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

mEPP, EEP and AP

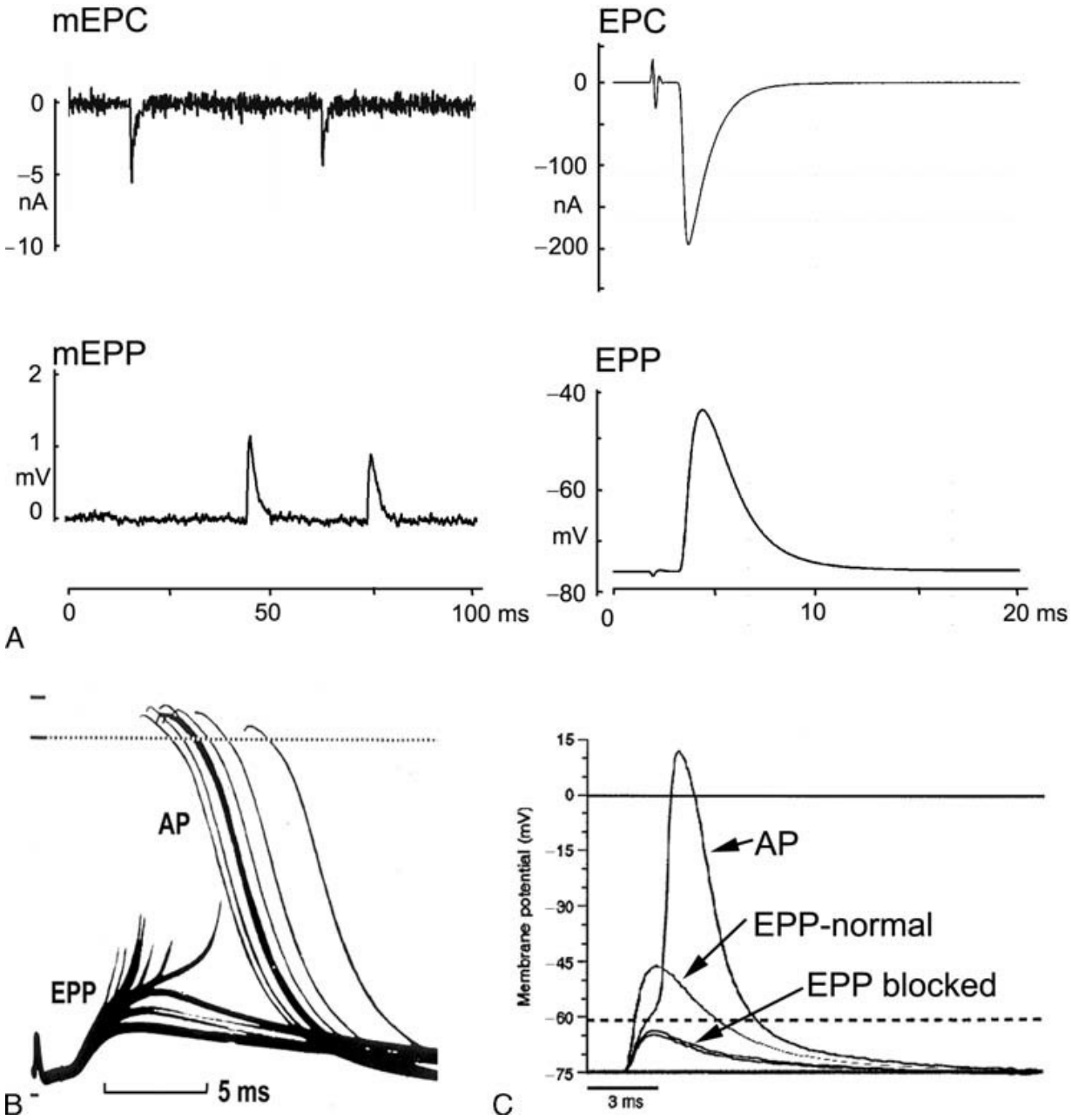
mEPP: **miniature endplate potential** is the postsynaptic depolarization produced by the content of a single vesicle

EPP: **endplate potential** is the net postsynaptic depolarization produced by the release of ACh from the synaptic vesicles

AP: **action potential**

Electrical events at the NMJ

Quantal content
m=EPC/mEPC



Neuromuscular transmission

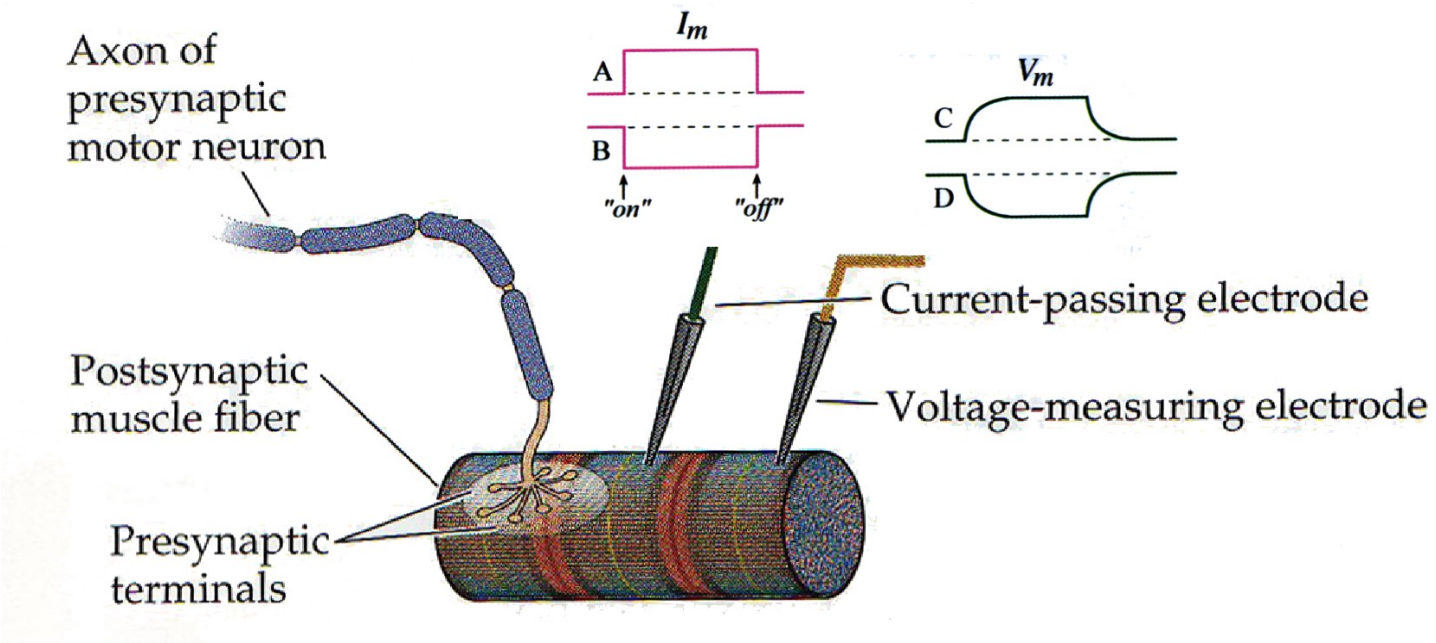
"is the process that translates a motorneuron action potential to action potentials in all type of fibers contacted by that motoneuron".

The steps of NM transmission are as follows:

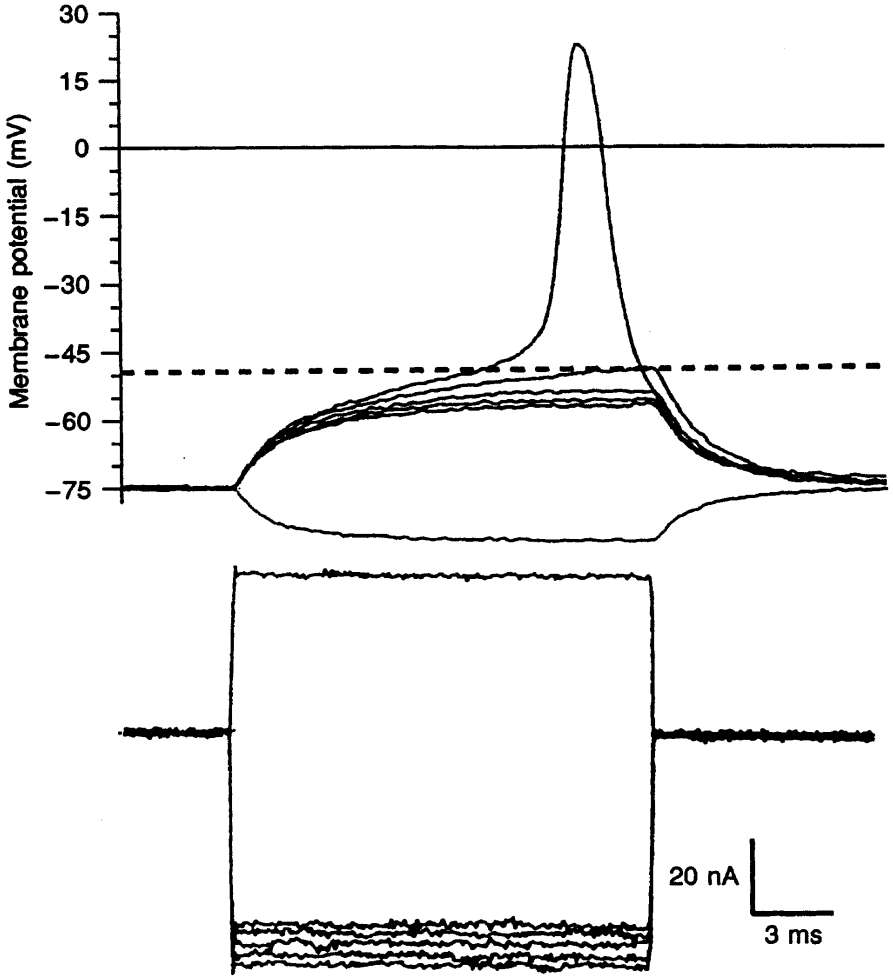
- an action potential invades the presynaptic motor nerve terminal
- depolarization of the nerve terminal opens calcium channels
- influx of calcium triggers fusion of synaptic vesicles to the membrane and release of ACh
- ACh diffuses across the synaptic cleft, binds AChRs, open them and causes depolarization of the postsynaptic muscle fiber
- depolarization of the muscle fiber triggers the opening of voltage-gated sodium channels which trigger an action potential in the muscle fibers

How would you measure the action potential threshold?

Smallest depolarization from the resting potential needed to trigger an action potential



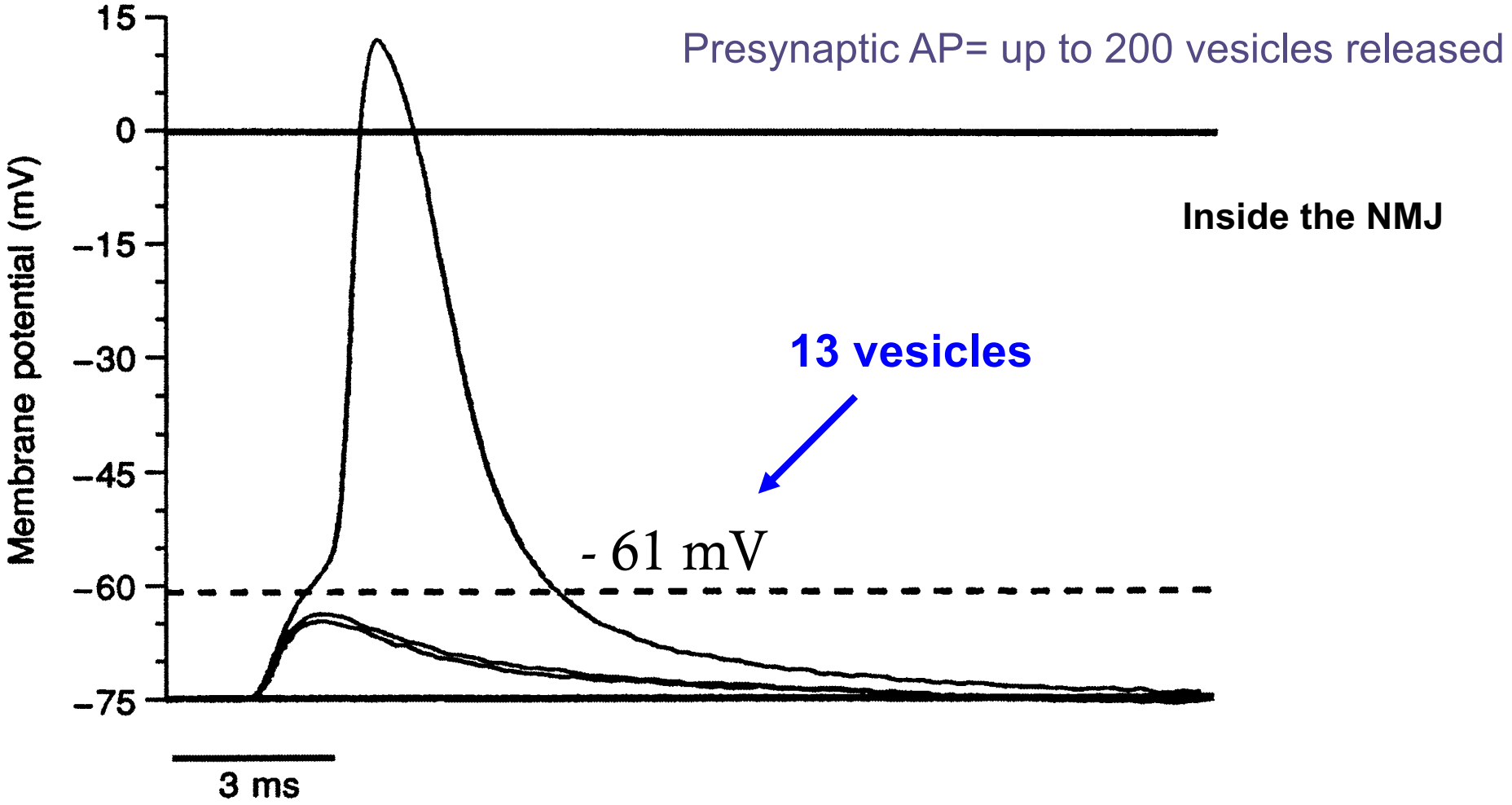
…. calculating the threshold



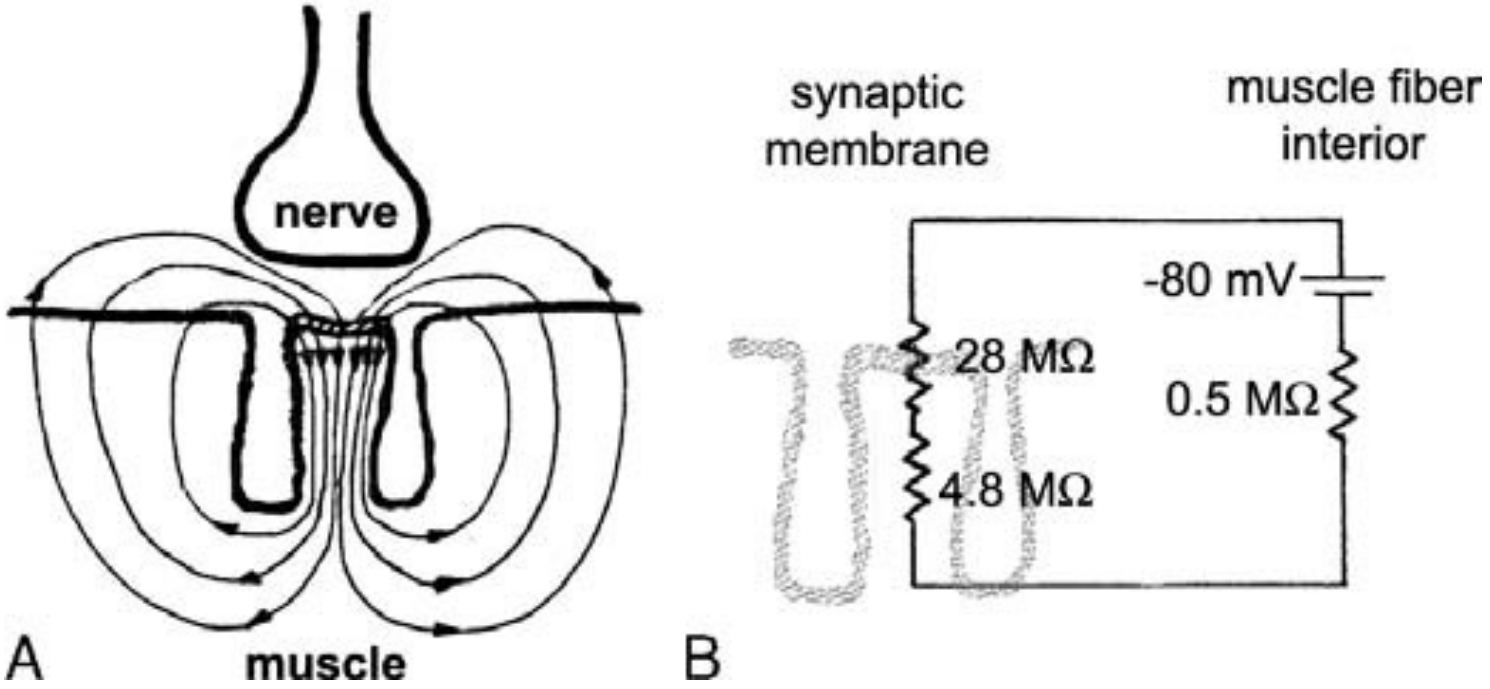
Out side the NMJ

Determination of the threshold for muscle fibre action potential generation. These properties can be determined by inserting two microelectrodes into the muscle fibre. Pulses of current are passed through one (lower traces) while the resulting changes in membrane potential are recorded with the other (upper traces). Note that outward current, shown increasing upwards, causes a hyperpolarisation of the muscle fibre, shown increasing downwards. For changes of membrane potential that do not reach the threshold, the ratio of the change in voltage to the change in current defines the **'input resistance'** of the muscle fibre. As the depolarisation of the muscle fibre approaches threshold, about -50 mV in this example, this relationship becomes non-linear and an action potential is soon generated (from Wood and Slater, 1995).

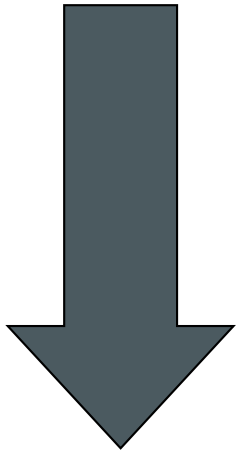
…. calculating the threshold



Few ACh-channels opened are needed to generate an AP

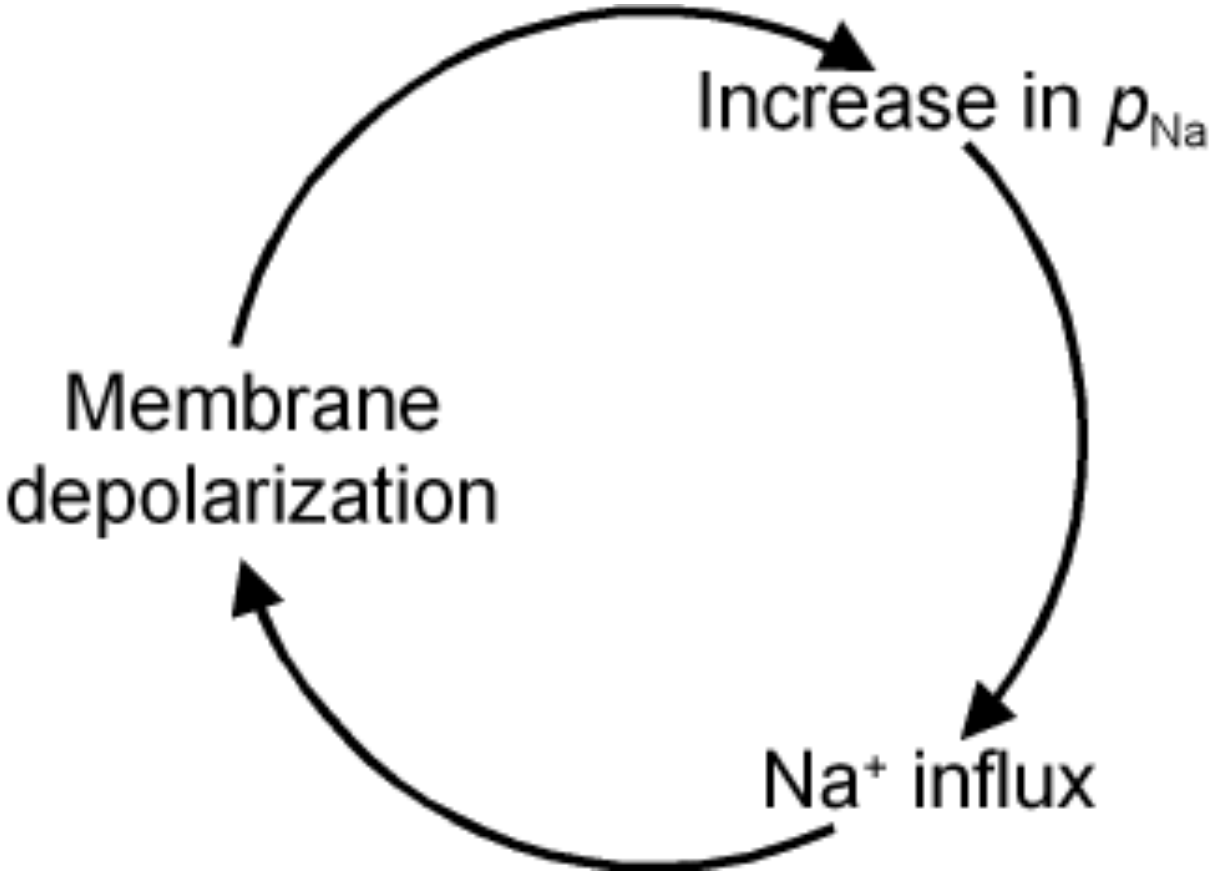


Depolarization to an absolute membrane potential of - 55 mV is adequate to trigger an action potential

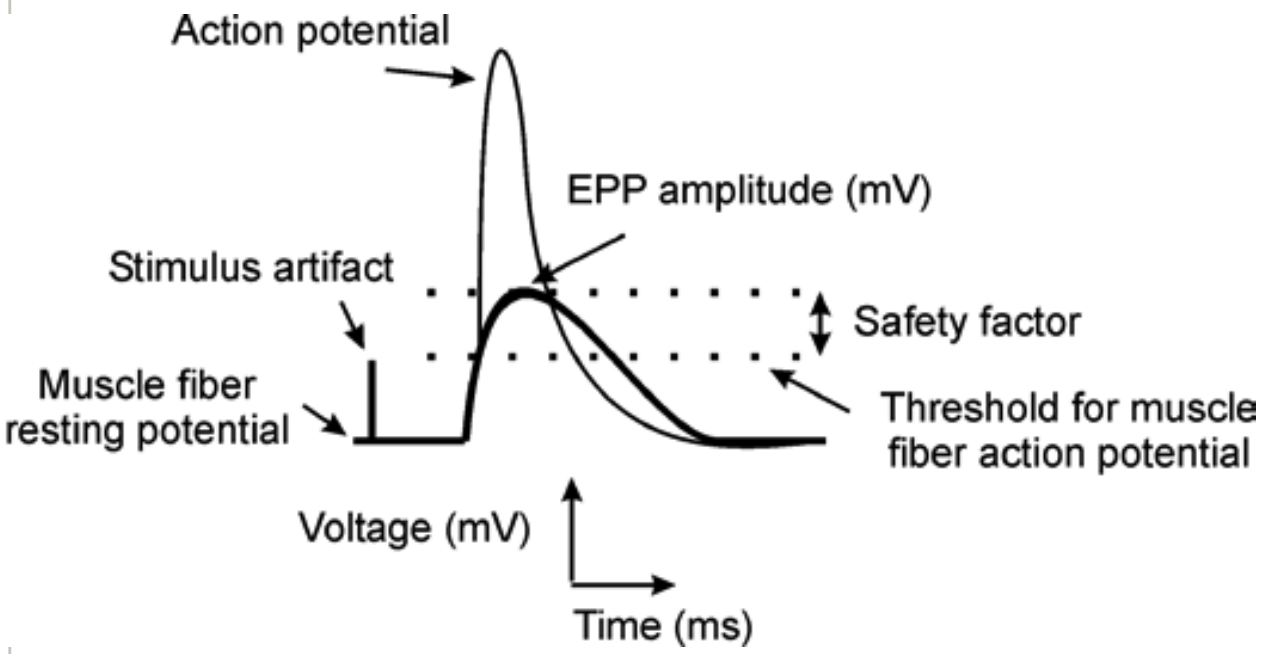


- 55 mV is the activation threshold for voltage-gated sodium channels at the endplate

“Hodgkin cycle”



3. The Safety Factor



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.

Definitions of the SF for neuromuscular transmission:

1. Ratio of the estimated peak amplitude of the endplate potential to the threshold depolarization required to generate an action potential.

2. The excess current generated in response to a nerve impulse over that required to reach the action potential threshold.

3. Number of ACh quanta actually released compared to the number which must act to generate an action potential.

Safety factor:

$$SF=(EPP)/(E_{AP}-E_M)$$

EPP = endplate potential

E_{AP} = threshold action potential

E_M = membrane potential

Low frequency (0.1-1 Hz)

Table 1
The range of reported safety factor for neuromuscular transmission in different muscles[a]

Species	Muscle	Safety factor	Source
Rat	Soleus	1.8	Gertler and Robbins, 1978
		3.5	Wood and Slater, 1997
	EDL	2.0	Gertler and Robbins, 1978
		5.0	Wood and Slater, 1997
		6.0	
	Diaphragm	3.0–5.0	Chang et al., 1975
		2.0–3.6	Kelly, 1978
		1.7–2.8	Wareham et al., 1994
Mouse	Soleus	4.6–5.8	Banker et al., 1983
	EDL	2.4–2.8	Harris and Ribchester, 1979
		3.2–5.8	Banker et al., 1983
Human	Intercostal	2.0	Elmqvist et al., 1964
Cat	Tenuissimus	2.0–4.0	Boyd and Martin, 1956
	Tibialis	4.0–12.0	Paton and Waud, 1967
	Sartorius	4.0–12.0	Paton and Waud, 1967
Frog	Cutaneous pectoris	4.0	Grinnell and Herrera, 1980
	Sartorius	1.0	Grinnell and Herrera, 1980
		3.1–5.5	Adams, 1989

[a] In some cases the values are derived from data presented in the paper referred to. These values are not directly comparable with one another since different methods have been used to estimate safety factor (see Section 4) and different experimental conditions such as temperature, pattern of activity and age of animals may influence the reported value (see Section 6)

Features of the NMJ that influence SF

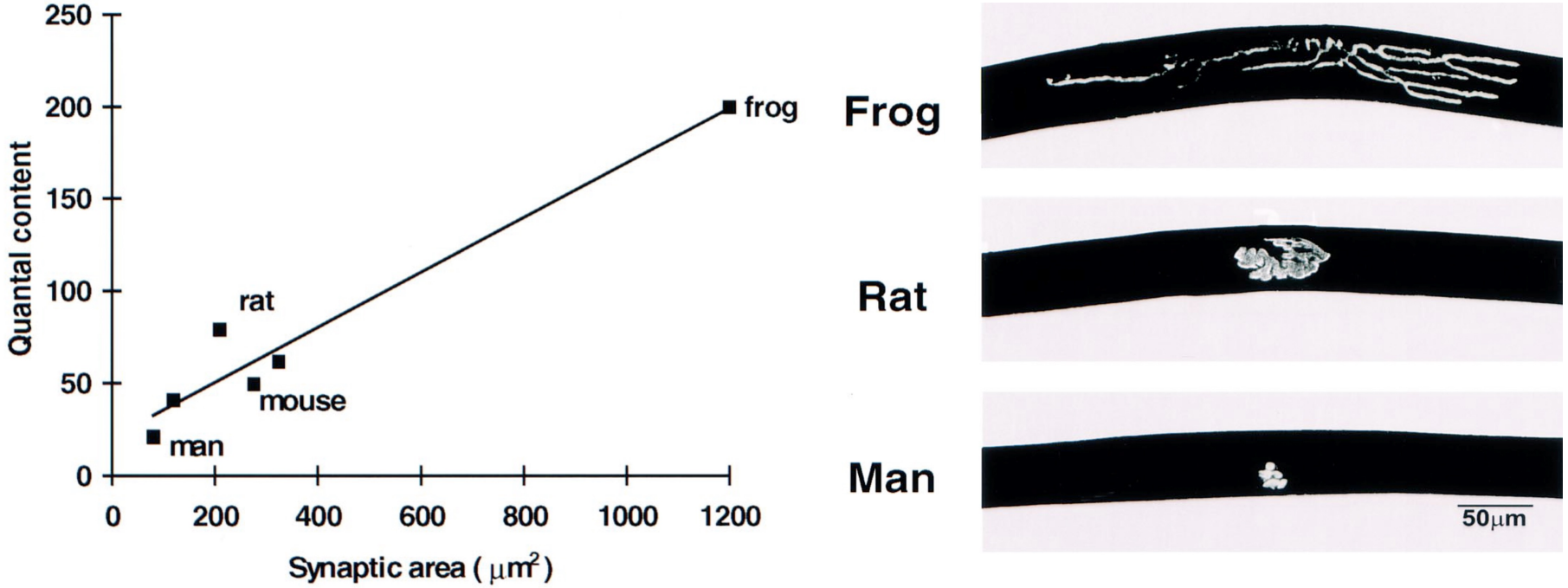
Presynaptic features influencing transmitter release:

1. Nerve terminal size: large motor nerve terminals release more transmitters than small terminal.

2. Pattern of activity: repetitive activity of 100 Hz induced an initial increase (*facilitation*) followed by a decrease (*depression*).

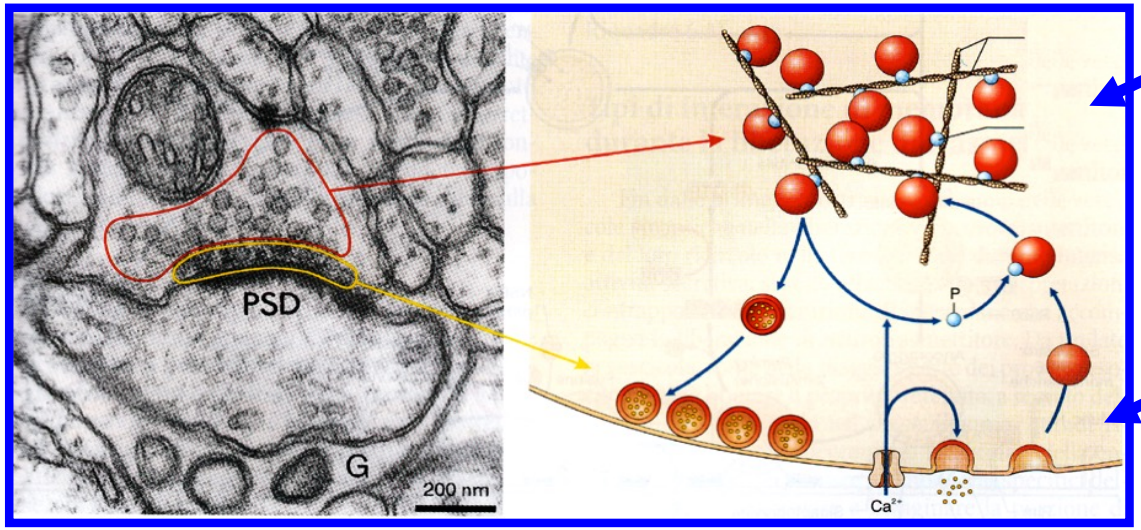
3. Short term modulation of release: due to the activation of Schwann cell and presynaptic receptors.

1. Nerve terminal size
(number of active zone & density of VOCC)



The quantal content is related to the size of NMJs in different species. Upper panels represent individual muscle fibres in (from above) frog (cutaneous pectoris), rat (soleus) and man (vastus lateralis). The NMJs have been visualised in these fluorescence micrographs by labelling the AChRs with a fluorescent conjugate of alpha-BgTx. The graph shows that the quantal content in these muscles and in a mouse muscle (epitrochleoanconeus) is approximately proportional to the synaptic area. The line is the best-fit determined by least squares ($r=0.973, p<0.001$).

2. Pattern of activity



Reserve pool

Releasable pool

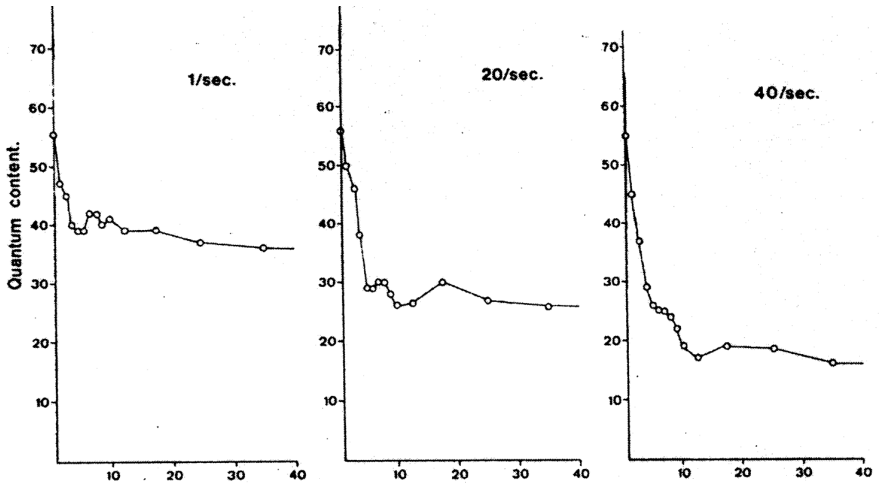


Fig. 8. The quantal content depends on the frequency of activation. Isolated human intercostal nerve-muscle preparations were stimulated at different frequencies. The muscle fibres were pretreated with glycerol to block action potentials. During stimulation, there is an initial rapid decline of quantal content. This is followed by a slower decline which is not seen in this figure (from Kamenskaya et al., 1975).

“Slow” motor units (es. rat *Soleus*):

- active for long periods at a continuous frequency of 10-20 Hz. SF=3.5

“Fast” motor neuron units (es. rat *extensor digitorum longus*):

- active for short periods at frequency of 50-100Hz. SF=5.0

3. Short term modulation of release

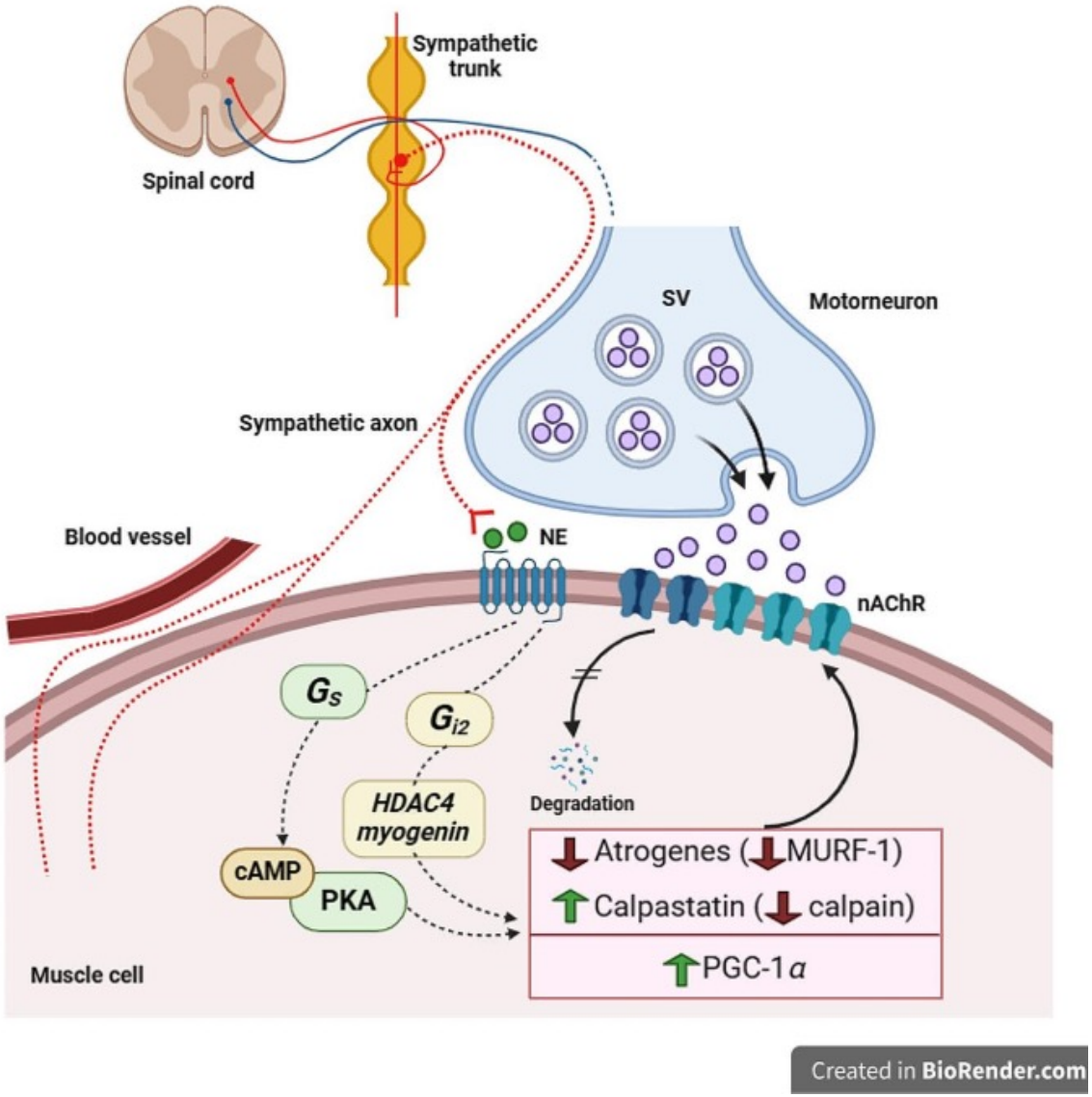
The quantal content can be modulated by the activation of a variety of receptors located on presynaptic membrane terminal, by the Schwann cells and by the sympathetic nervous system.

Cholinergic modulation through AChRs

Purinergic modulation through ATP/adenosine receptors

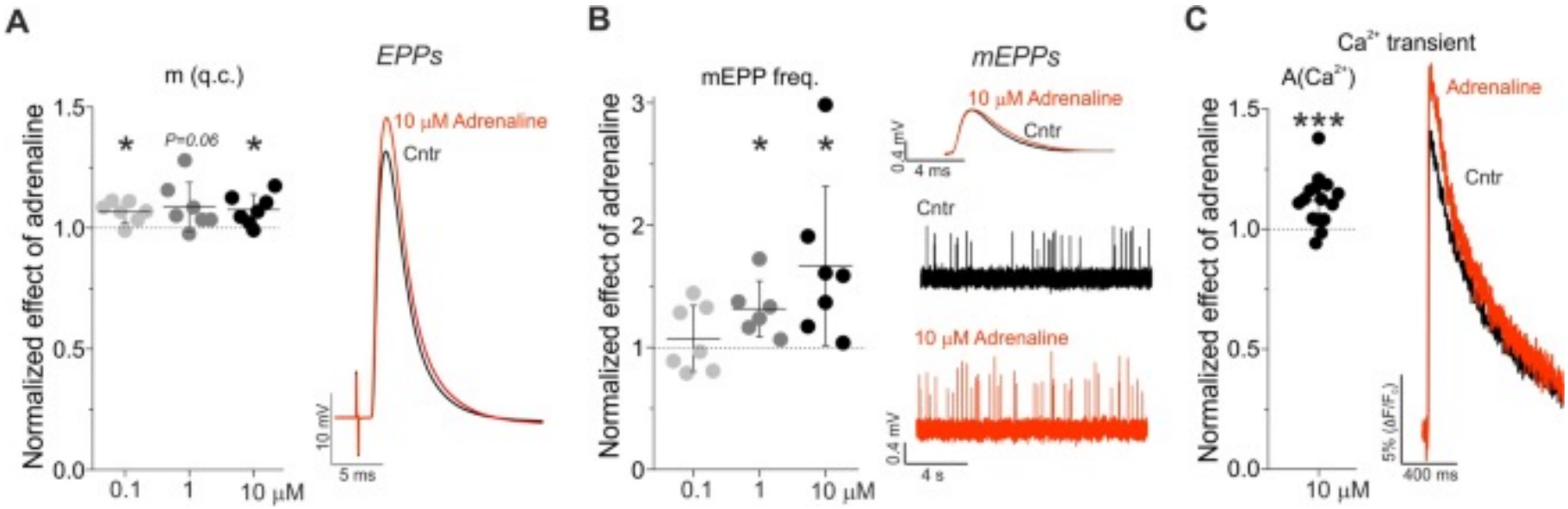
Adrenergic modulation through adrenoreceptors

Rudolf R, Kettelhut IC, Navegantes LCC. Sympathetic innervation in skeletal muscle and its role at the neuromuscular junction. J Muscle Res Cell Motil. 2024 Jun;45(2):79-86. doi: 10.1007/s10974-024-09665-9



Sympathetic nervous system acts upon multiple targets within skeletal muscle tissue. The schematic drawing illustrates that sympathetic innervation in skeletal muscle as a functional unit affects not only vasomotor activity, but impacts also on muscle fiber and motor neuron physiology, e.g., by controlling muscle force production and synchrony of synaptic vesicle (SV) release, respectively. At the NMJ postsynapse, sympathetic release of norepinephrine (NE) affects the protein turnover in general and that of nicotinic acetylcholine receptors (nAChR), in particular. Mechanistically, this appears to involve downregulation of proteolytic systems (atrogenes, calpain). Control of these systems might occur via adrenoceptor-mediated tuning of cAMP/PKA and/or HDAC4/myogenin axes. In addition, sympathetic innervation positively affects PGC-1alpha signaling

Adrenaline increased spontaneous and evoked ACh release as well as action potential-induced Ca^{2+} influx into the motor nerve terminals.



Adrenaline action in the neuromuscular junctions. **A**, **B**—Effects of adrenaline at 0.1, 1 and 10 μM on quantal content of EPPs (q.c. or m) and frequency (freq.) of mEPPs, respectively. Right, representative traces of EPPs (A) and mEPPs (B). **C**—Effect of 10 μM adrenaline on intraterminal Ca^{2+} transient. Quantification of Ca^{2+} transient amplitudes and native traces are shown. A–B: Y-axis: Normalized effect, where values before adrenaline application were taken as 1.0. n = 6–15 *per* group; *P < 0.05, ***P < 0.001 by paired two-tailed Student's *t*-test

Arkhipov A, Zhilyakov N, Sibgatullina G, Nevsky E, Bukharaeva EA, Petrov AM. Adrenergic Modulation of Acetylcholine Release at the Mouse Neuromuscular Junctions of Fast-Twitch Skeletal Muscle. Neurochem Res. 2025 May 12;50(3):162. doi: 10.1007/s11064-025-04415-w.

Features of the NMJ that influence SF

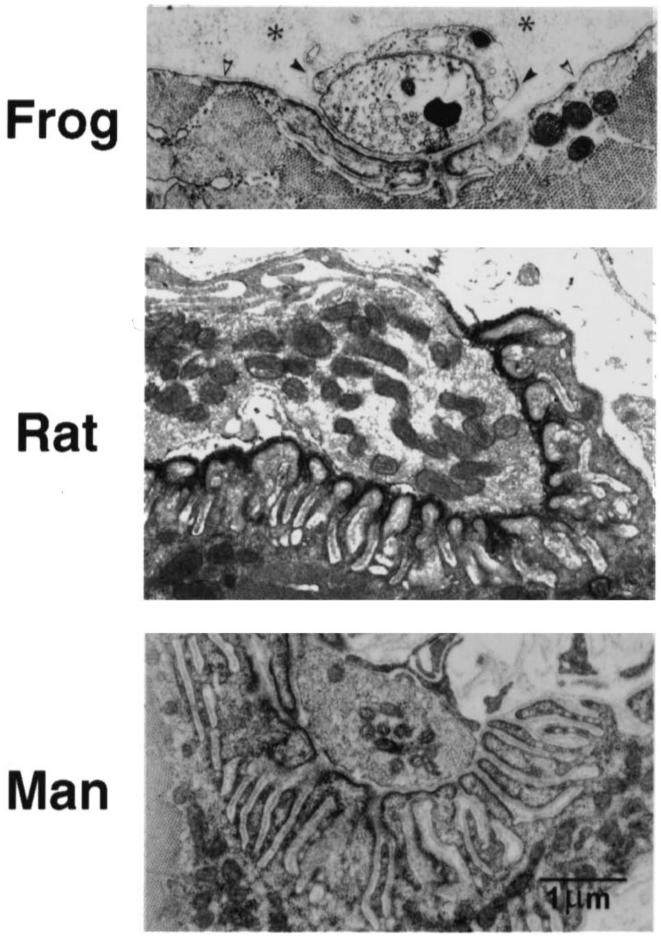
Postsynaptic features influencing the response to transmitter:

1. Density and distribution of nAChRs: it is generally assumed that the density of nAChRs is similar in all NMJ

2. The Junctional Folds

3. AChE

2. Voltage gated sodium channels/Folds: in the depths of postsynaptic folds the VGSC are 10 times as concentrated as in the membrane far from the NMJ.



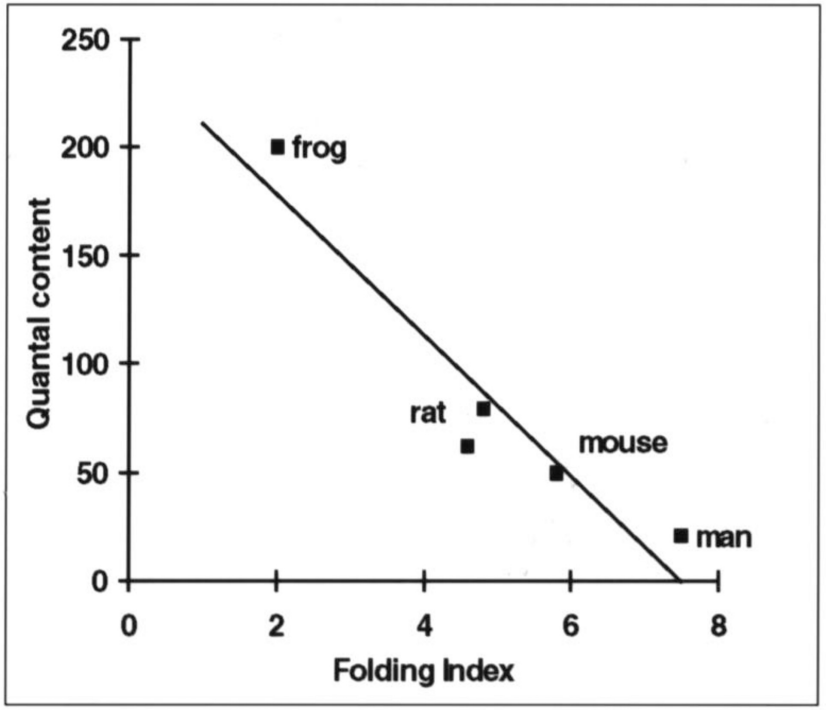


Fig. 18. The extent of postsynaptic folding is inversely related to quantal content in NMJs from a number of species. There is much more folding at human NMJs than at those in frog. This is consistent with the hypothesis that the folds, and the sodium channels within them, serve to amplify the effect of the transmitter released from the nerve.

3. **AChE:** the enzyme activity restricts both the spatial and temporal extent of the action of a single quantum

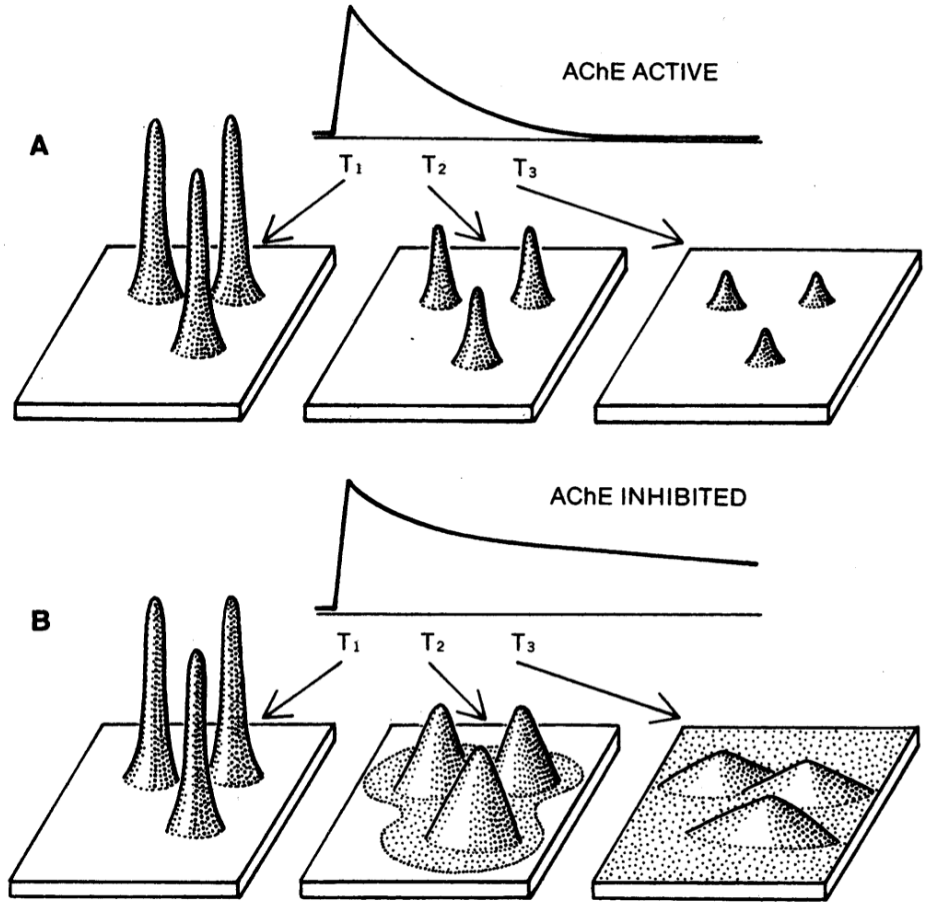


Fig. 14. The action of AChE at the NMJ. In the presence of normal AChE activity (A), the ACh molecules released as a 'quantum' from a given site on the nerve terminal are digested before they have spread into the 'territory' of another quantum. Thus each quantum acts independently of the others. When AChE activity is blocked (B), ACh diffuses much further and the territories of adjacent quanta overlap. As a result, the ACh concentration rises in a much larger region of the synaptic cleft, and more AChR are activated (from Hartzell et al., 1975).

Can the safety factor be reduced?

1. Reduction in sensitivity of the postsynaptic membrane to ACh

1. Reduction in ACh release

3. Elevation in the threshold for muscle fiber action potential initiation

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