The neuromuscular junction (NMJ) of the frog

(A) Stimulate axon

Record postsynaptic membrane potential
(the end plate potential)

Muscle cell
The endplate potential (EPP)

Recording from a muscle fiber

Control conditions

Curare

Action potential

endplate potential

threshold
Strychnos Toxifera
The discovery of miniature endplate potentials; “minis”

Sir Bernard Katz

spontaneous

Nerve stim.

evoked
Low Ca\(^{++}\) in the perfusion medium

Ca\(^{++}\) is necessary for transmitter release

Fluctuation of EPP amplitudes
Very low Ca++

The amplitude of evoked EPP decreases when reducing external [Ca2+]. The amplitude of minis does not.
Transmitter release is stochastic

Very low Ca^{++}

EPP amplitude = n*p*q
Release is vesicular

Synaptic Vesicle exocytosis captured by quick freezing; Hauser & Reese

One miniature EPP correspond to the release of one vesicle. AP synchronize vesicular release.
Vesicular Cycling

Load:
- Releasable Unstained
- Exocytosis Staining
- Endocytosis Stained

Test:
- Repriming
- Exocytosis Destaining
- Endocytosis Destained
- Releasable Stained
Cycling Kinetics
Two Ways to Release Neuroransmitter
Extracellular recording at NMJ without Ca\textsuperscript{2+} in the perfusion solution

- stimulation artifact
- Ca\textsuperscript{2+} leak from recording pipette
- presynaptic action potential
- No Ca\textsuperscript{2+} leak from recording pipette

End plate potential

Where is Ca\textsuperscript{2+} necessary?
When is Ca\textsuperscript{2+} necessary?

Interval between Ca\textsuperscript{2+} pulse and stim
What is the relationship between Ca$^{2+}$ and transmitter release?

Highly nonlinear relationship: $EPP = k[Ca^{2+}]^4$
Is an action potential essential?

(A) Squid giant synapse

In the presence of TTX

Post V_m
Pre I
Pre V_m

Postsynaptic Potential

Ca^2+ Current

Post V_m
Pre I
Pre V_m

Post V_m
Pre I
Pre V_m

Post V_m
Pre I
Pre V_m
How far away from the release site are the Ca2+ channels?

EGTA: Slow Ca2+ buffer injected in the presynaptic terminal

BAPTA: Fast Ca2+ buffer injected in the presynaptic terminal
Which Ca\textsuperscript{2+} channel is responsible for Transmitter release?

**Western Grass Spider**

*Agelenopsis Aperta*

**Conus Granulatus**

*conotoxins*

**Agatoxins**
Which Ca\textsuperscript{2+} channel is responsible for Transmitter release?

\omega conotoxin MVII A: Blocks N-type Ca\textsuperscript{2+} channels

\omega Agatoxin IVa: Blocks P-type Ca\textsuperscript{2+} channels
**N-Type Ca^{2+} Channel**

**Channel name** CaV2.2  
**Description** voltage-gated calcium channel 1 subunit  
**Other names** N-type, 1B; rbB-I, rbB-II (in rat)1,2, BIII (in rabbit)3  
**Molecular information** human: 2339aa, M94172, 2237aa, M94173 (ref. 4), chr. 9q34, CACN1B  
rat: 2336aa, M92905 (ref. 1)  
mouse: 2329aa, NM007579, NP031605  
**Associated subunits** 2/1, 3, 4 (ref. 5) possibly  
**Functional assays** voltage clamp, patch clamp, calcium imaging, neurotransmitter release, 45Ca uptake into synaptosomes  
**Current** ICa,N  
**Conductance** 20pS (bullfrog sympathetic neurones)6; 14.3pS (rabbit BIII cDNA in skeletal muscle myotubes)3  
**Ion selectivity** Ba^{2+} > Ca^{2+}  
**Activation**  
\[ V_a = +7.8\text{mV}, \tau_a = 3\text{ms at } +10\text{mV (human 1B /2/1-3 in HEK 293 cells, 15mM Ba}^{2+} \text{ charge carrier)}4,7; V_a = +9.7\text{mV}, \tau_a = 2.8\text{ms at } +20\text{mV (rat 1B-II/1b, in } \text{Xenopus} \text{ oocytes, } 40\text{mM Ba}^{2+} \text{ charge carrier)}2 \]  
**Inactivation**  
\[ V_h = 61\text{mV}, \tau_h \approx 200\text{ms at } +10\text{mV (human 1B /2/1-3 in HEK 293 cells, 15mM Ba}^{2+} \text{ charge carrier)}4,7; V_h = 67.5\text{mV}, \tau_h = 112\text{ms at } +20\text{mV (rat 1B-II/1b in } \text{Xenopus} \text{ oocytes, } 40\text{mM Ba}^{2+})2 \]  
**Activators** none  
**Gating inhibitors** none  
**Blockers** -conotoxin GVIA (1–2M, irreversible block), -conotoxin MVIIA (SNX-111, ziconotide), -conotoxin MVIIC (ref. 8)  
**Channel distribution** neurones (presynaptic terminals, dendrites, cell bodies)9  
**Physiological functions** peptide toxins that selectively inhibit N-type channels block a significant fraction of neurotransmission release in the mammalian peripheral and central nervous systems (ref. 10)
P-Type Ca2+ Channel

**Channel name** CaV2.1  
**Description** voltage-gated calcium channel 1 subunit  
**Other names** 1A, P-type, Q-type, rbA-I (in rat)1; Bl-1, Bl-2 (in rabbit)2  
**Molecular information** human: 2510aa, AF004883, 2662aa, AF004884, chr. 19p13, CACNA1A  
rat: 2212aa, M64373  
mouse: 2165aa, NM007578, NP031604  
(see Comments)  
**Associated subunits** 2, , possibly  
**Functional assays** voltage clamp, patch clamp, calcium imaging, neurotransmitter release  
**Current** ICa,P, ICa,Q  
**Conductance** 9, 14, 19pS (P-type, cerebellar Purkinje neurones)4; 16–17pS (for 1A/2/ in Xenopus oocytes)2,5,6  
**Ion selectivity** Ba2+ > Ca2+  
**Activation**  
- Va = 5mV for native P-type, Va = 11mV for native Q-type (with 5mM Ba2+ charge carrier)7  
- Va = 4.1mV for rat 1A-a/2/4  
- Va = +2.1mV for rat 1A-b/2/4 (with 5mM Ba2+ charge carrier)6  
- Va = +9.5mV; a = 2.2ms at +10mV for human 1A-1/2/1b in HEK 293 cells (with 15mM Ba2+ charge carrier)3  
**Inactivation**  
- Vh = 17.2mV for 1A-a/2/4, Vh = 1.6 mV for 1A-b/2/4 (with 5mM Ba2+ charge carrier); Vh = 17mV, th = 690ms at +10mV human 1A-1/2/1b in HEK 293 cells (with 15mM Ba2+ charge carrier)3; h > 1s at 0mV native P-type (with 5mM Ba2+ charge carrier)7  
(see Comments)  
**Activators** none  
**Gating inhibitors** -agatoxin IVA (P-type Kd =1–3nM (ref. 8); Q-type Kd ~ 100–200nM (refs. 5,9)), -agatoxin IVB (ref. 6)  
**Blockers** -conotoxin MVIIC (ref. 8)  
(see Comments)  
**Radioligands** [125I]--conotoxin MVIIC  
**Channel distribution** neurones (presynaptic terminals, dendrites, some cell bodies), heart, pancreas, pituitary  
**Physiological functions** neurotransmitter release in central neurones and neuromuscular junction; excitation-secretion coupling in pancreatic cells