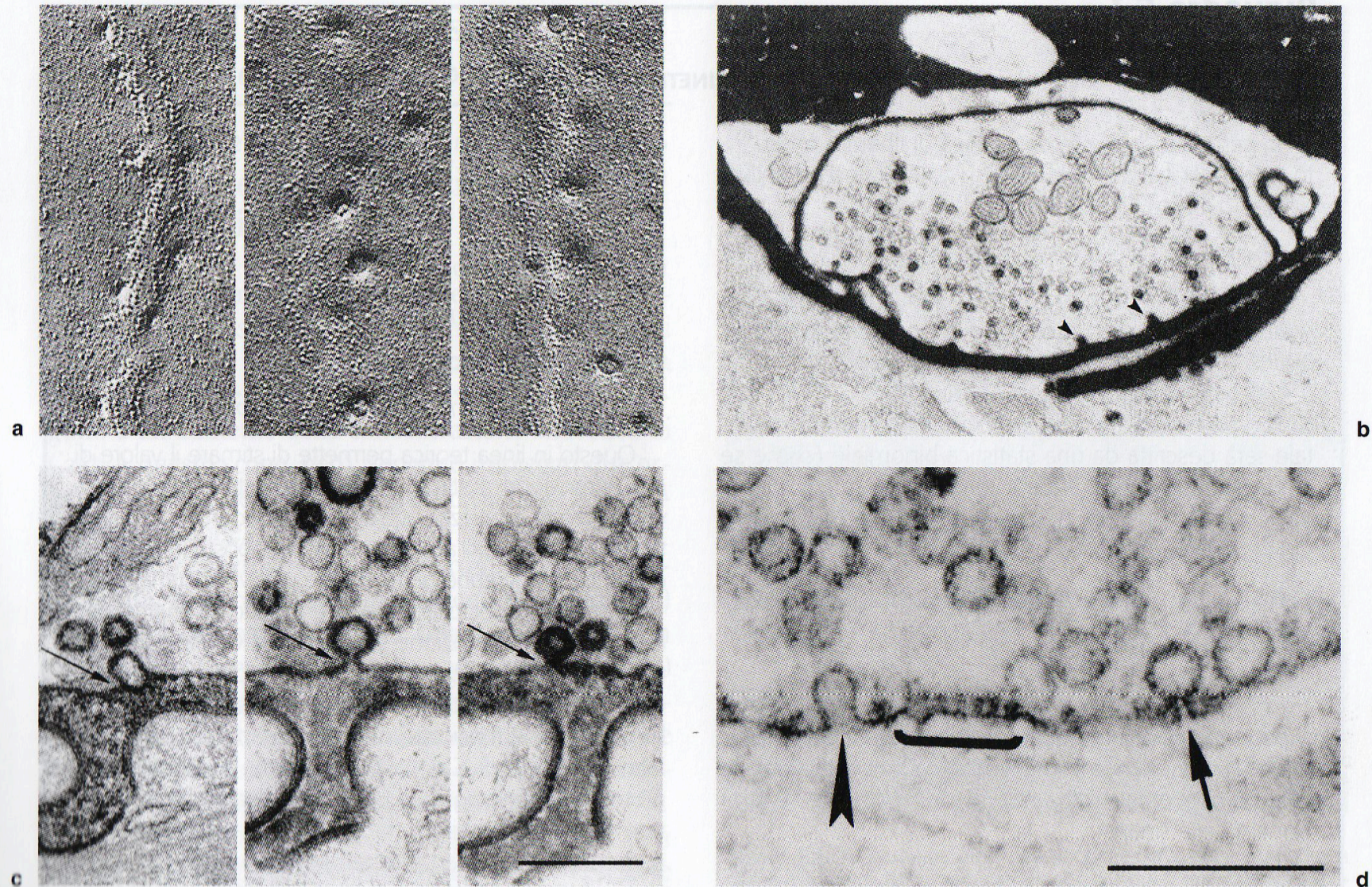
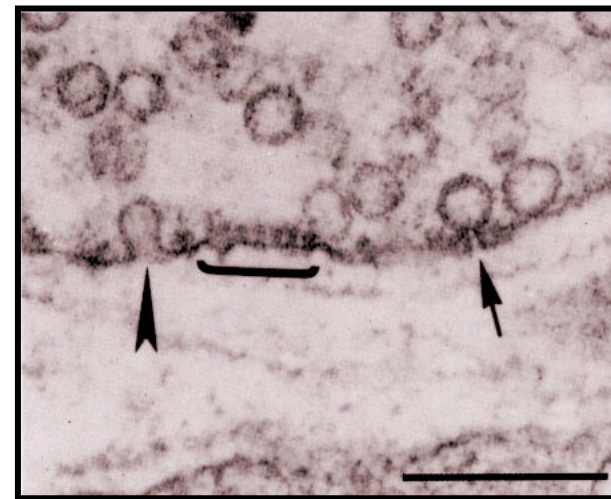
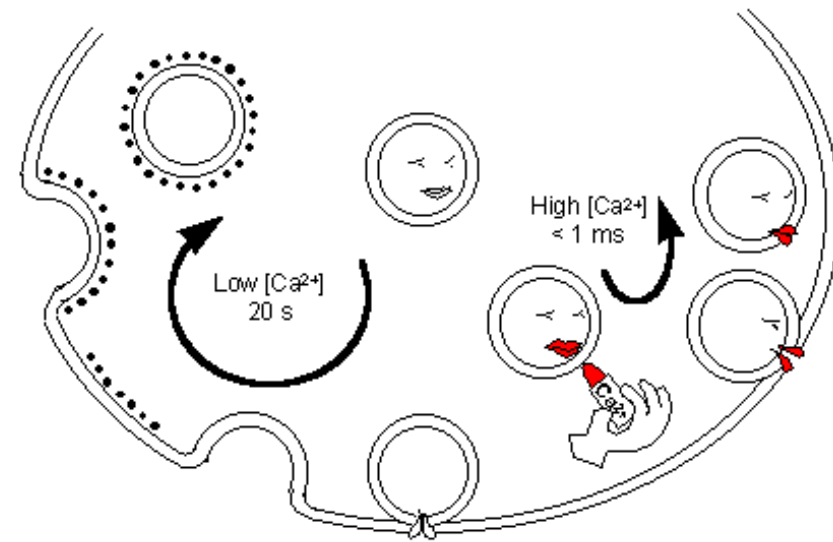
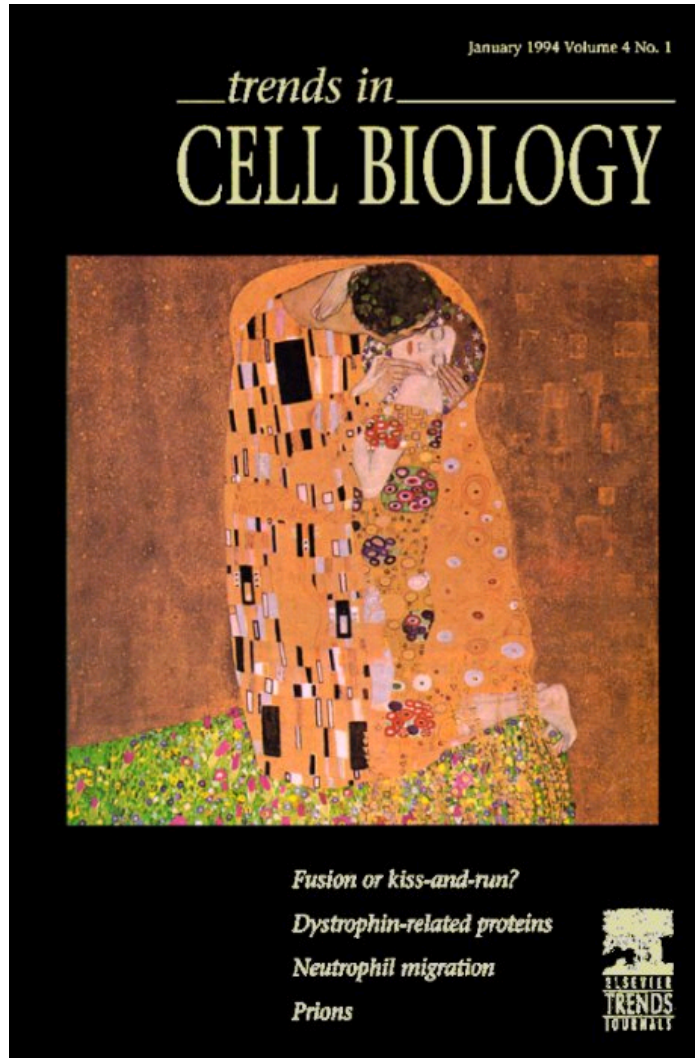


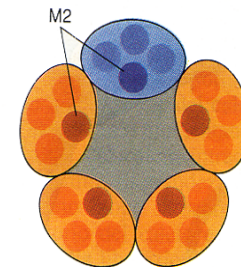
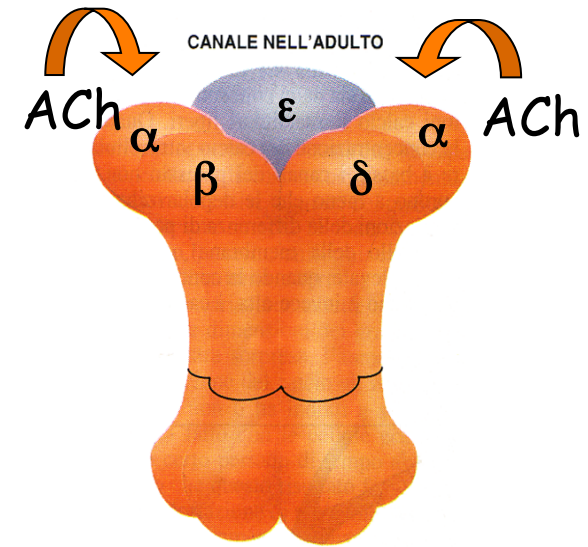
Figura 6.4 a, Immagini di *freeze-fracture* della membrana presinaptica di una giunzione neuromuscolare, che mostrano la fusione di vescicole sinaptiche presso la zona attiva, durante intensa attivazione della liberazione di trasmettitore. In queste condizioni le vescicole riciccolano attivamente come dimostrato in **b** dal fatto che molte vescicole appaiono marcate dopo stimolazione in presenza di un tracciante extracellulare (perossidasi del rafano). **c**, Nelle microfotografie sono illustrati vari momenti dell'interazione della vescicola con la membrana, prima e dopo che la vescicola carichi il tracciante. **d**, Nella microfotografia si vedono immagini di fusione (frecce) prodotte in risposta a un singolo stimolo e catturate con la tecnica del *quick-freezing*; la zona attiva è evidenziata dalla sottolineatura (pgc B. Ceccarelli, F. Grohovaz, R. Fesce, F. Torritarelli – barre di calibrazione, 0,2 μ m).

“Kiss and Run”

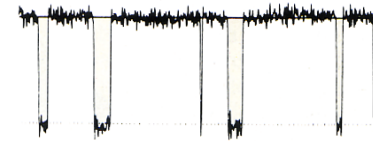


Il canale acetilcolinico nicotinico muscolare

(muscular nAChR)

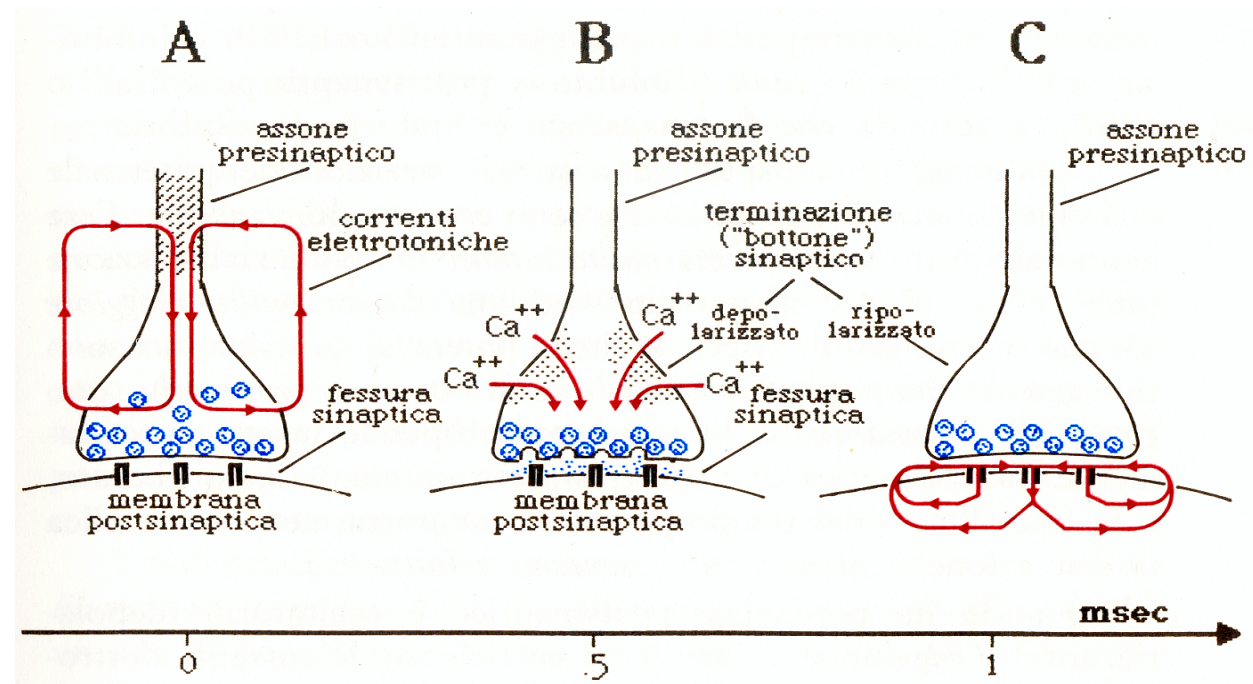


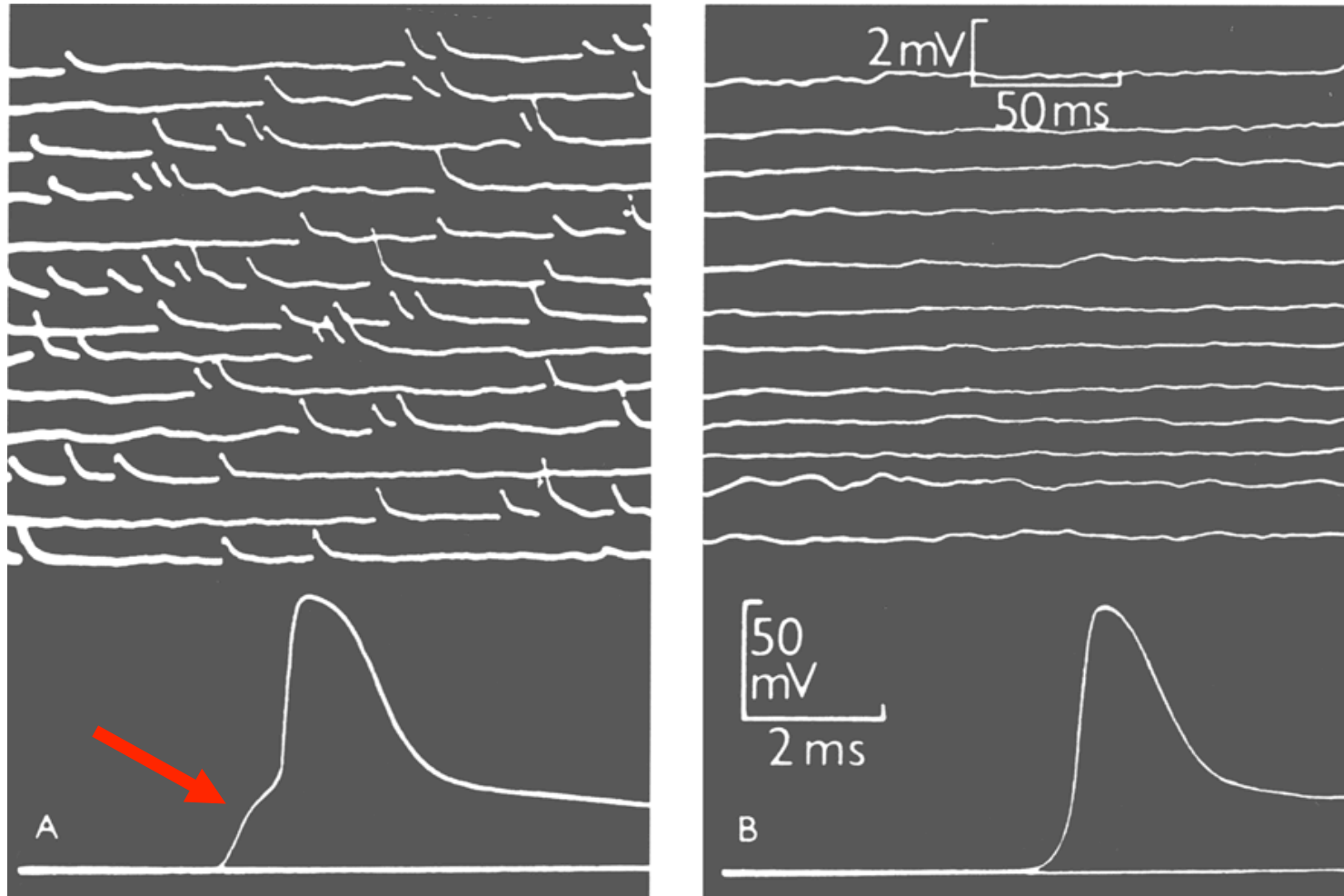
TRACCIATO DELLA CORRENTE
NEL CANALE DELL'ADULTO



Il neurotrasmettitore genera una depolarizzazione

Fig. 7.10 - I principali eventi che si succedono nelle sinapsi chimiche. **A:** Depolarizzazione della terminazione assonica ad opera delle correnti elettrotoniche generate dal potenziale d'azione. **B:** Attivazione dei canali voltaggio-dipendenti per il Ca^{++} , esocitosi del neurotrasmettitore e sua diffusione nella fessura sinaptica. **C:** Attivazione di canali ionici chemio-dipendenti presenti nella membrana post-sinaptica. Nella figura si immagina che le correnti ioniche che attraversano i canali aperti abbiano una direzione entrante, e siano quindi depolarizzanti (sinapsi eccitatoria). Non è rappresentato nessuno dei processi che mettono capo all'allontanamento del neurotrasmettitore dalla fessura sinaptica o alla sua demolizione.





Intracellular recording from single muscle fibre of frog. (A) at the motor end-plate. Upper part shows spontaneous 'miniature end-plate potentials' which are localized at junction and arise from sudden discharge by motor nerve ending of packets of acetylcholine, each containing thousands of molecules. Lower part shows single response to nerve impulse which was started by electric shock at the beginning of trace; first step of response is large 'end-plate potential' resulting from synchronous delivery of few hundred packets of acetylcholine, this leading to full size action potential. (B) Traces recorded in same muscle fibre, 2mm away from end-plate. Upper part shows much attenuated and barely recognizable residues of miniature end-plate potentials. Lower part shows propagated action potential, delayed by conduction over 2 mm distance and not preceded by end-plate step. (From: P. Fatt and B. Katz, *Nature*, 166, 597 (1950)).

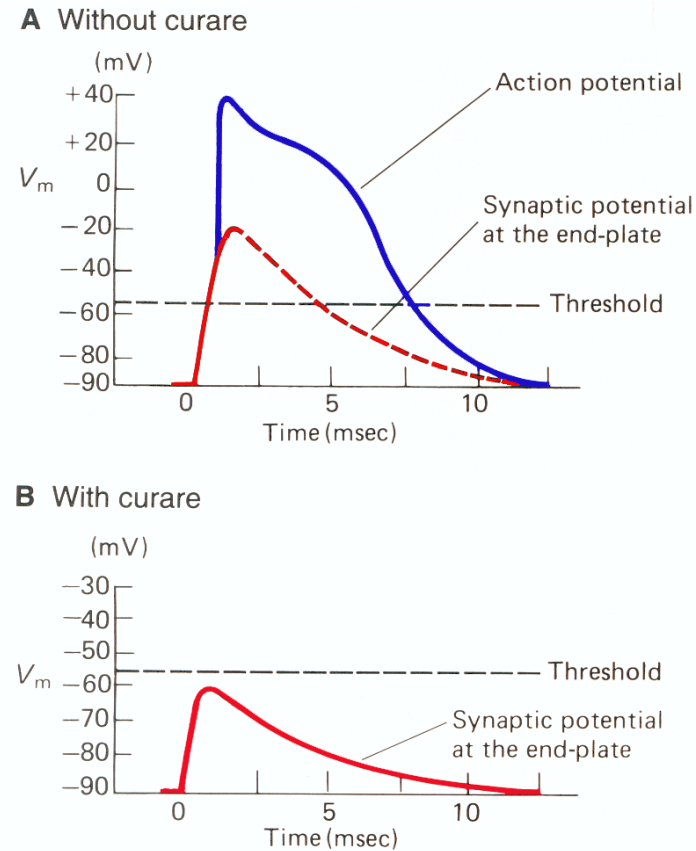
Il potenziale di placca innesca il potenziale d'azione

FIGURE 10-4

The end-plate potential (EPP) can be isolated pharmacologically for study.

A. Under normal circumstances stimulation of the motor axon produces a large EPP in the muscle fiber that surpasses threshold and triggers an action potential (**solid trace**). The **dashed trace** shows the inferred time course of the underlying EPP.

B. The EPP can be isolated in the presence of curare. Curare blocks the binding of ACh to its receptor and so reduces the amplitude of the EPP below threshold. This technique is used to study the currents and channels that contribute to the EPP, which are different from those producing an action potential. The values for the resting potential, synaptic potential, and action potential shown in these intracellular recordings are typical of a vertebrate skeletal muscle.



Gli eventi elettrici nella sinapsi chimica

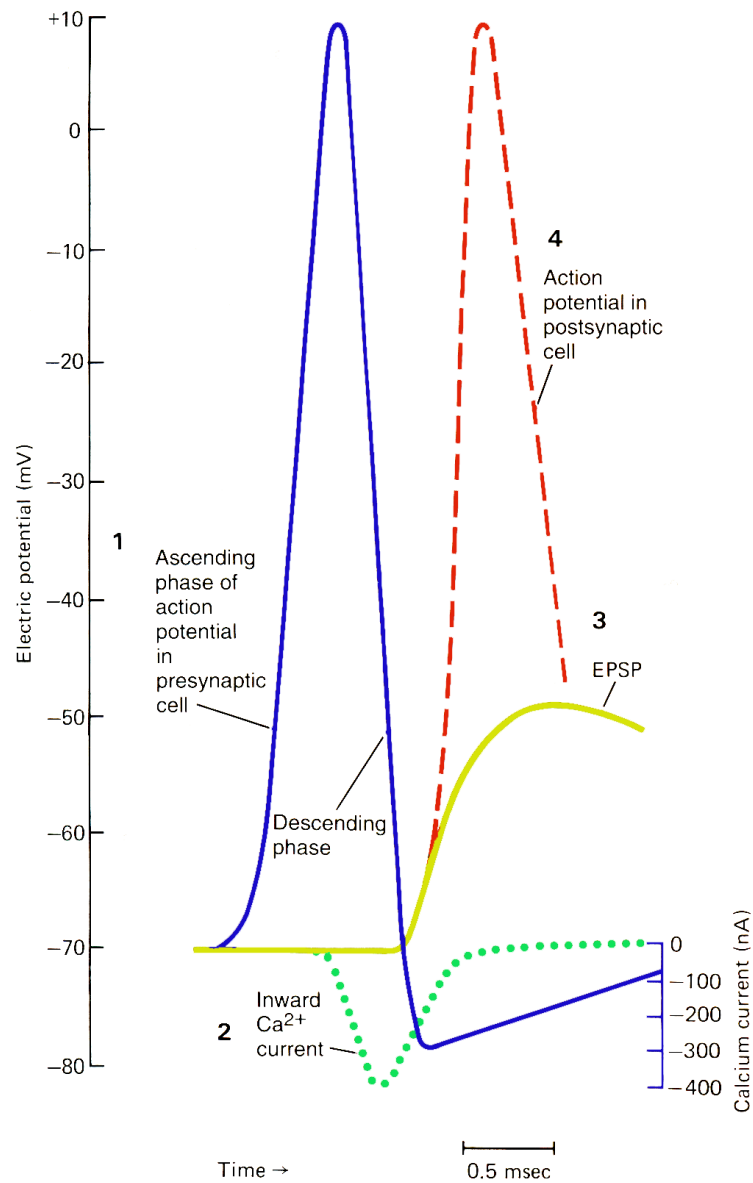
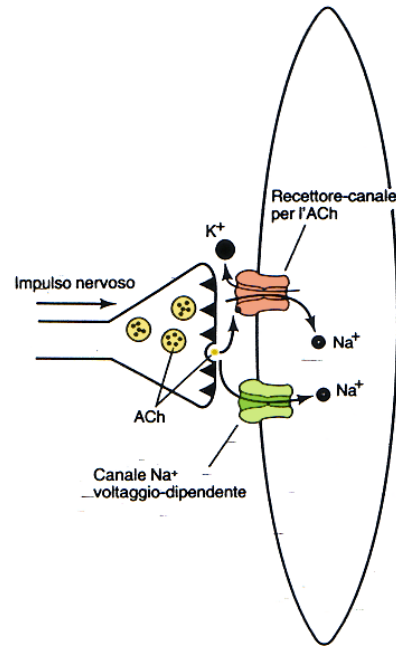


FIGURE 13-5

Time course of four events related to synaptic transmission. An action potential in the presynaptic cell (**1**) causes presynaptic Ca^{2+} channels to open and a Ca^{2+} current (**2**) to flow into the terminal leading to the release of neurotransmitter from the terminal. (Note that the Ca^{2+} current is turned on late during the falling phase of the presynaptic action potential.) The postsynaptic response to the transmitter begins soon afterward (**3**), and, if sufficiently large, will trigger an action potential in the postsynaptic cell (**4**). (Adapted from Llinás, 1982.)

Dal potenziale di placca al potenziale d'azione



A₁ Canali controllati dai neurotrasmettitori

Legame con l'ACh

Apertura del canale

Ingresso di Na⁺
e fuoriuscita di K⁺

Depolarizzazione

Risultato finale: potenziale di placca

A₂ Canali ad accesso regolato dal voltaggio

Apertura del canale Na⁺

Ingresso di Na⁺

Depolarizzazione

Risultato finale: potenziale d'azione

La patologia: la miastenia grave

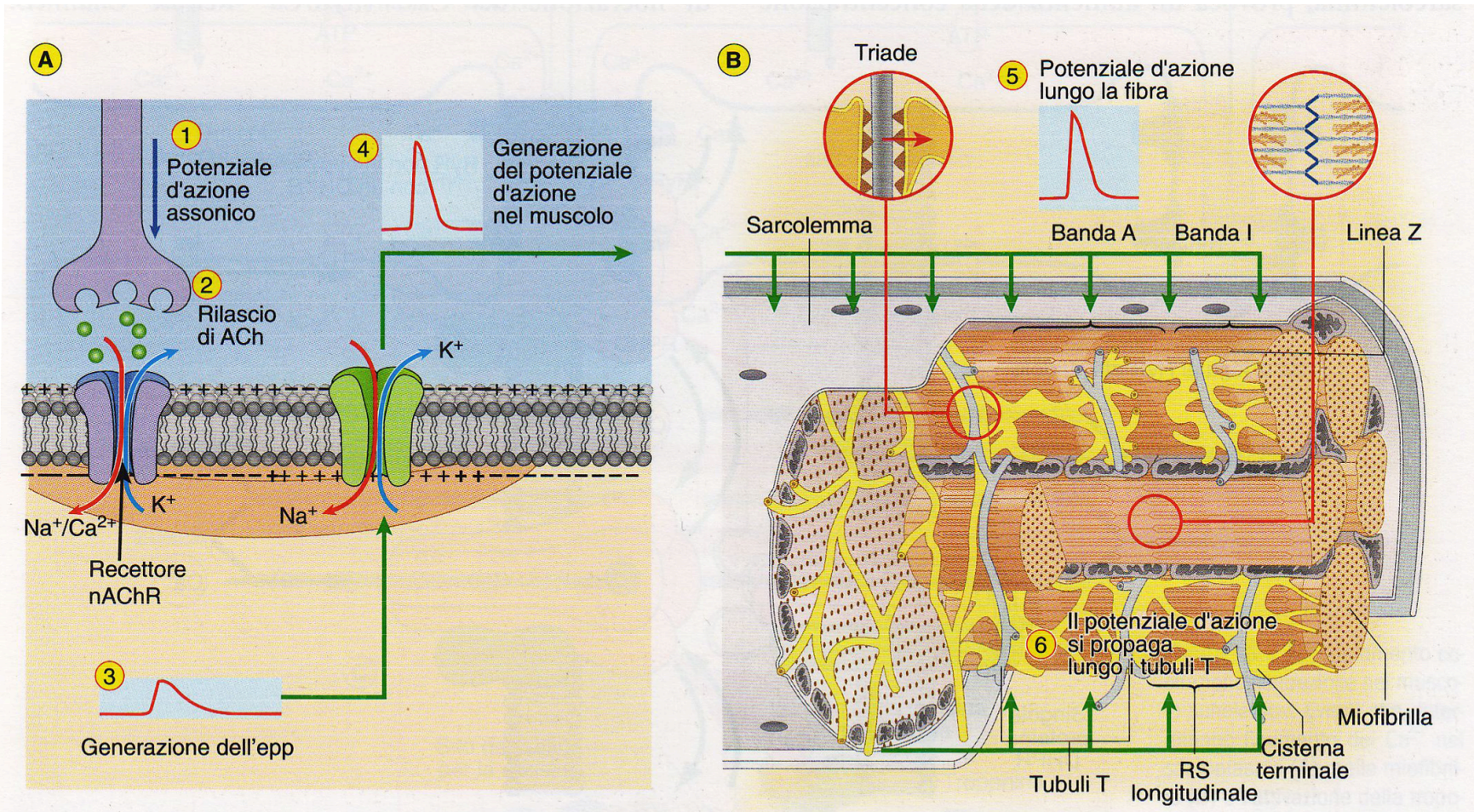
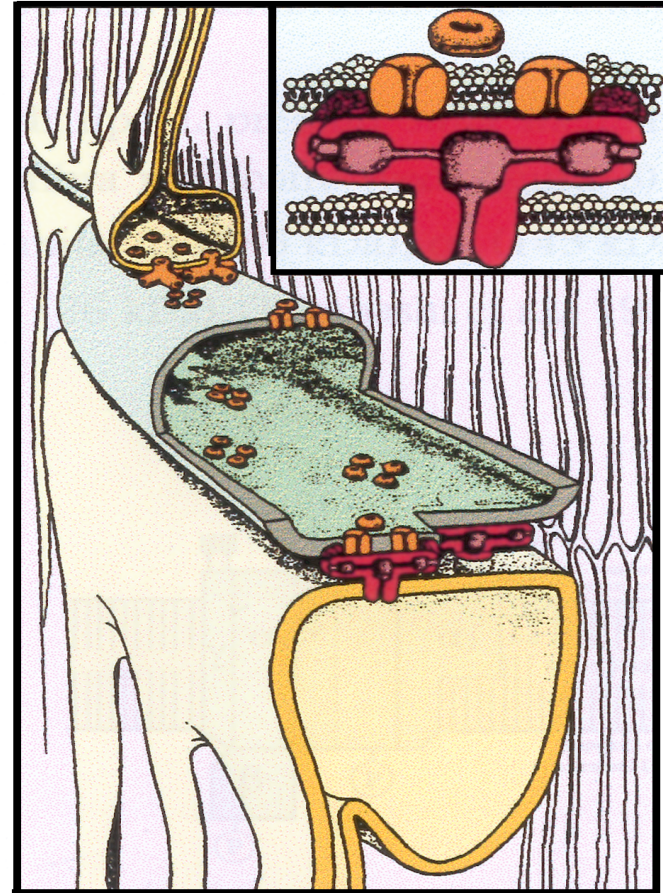


Figura 18.7 Eventi che determinano la generazione del potenziale d'azione (PA) nel sarcolemma (A) e la sua propagazione lungo il sarcolemma e nei tubuli T (B). In (A) il canale verde rappresenta simbolicamente i canali del Na⁺ che fanno entrare Na⁺ e i canali del K⁺ che fanno uscire K⁺. La loro apertura sequenziale dà origine al PA che si propaga lungo la fibra muscolare.

La triade ed i canali ionici:

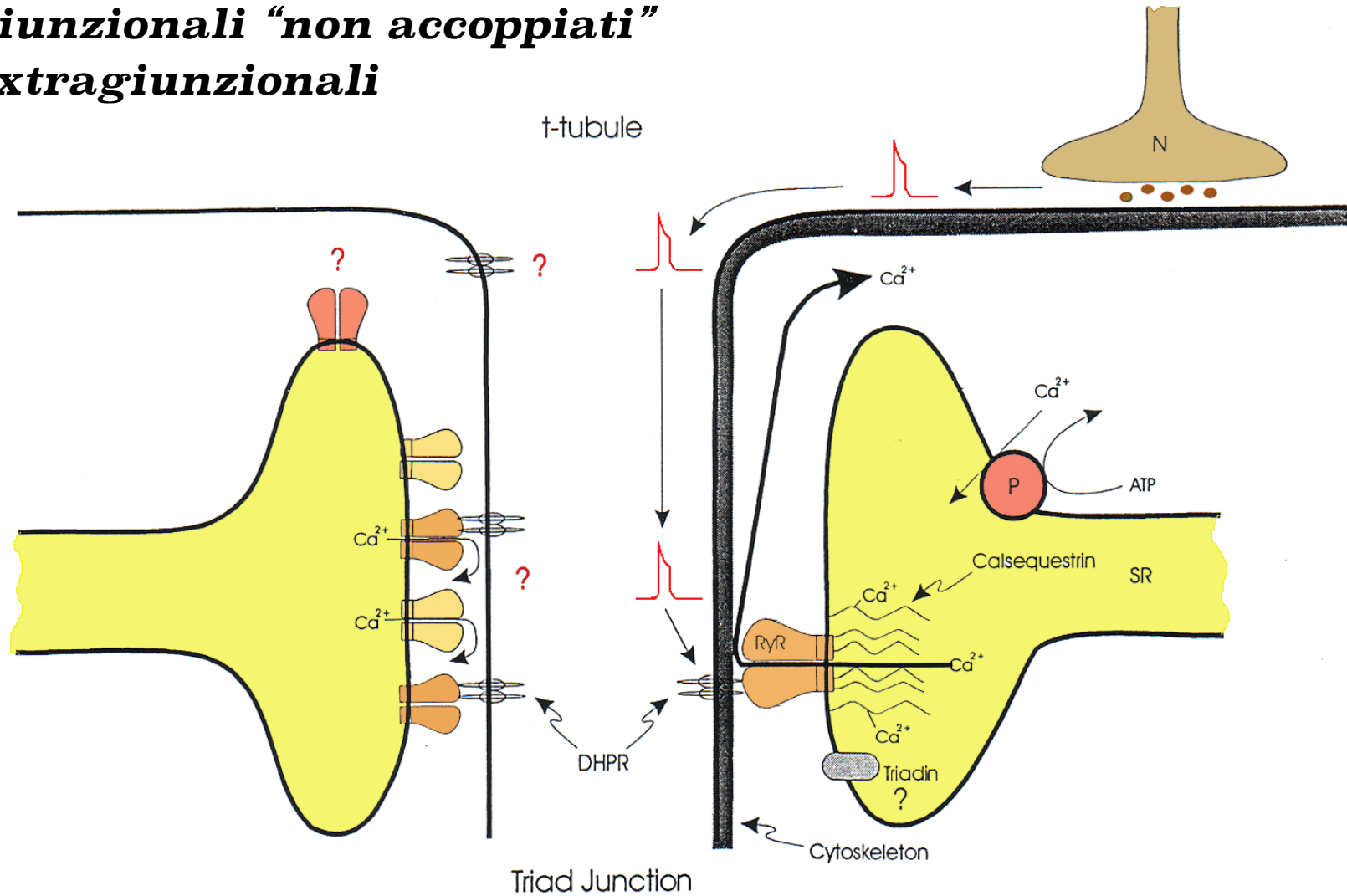
- canali voltaggio-dipendenti per il Ca^{2+} (DHPR)
- canali per il Ca^{2+} rianodina-sensibili o canali caffeina-sensibili (RyR)



(modificata da Ríos and Pizarro, 1991)

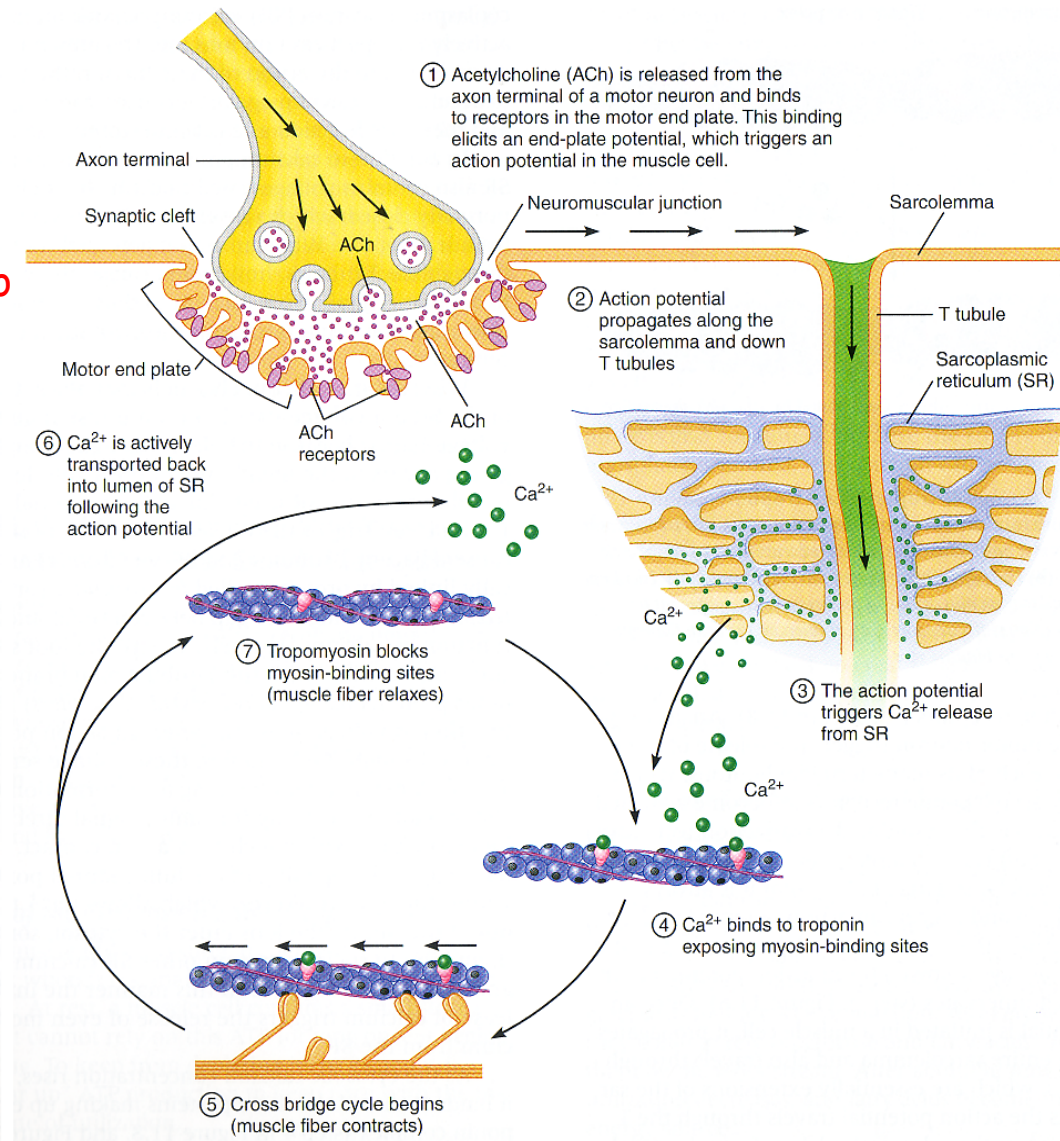
I diversi tipi di RyR

- Giunzionali "accoppiati"
- Giunzionali "non accoppiati"
- Extragiunzionali



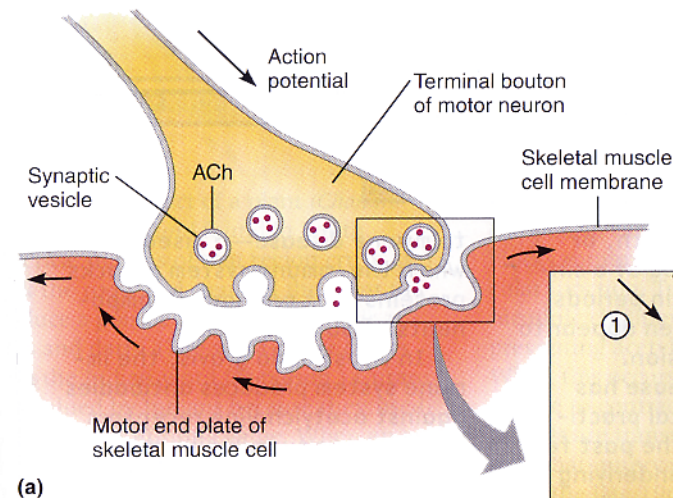
Il meccanismo di accoppiamento eccitazione-contrazione

- 1) Rilascio di acetilcolina
- 2) Potenziale d'azione muscolare
- 3) Liberazione di Ca^{2+} dal reticolo
- 4) Interazione Ca^{2+} -troponina
- 5) Interazione actina-miosina
- 6) Contrazione



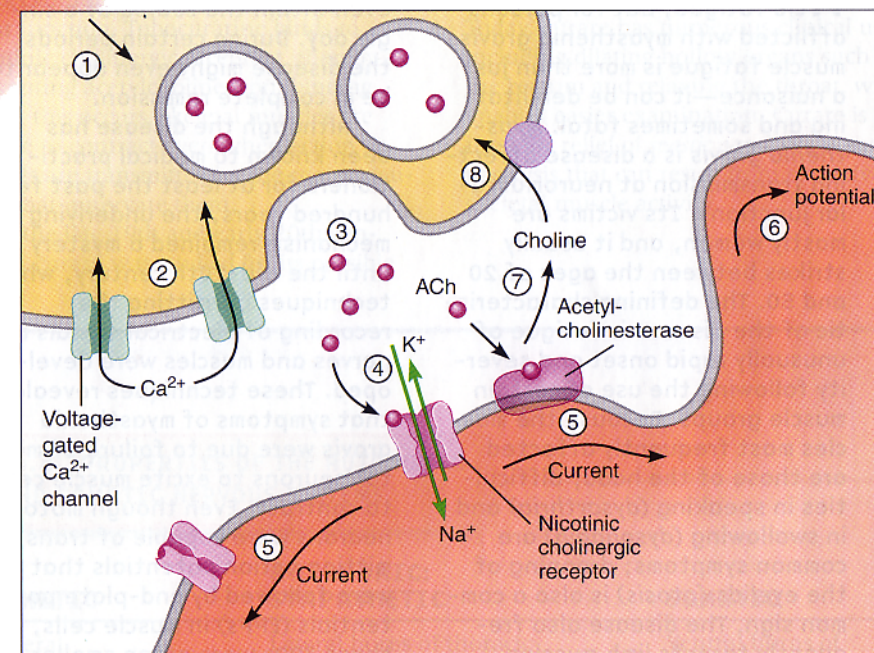
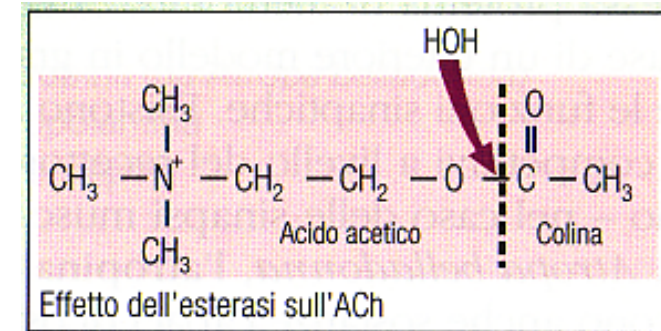
Lo spegnimento della sinapsi chimica

- desensibilizzazione del recettore e/o canale
- diffusione del neurotrasmettitore
- inattivazione chimica o riassorbimento del neurotrasmettitore



(a)

FIGURE 10.15 Functional anatomy of the neuromuscular junction. (a) Both the axon terminal of the motor neuron and the portion of the plasma membrane of the skeletal muscle called the motor end plate are specialized at the neuromuscular junction. (b) Communication at the neuromuscular junction. When an action potential arrives at the axon terminal of a motor neuron (1), voltage-gated calcium channels open and calcium enters the cytoplasm (2). The entry of calcium triggers the release by exocytosis of acetylcholine (3), which diffuses to and binds to nicotinic cholinergic receptors at the motor end plate, opening small cation channels. Sodium enters the cell (4), producing an end-plate potential that generates currents throughout the plasma membrane of the skeletal muscle cell (5), depolarizing the membrane to threshold to generate an action potential (6). The action potential spreads along the skeletal muscle cell membrane, ultimately stimulating contraction. Acetylcholinesterase degrades acetylcholine to produce acetate and choline (7), which is actively transported into the terminal bouton (8), where it can be used to synthesize more acetylcholine.



(b)

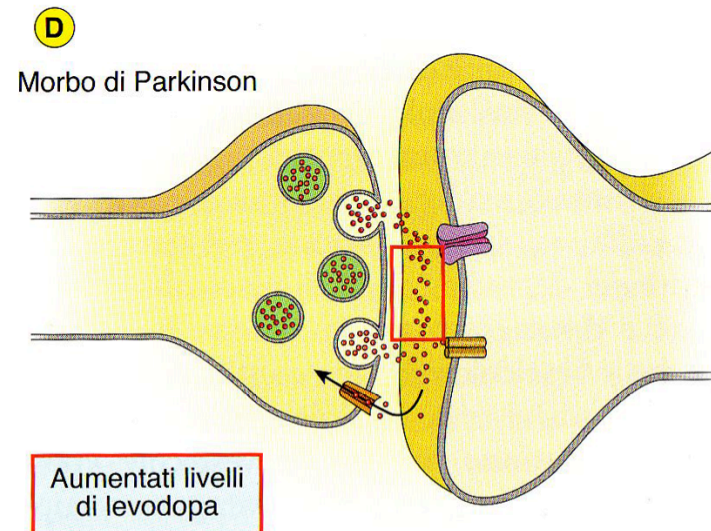
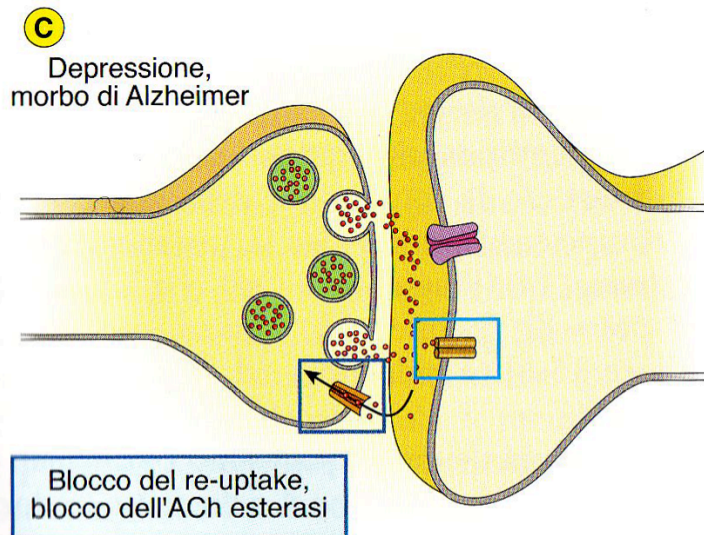
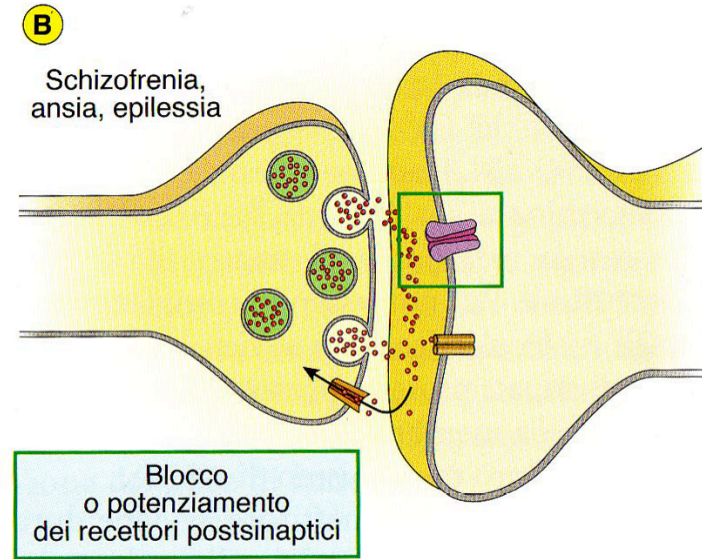
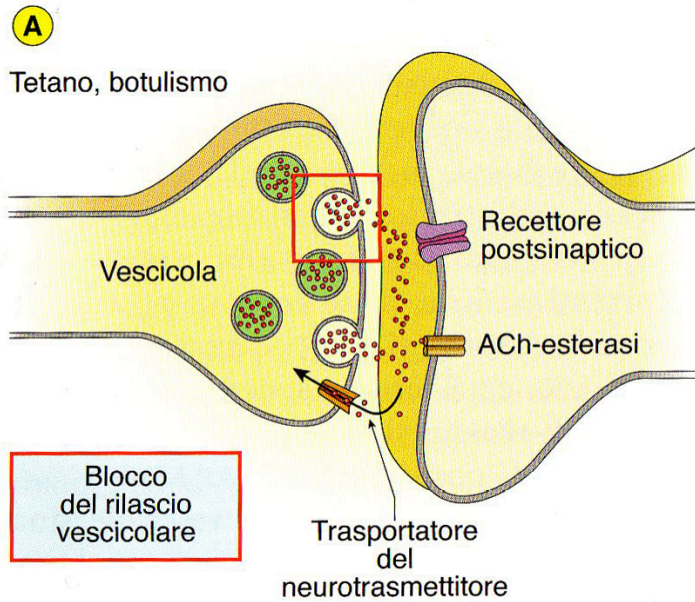
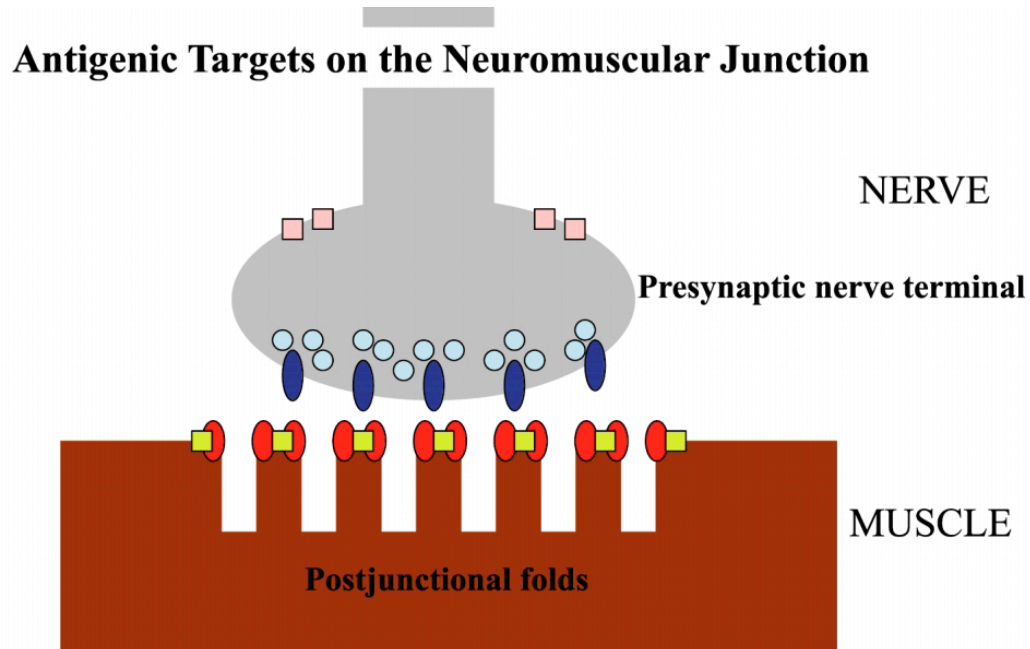


Figura 4.15 Disfunzioni della sinapsi chimica del sistema nervoso centrale ed azione farmacologica dei farmaci usati nelle diverse terapie. In ciascun pannello sono rappresentati il terminale presinaptico e la regione postsinaptica di una sinapsi chimica dove agisce il neurotrasmettitore rilasciato per esocitosi. I riquadri colorati dei vari pannelli indicano la zona in cui o è presente la disfunzione (tetano e botulismo) oppure è localizzata l'azione dei farmaci che caratterizzano il trattamento terapeutico.

Tabella 4.2 Sostanze modulatrici della trasmissione sinaptica e loro meccanismo d'azione. Molte molecole sono in grado di modulare la trasmissione sinaptica. In virtù di questa loro proprietà alcune di esse sono impiegate nel trattamento farmacologico di malattie che derivano da alterazioni della trasmissione sinaptica. Esse possono essere distinte principalmente in base al loro sito d'azione: presinaptico o postsinaptico.

Tipo di farmaco	Sito d'azione	Meccanismo d'azione	Impiego terapeutico
Inibitori del trasporto di neurotrasmettitori (es. reserpina)	Presinaptico	Inibiscono il trasporto della noradrenalina nelle vescicole sinaptiche del sistema nervoso simpatico	Iperensione
Tossina botulinica	Presinaptico	Inibisce il processo di esocitosi nella giunzione neuromuscolare (paralisi flaccida)	Distonie (blefarospasmo, emispasmo facciale)
Tossina tetanica	Presinaptico	Inibisce il processo di esocitosi degli interneuroni inibitori spinali (paralisi spastica)	
Inibitori del re-uptake (es. fluoxetina o Prozac)	Presinaptico	Inibiscono il meccanismo di up-take della serotonina e della noradrenalina nei neuroni centrali	Depressione e altri disturbi dell'umore
Precursori dei neurotrasmettitori (es. levodopa)	Presinaptico	Aumentano la concentrazione del neurotrasmettitore rilasciato; potenziano l'azione delle sinapsi dopaminergiche	Malattia di Parkinson
Antagonisti recettoriali (es. clorpromazina e aloperidolo)	Postsinaptico	Bloccano i recettori dopaminergici e riducono in questo modo gli effetti dell'eccessivo rilascio di dopamina nei neuroni centrali	Schizofrenia
Agonisti recettoriali: barbiturici (es. fenobarbitale)	Postsinaptico	Potenziano l'azione inibitoria del recettore GABA _A	Ansiolitici
Agonisti recettoriali: benzodiazepine (es. diazepam)	Postsinaptico	Potenziano l'azione inibitoria del recettore GABA _A	Ansiolitici, antiepilettici
Anticolinesterasici (es. fisostigmina, edrofonio, galantamina)	Postsinaptico	Inibiscono l'idrolisi dell'ACh; potenziano l'azione eccitatoria del recettore nAChR	Miastenia grave, malattia di Alzheimer

Disorders of the NMJ



- Voltage-gated potassium channels (VGKC)
- Voltage-gated calcium channels (VGCC)
- Acetylcholine receptors (AChR)
- Muscle specific kinase (MuSK)

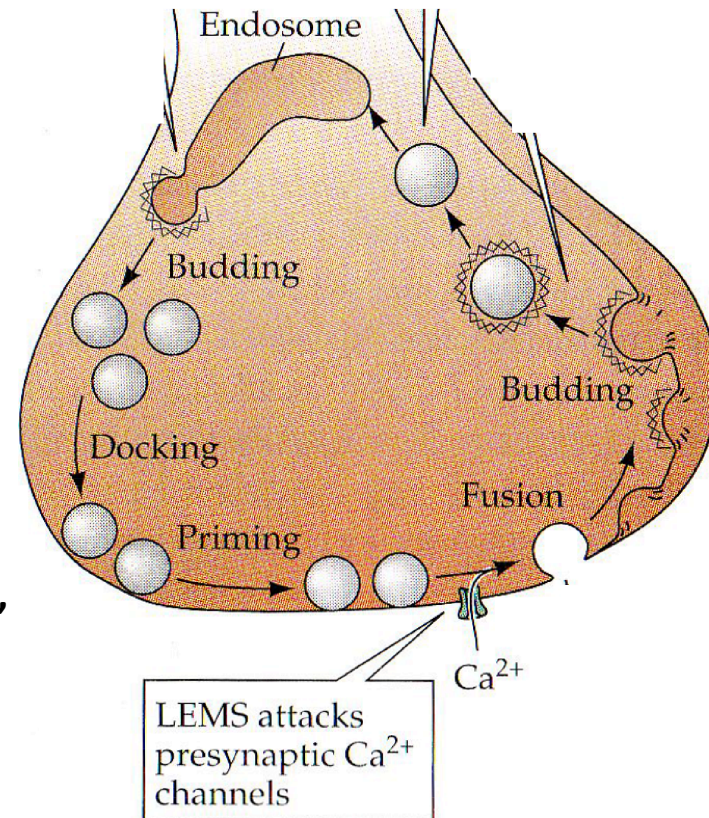
Antigenic targets on the mammalian neuromuscular junction; AChR (red) and muscle-specific kinase (MuSK, yellow) situated on the postsynaptic membrane and voltage-gated potassium (VGKC, pink) and calcium channels (VGCC, blue) present on the presynaptic nerve terminal. The presynaptic nerve terminal (grey), 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.

Reduction in release of ACh

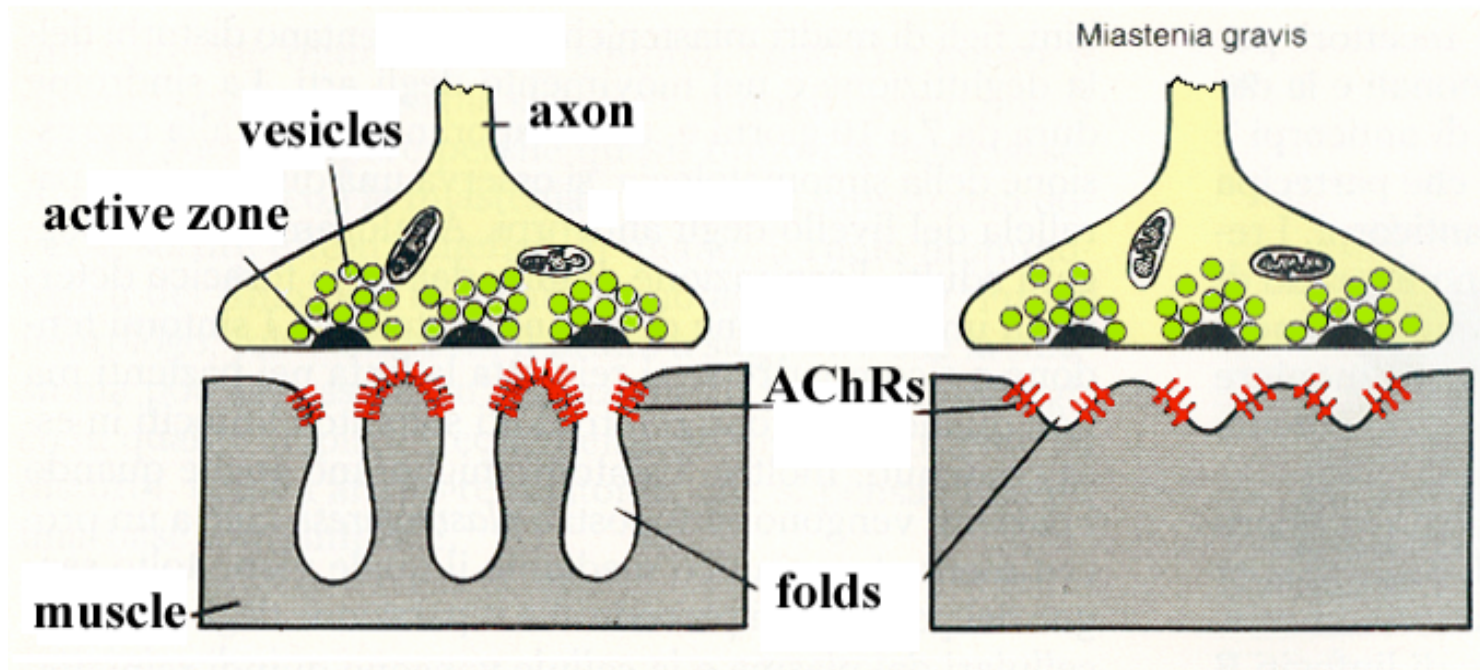
Leamber-Eaton myasthenic syndrome (LEMS)

* 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



Reduction in sensitivity of the postsynaptic membrane to ACh



Myasthenia gravis

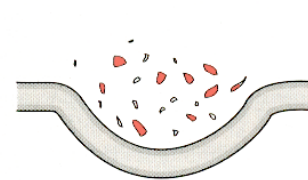
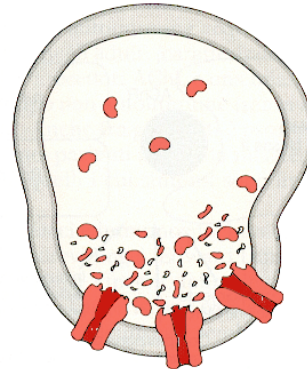
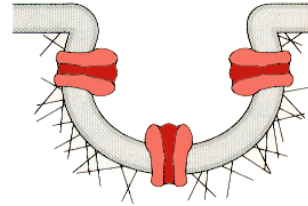
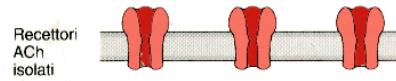


- Asymmetrical ptosis (a drooping of one or both eyelids),
- Diplopia (double vision) due to weakness of the muscles that control eye movements

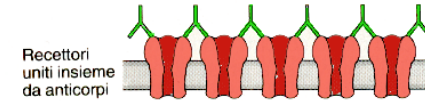
“grave muscle weakness”

**L'effetto
degli
anticorpi:
la rapida
internalizzazione
del nAChR
(o turnover)**

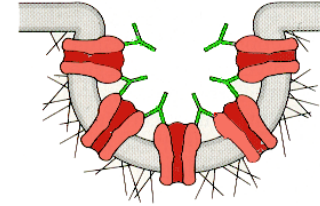
A Turnover normale, 5-7 giorni



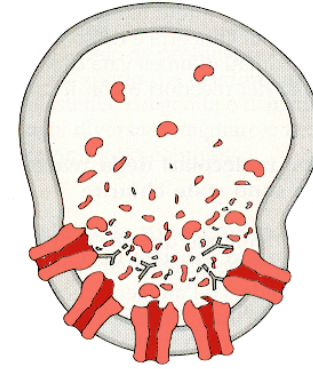
B Turnover rapido per azione antigenica nella miastenia gravis, 2,5 giorni



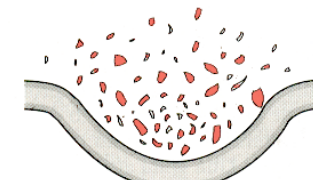
Endocitosi dovuta a meccanismi che interessano le strutture del citoscheletro e che richiedono dispendio energetico



Distruzione delle proteine nei lisosomi



Liberazione di residui aminoacidici da parte della cellula





Nature Reviews | Immunology

Figure 1 | Notable faces in myasthenia gravis research. a | Thomas Willis (circa 1667) from the Poole collection of the Bodleian Library, University of Oxford. A London physician who made pertinent observations of many neurological diseases, including arguably the first description of myasthenia gravis. Reproduced, with permission, from REF. 88 © 1991, RSM Press Ltd. b | Mary Walker (circa 1950), a resident at St Alfege's Hospital in London, who, in 1934, was the first doctor to try acetylcholinesterase inhibitors for the treatment of myasthenia gravis. Photograph courtesy of Mrs Pamela Furtek (reproduced from the Mary Walker website, courtesy of the Medical Photography Department, Queen Elizabeth Hospital, UK).

Prevalence & Mortality during 1900-2000

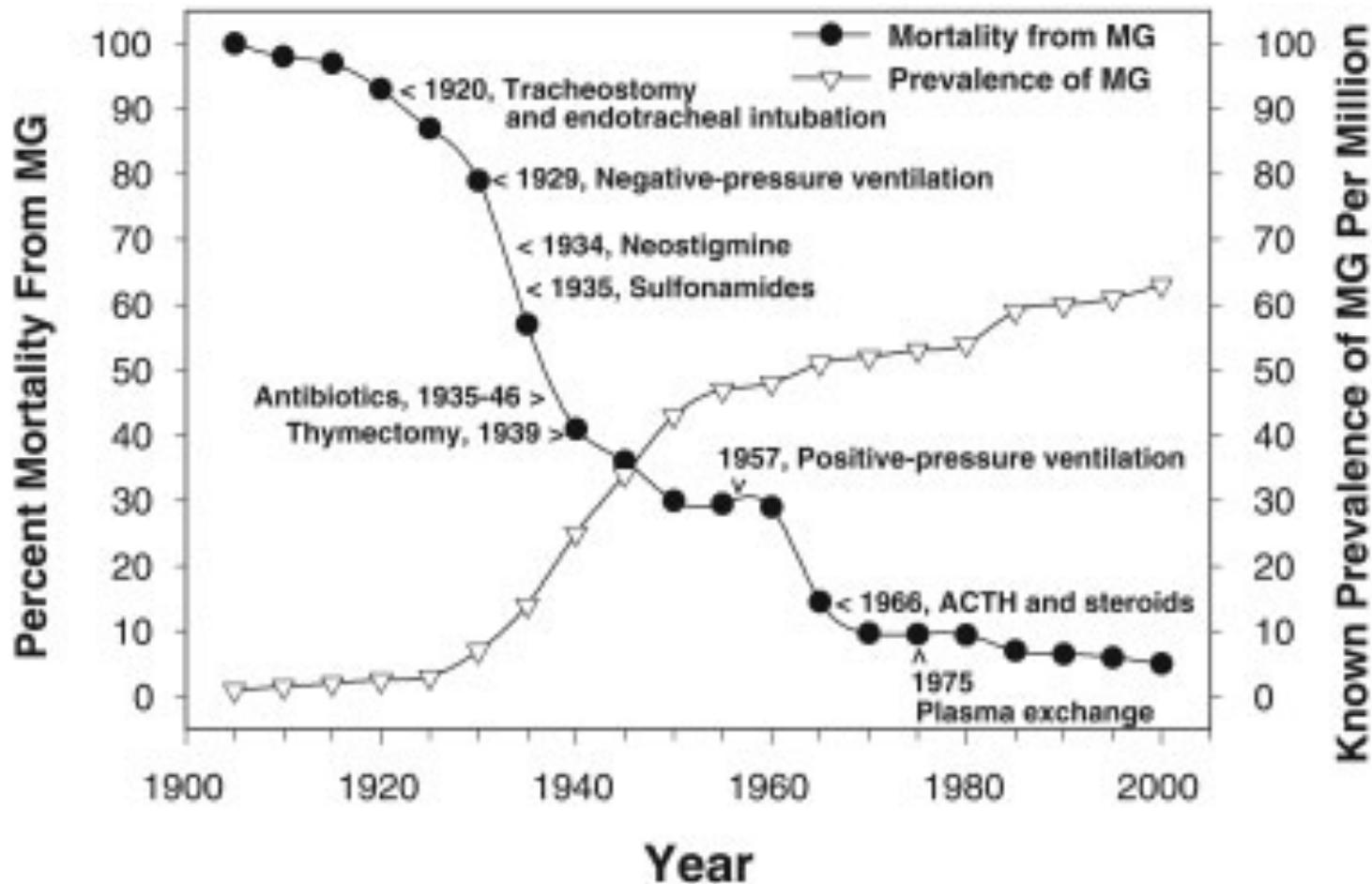


Table 2

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

	<u>MEPP</u>	<u>EPP</u>
	(mV)	(mV)
Control	0.91	40.2
(<i>n</i> =38 fibers)	± 0.03	± 1.3
MG	0.48	23.5
(<i>n</i> =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.

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

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

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

Congenital Myasthenic Syndromes

Fast-channel syndrome

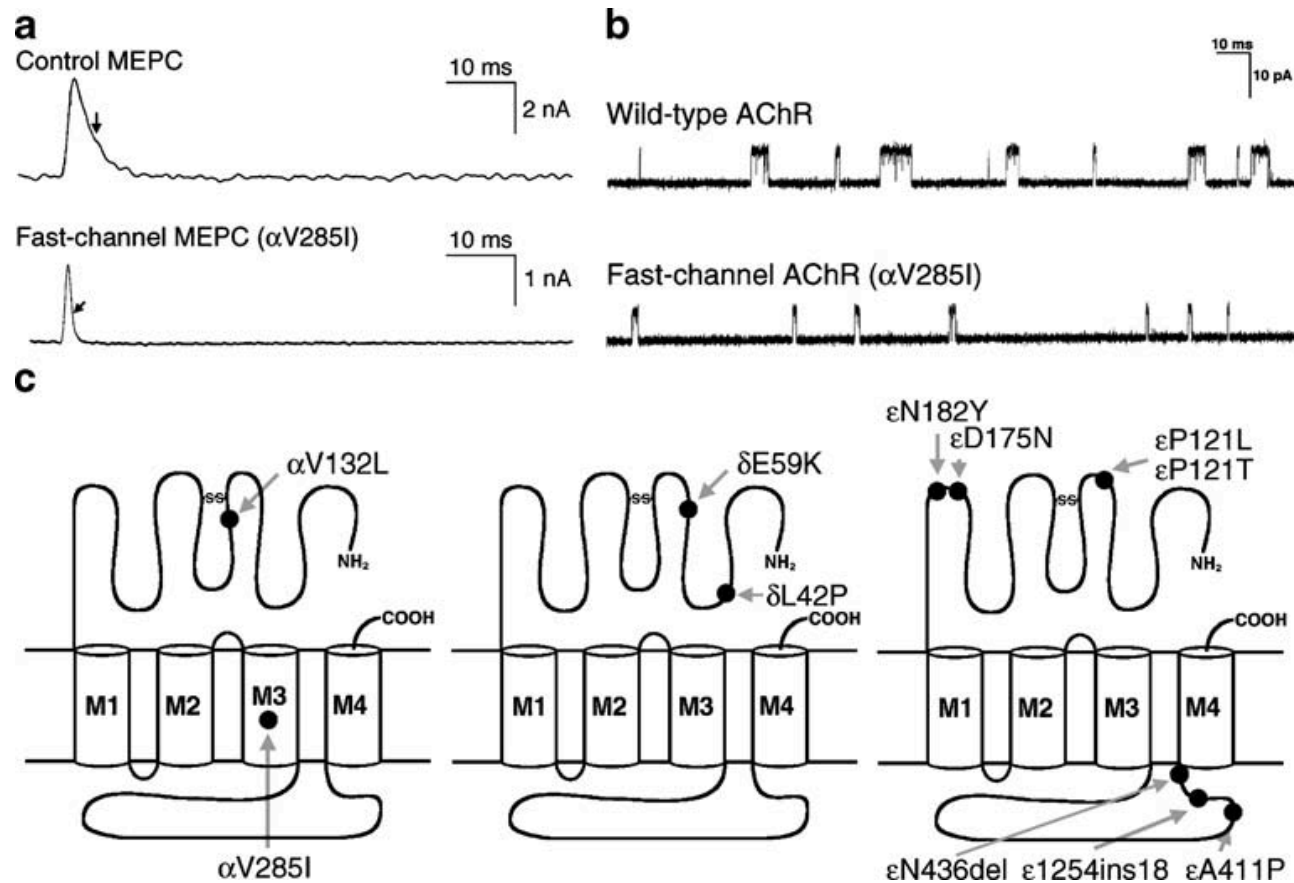


Figure 4 Fast-channel syndromes. **a** MEPC recorded from EPs of a control subject and a patient harboring the α V285I fast-channel mutation. Arrows indicate decay time constants. **b** Single-channel currents from wild-type and fast-channel (α V285I) AChRs expressed in HEK cells. **c** Schematic diagram of fastchannel mutations in the AChR α , β , and δ subunits. (From Engel 2004, by permission)

Slow-channel syndrome

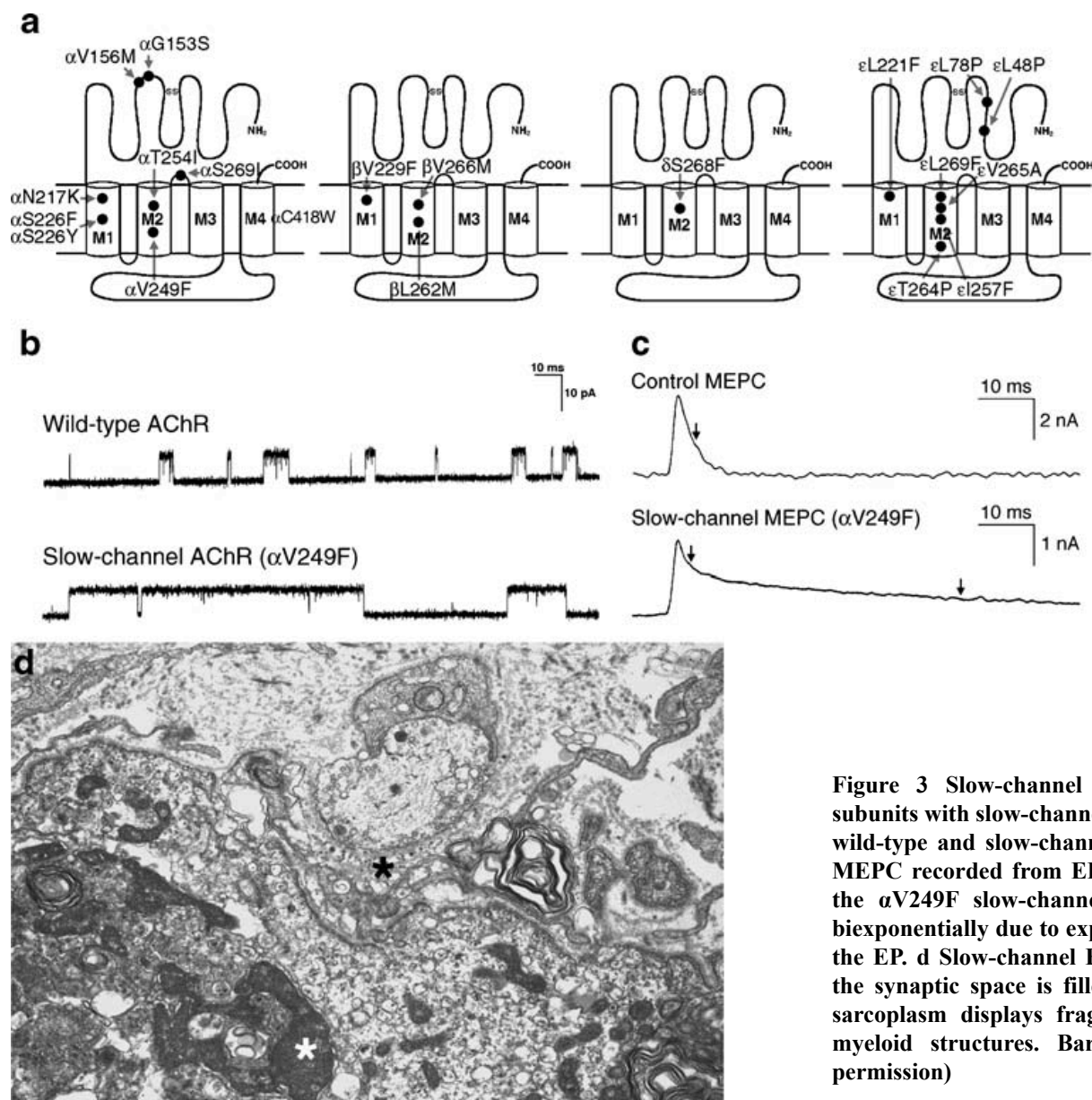


Figure 3 Slow-channel syndromes. **a** Schematic diagram of AChR subunits with slow-channel mutation. **b** Single-channel currents from wild-type and slow-channel (α V249F) AChRs expressed in HEK cells. **c** MEPC recorded from EPs of a control subject and a patient harboring the α V249F slow-channel 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 μ m. (a to c are from Engel 2004, by permission)