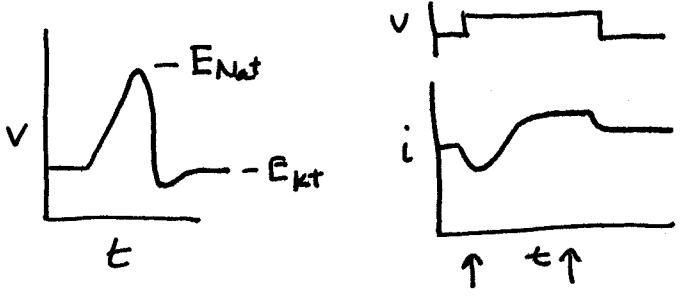


CLONING VOLTAGE SENSITIVE ION CHANNELS - Na⁺ / K⁺ NEURO - AP 2 NOTES

CLONE Na⁺ CHANNEL

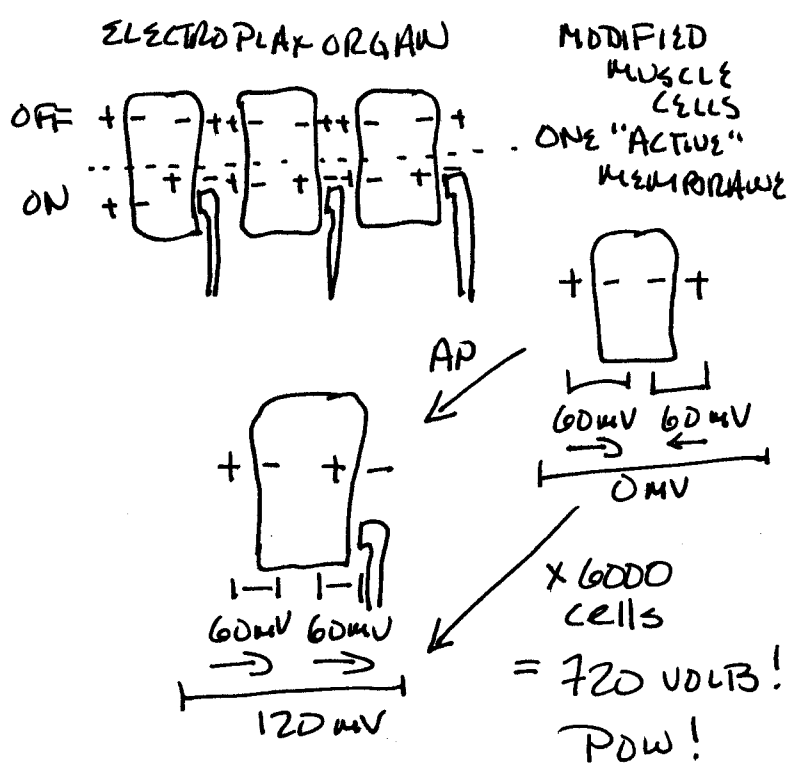
HILL PREDICT ROLES OF Na⁺ & K⁺



INWARD Na⁺ FAST SELF INACTIVATING
OUTWARD K⁺ DELAYED

HILLE CONFIRMS w/ TTX, TEA

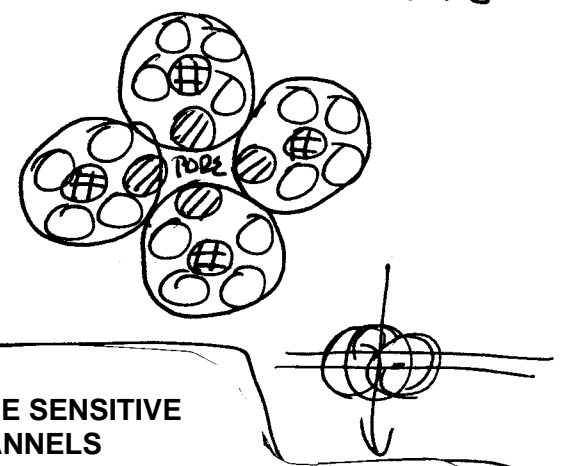
CLONE Na⁺ CHANNEL - ELECTRIC FISH



ACTIVE MEMBRANE RICH IN ACh RECEPTORS Na⁺ CHANNELS

- Noda et al 1984
- PURIFY TTX BINDING PROTEIN PARTIAL SEQUENCE MAKE ANTIBODY
 - MAKE cDNA LIBRARY FORCE BACTERIA / PLASMIDS TO EXPRESS PROTEINS SCREEN FOR TTX-PROTEIN USING ANTIBODY SEQUENCE CLONE - CONFIRM PRESENCE OF PEPTIDE SEQUENCES
 - CHARACTERIZE

KNOWN MEMBRANE PROTEIN 10 TRANSMEMBRANE REGIONS "HYDROPHOBICITY ANALYSIS" 4-SUBDOMAINS - EACH w/ 6 TRANSMEMBRANE REGIONS



Na⁺: gene encodes all 4 domains
K⁺: gene encodes single domain, channel is formed by 4 subunits comprising a tetramer.
Ca⁺⁺: gene encodes all 4 domains

Na⁺ CHANNEL PURIFICATION
 SUCCESSFUL ⇒ TTX BINDING

K⁺ CHANNEL - TEA BINDING
 TOO WEAK



DROSOPHILA SHAKER MUTANT

TROUT & KAPLAN (1973) -

FLY SHAKES UNDER ETHER
 "SHAKER"

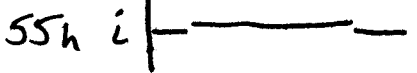
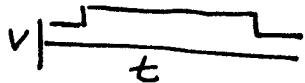
EVENTUALLY THOUGHT TO
 HAVE SOMETHING TO DO W/
 MUSCLE ION CONDUCTANCE

FLY MUSCLES (ARTHROPODS IN GEN.)
 NO - APs → NO Na⁺ CURRENTS

SALKOFF & WYMAN (1981a)

VOLTAGE CLAMP STUDY OF
 DEVELOPING FLIGHT MUSCLE

PUPA - 100 hrs



t

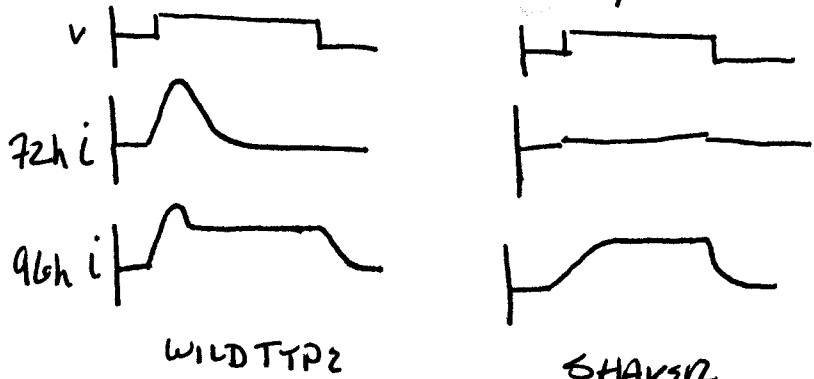
Z CURRENTS

{ A-CURRENT
 FAST, SELF INACTIVATING

{ A + DR CURRENTS

SALKOFF & WYMAN (1981b)

VC STUDY OF SHAKER MUTANT



WILDTYPE

SHAKER

EARLY / INACTIVATING
 CURRENT MISSING

K-CHANNEL

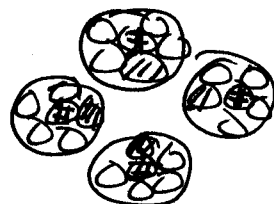
TEMPZ et al 1988

CLONE / ID GENE THAT
 IS SHAKER MUTATION

= VOLTAGE SENSITIVE ION
 CHANNEL

ONE DOMAIN OF
 4 DOMAIN CHANNEL

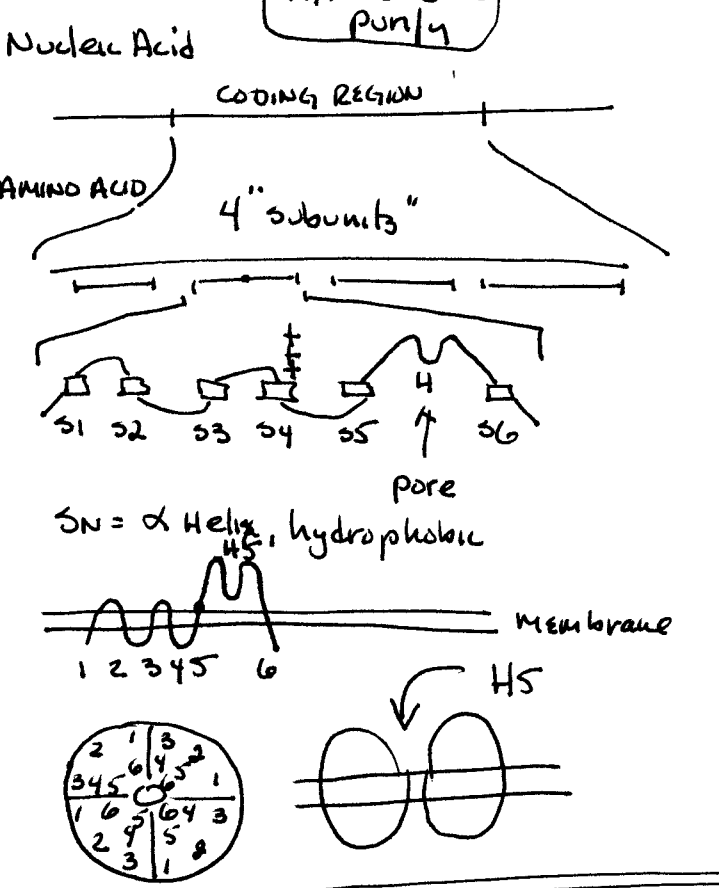
- TETRAMER



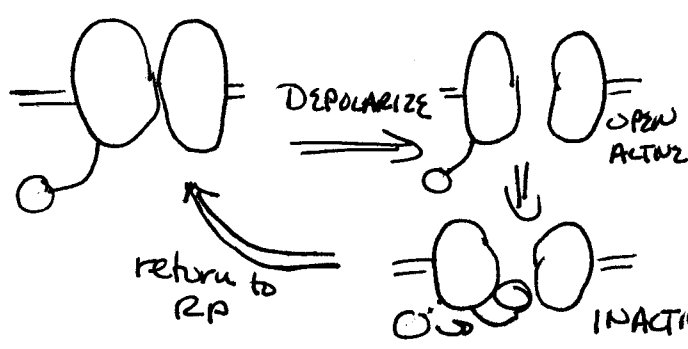
3 MOLECULAR BIOLOGY
 VOLT. SENS. ION CHANNELS

AXONS (APs):
 VOLTAGE SENSITIVE ION CHANNELS [GATED]
 DENDRITES (SYNAPSE)
 LIGAND SENSITIVE ION CHANNELS [GATED]

VOLTAGE SENSITIVE Na⁺ channel Noda et al 1984
 TTX used to purify



- ① ION SELECTIVE - H5, AMINO ACIDS
- ② VOLTAGE SENSITIVE - 54 ↑↑↑
- ③ SELF INACTIVATION - N-terminus "BALL & CHAIN"



VOLTAGE SENSITIVE K⁺ channel

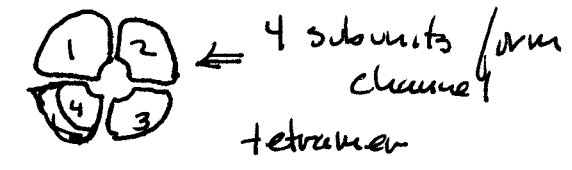
12/8/00

Wyman / Salkoff -

- Shaker - fast, inactivating (A-current)
- Delayed Rectifier - slow - not inactivating

Shaker cloned → K⁺ channel

GENE = SINGLE "subunit"



Voltage Sensitive Ion Channels: Na⁺, K⁺, Ca⁺⁺
 Na⁺: one gene encodes entire channel (all 4 domains)
 K⁺: gene encodes only one domain - channel formed by 4 proteins coming together to form a tetramer.
 Ca⁺⁺: one gene encodes entire channel (all 4 domains)

EXPERIMENTS

- Reconstruct - frog oocytes
- SINGLE CHANNEL ANALYSIS - PATCH CLAMP (Neher & Sakmann, Nobel Prize)
- EXPERIMENTATION - SITE DIRECTED ALTER PROTEINS - MUTAGENESIS

