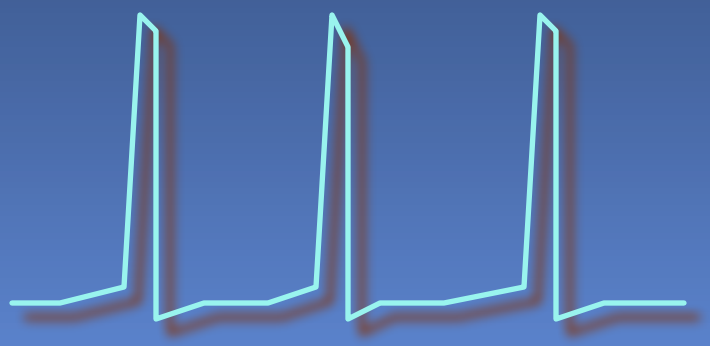
Oscillating Channels and Sensors

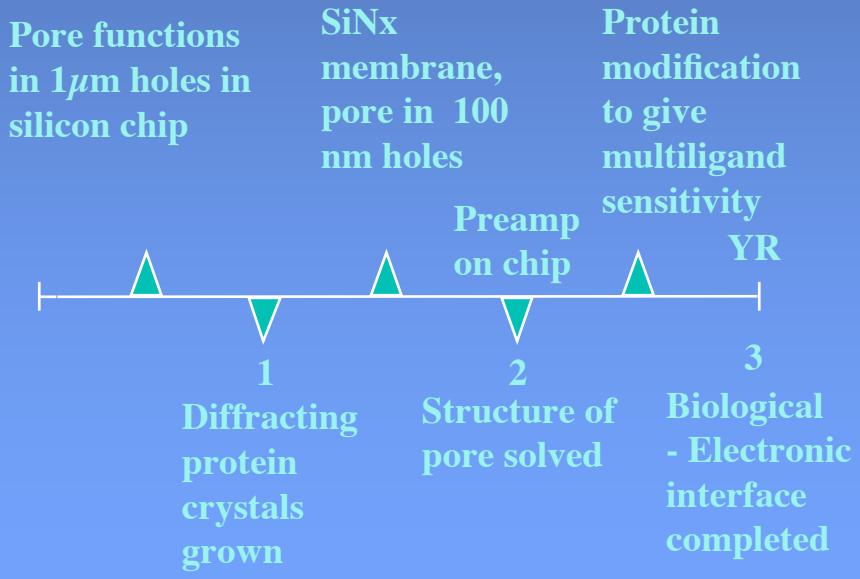
ONR/DARPA Julie E. M. McGeoch and Guido Guidotti 1998



New Ideas

- Simple oscillating mammalian protein.
- Protein subunits 'sense' cooperatively.
- Sharp chemical threshold discrimination.
- Engineered sensitivity to chemical agents.

Schedule



Impact

- New biological-electronic interface.
- Compact, rapid sensor of agents.
- Protection of personnel.
- Detection of threat.

HARVARD UNIVERSITY

ACKNOWLEDGEMENTS FOR RESEARCH ON OSCILLATING CHANNELS AND SENSORS

STRUCTURE OF PORE PROTEIN: SUBUNIT C

1) CRYSTALLOGRAPHY
Louise N. Johnson (University of Oxford UK)
Martin E. M. Noble (University of Oxford UK)
Richard J. Staples
Don C. Wiley
Lydia Mosyak

2) MASS SPECTROSCOPY and MICROCHEMISTRY TO DETERMINE IDENTITY OF 42 MASS ADDITI0N
Andrew N. Tyler
William Lane
John M. Neveu

SENSOR HARDWARE

Henry I. Smith (Nano Structures Lab MIT)
David Carter (Nano Structures Lab MIT)
Mark K. Mondol (Nano Structures Lab MIT)
James Carter (Nano Structures Lab MIT)
Robert M. Westervelt
Carol Livermore
Jeffrey P. Sercel & Rob Higley (JPSA Associates)
Eric Mazur
Chris Schaffer
Nozomi Nishimura
Hank Smith (MIT)
Harvard Biological Lab workshop
Harvard Physics Lab workshop
Malcolm W. McGeoch (PLEX Corp)
Winfield Hill (Rowland Institute)

PROTEIN MODIFICATION TO MAKE SENSOR PORE MULTILIGAND SENSITIVE

John E. Walker (MRC Cambridge UK) provided 2 genes of subunit c to allow protein expression in cell lines.

ASSAY OF OSCILLATOR IN INTACT NEURONS

David N. Palmer (Lincoln University NZ)

SEARCH FOR CALCIUM REGULATOR PROTEIN OF ION PORE, SUBUNIT C

Rose-Mary Boustany (Duke University Medical Center)
William Mobley (Stanford)
John Cooper (Stanford)

MODELING OF OSCILLATOR

Malcolm W. McGeoch (PLEX Corp)

CHARACTERISTICS OF THE ION PORE PROTEIN AND ITS OSCILLATING CURRENT.

MECHANISM OF OSCILLATIONS

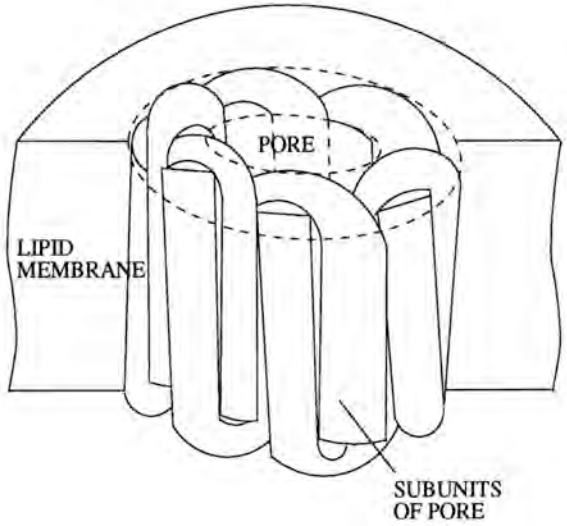
POSSIBLE FUNCTION FOR OSCILLATIONS IN THE BRAIN

NEXT 8 SLIDES

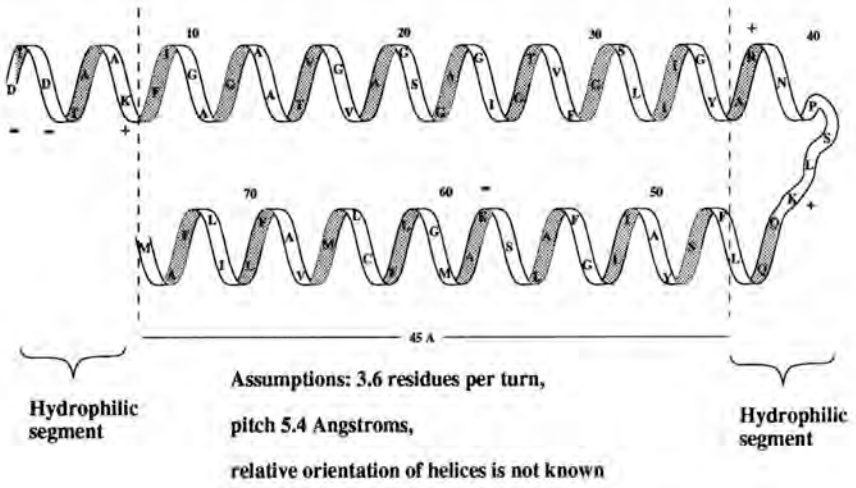
Predicted ion pore structure with 12 monomers as a ring -> of 6 dimers

Monomer of 75 amino acids->

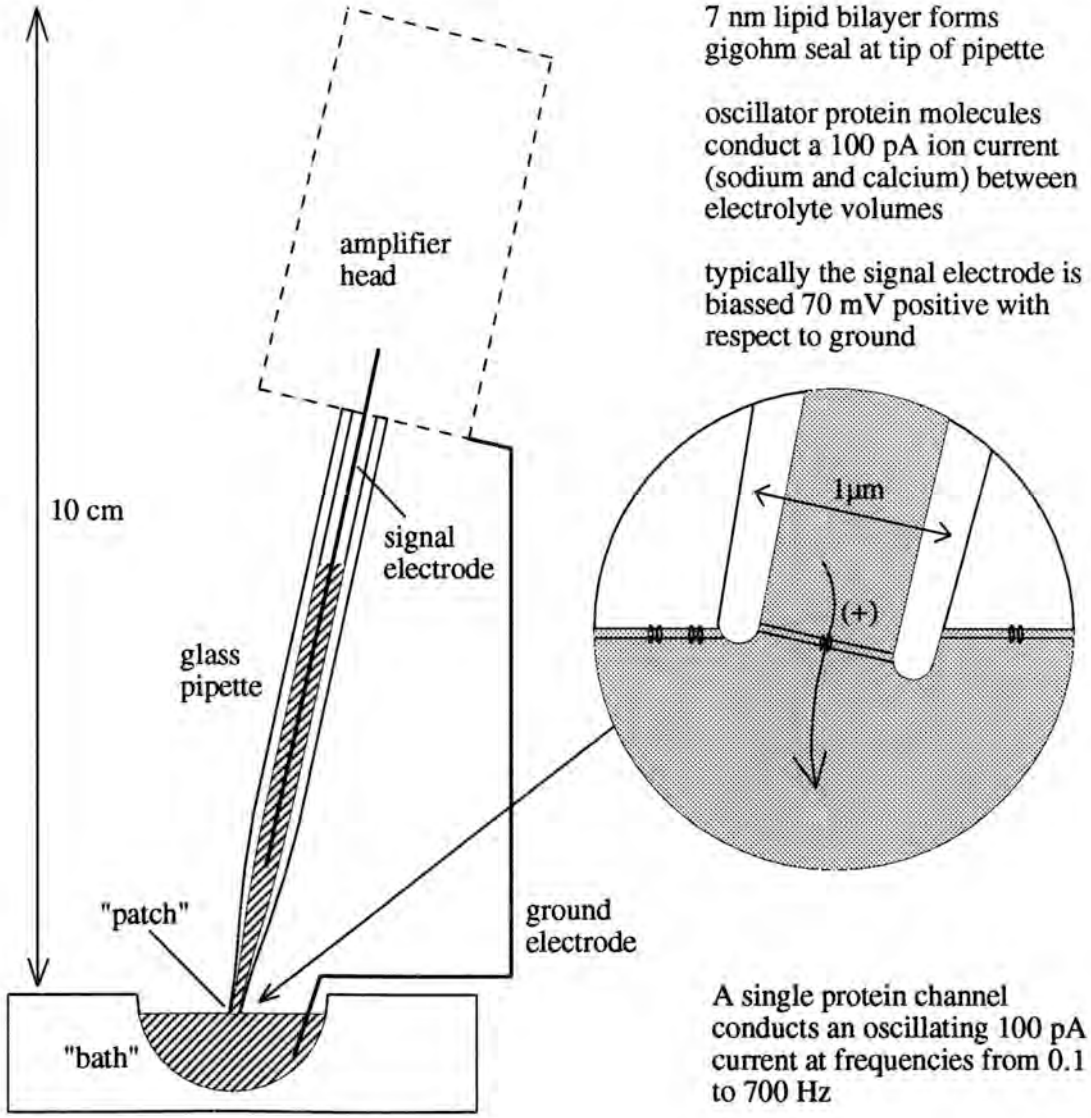
Oscillating ion current passes through a protein Pore containing possibly twelve subunits



Transmembrane Helices of Bovine Fo subunit c modeled from possible structure of E. Coli Fo subunit c, from R. H Fillingame, Biochim. Biophys. Acta, 1101 (1992) pp240-243



Conventional biological measurement of oscillating ion channel current ("patch clamp method")



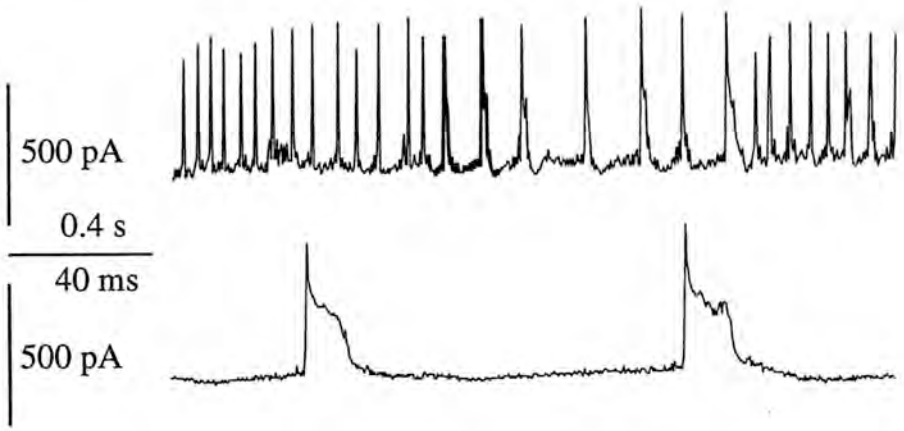
7 nm lipid bilayer forms gigohm seal at tip of pipette

oscillator protein molecules conduct a 100 pA ion current (sodium and calcium) between electrolyte volumes

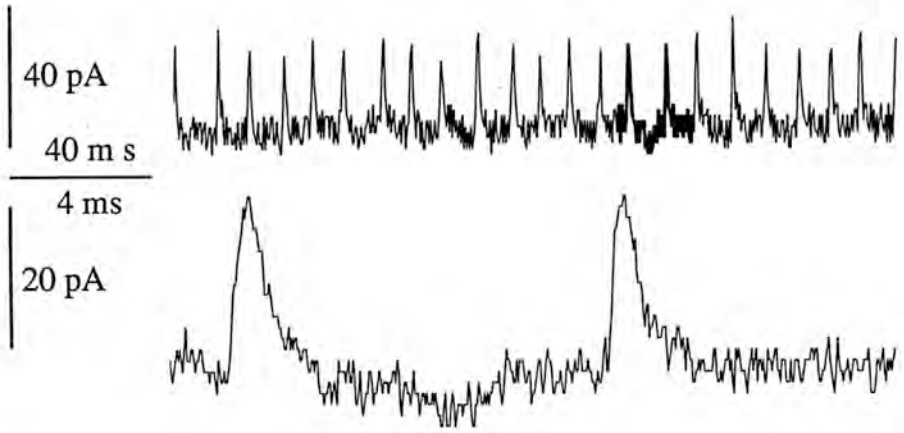
typically the signal electrode is biassed 70 mV positive with respect to ground

A single protein channel conducts an oscillating 100 pA current at frequencies from 0.1 to 700 Hz

A Sheep liver subunit c clamped at 70mV. *7.14nS, 25 Hz*



B Sheep liver subunit c clamped at 100mV. *0.25nS, 150Hz*

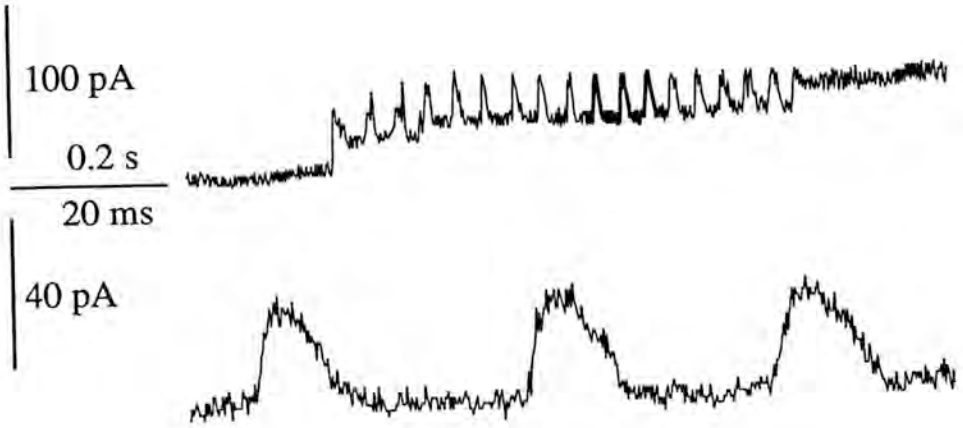


C: Experiment with only 20 molecules per patch. 2 pores are present. At start both are shut, then one open and one oscillating, then both open.

D: an increase in voltage increases the oscillation frequency

C

Sheep liver subunit c clamped at 70mV (high dilution, approx 20 molecules in the patch).
0.387nS, 30Hz

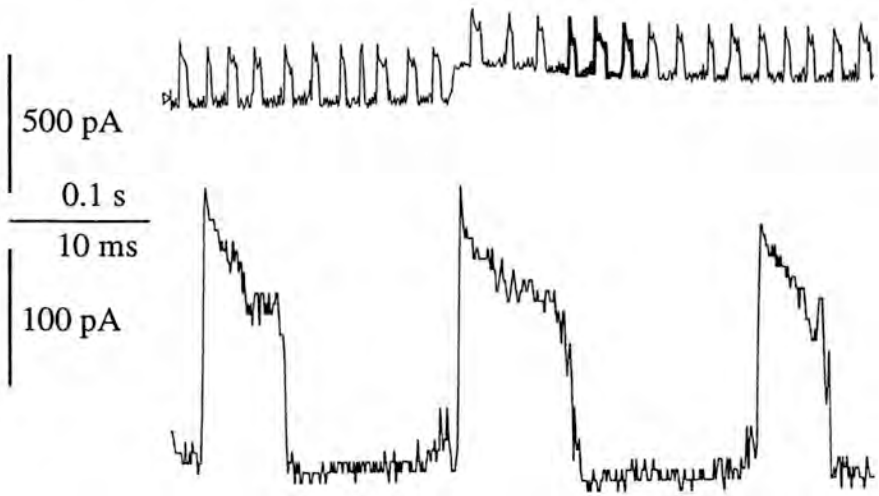


D

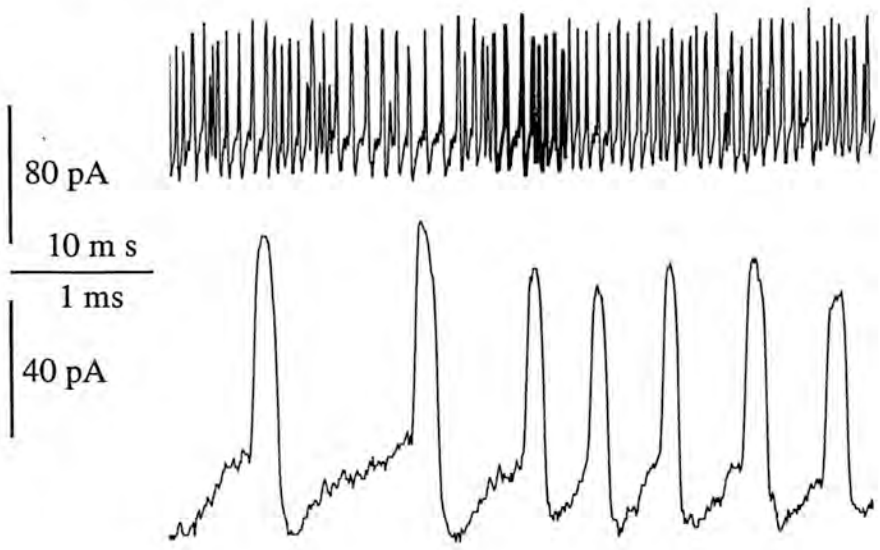
bovine brain crystallized subunit c, with voltage increasing from 60 mV to 70 mV at mid-trace
At 60mV 2.43nS, 4Hz, at 70mV 1.63nS, 20Hz

Fast oscillations->

E bovine brain crystallized subunit c clamped at 80mV. *2.60nS. 70Hz*

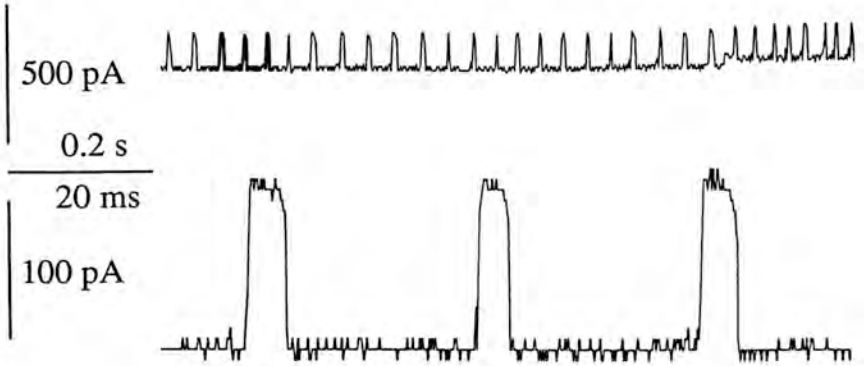


F bovine brain crystallized subunit c clamped at 80mV. *0.583nS, 700Hz*

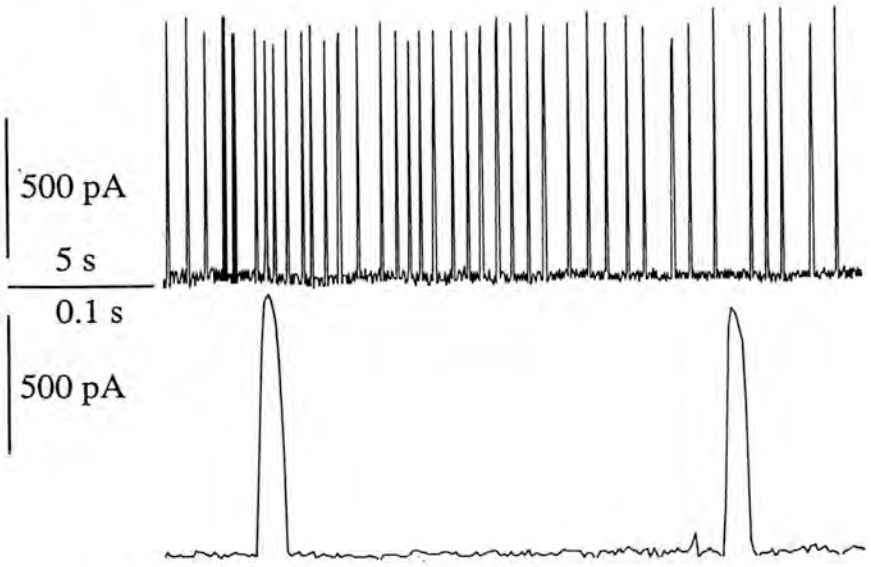


Slow oscillations->

G bovine brain crystallized subunit c clamped at 70mV *1.78nS, 30Hz*

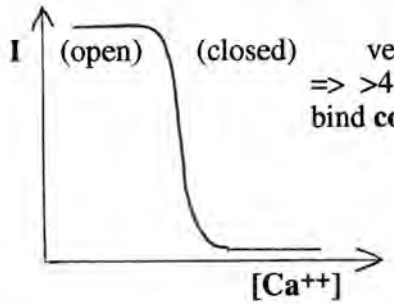


H rat hippocampal extract clamped at 60mV *15.62nS, 2Hz.*



Mechanism for Oscillation

Channel current is inhibited cooperatively by Ca^{++} ions
(M. W. McGeoch and J. E. M. McGeoch, Biophysical J. **66** 161 (1994))

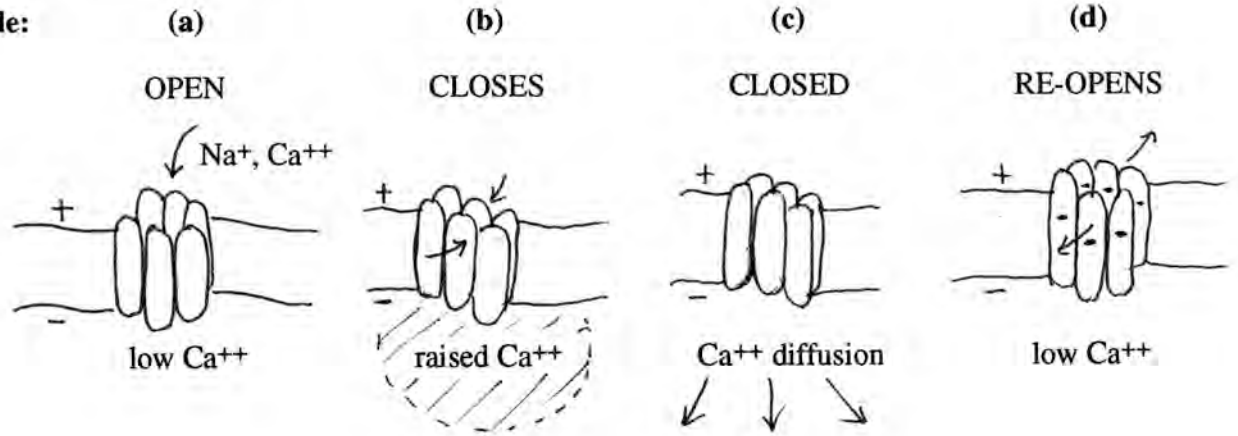


very sharp
=> >4 Ca^{++} ions bind **cooperatively**

=> sudden, **definite** changes of state
e.g. 6 Ca^{++} at 0.5 eV each => 3 eV chemical energy Δ

Opening force is generated by **-ve charge repulsion** when Ca^{++} off

Cycle:



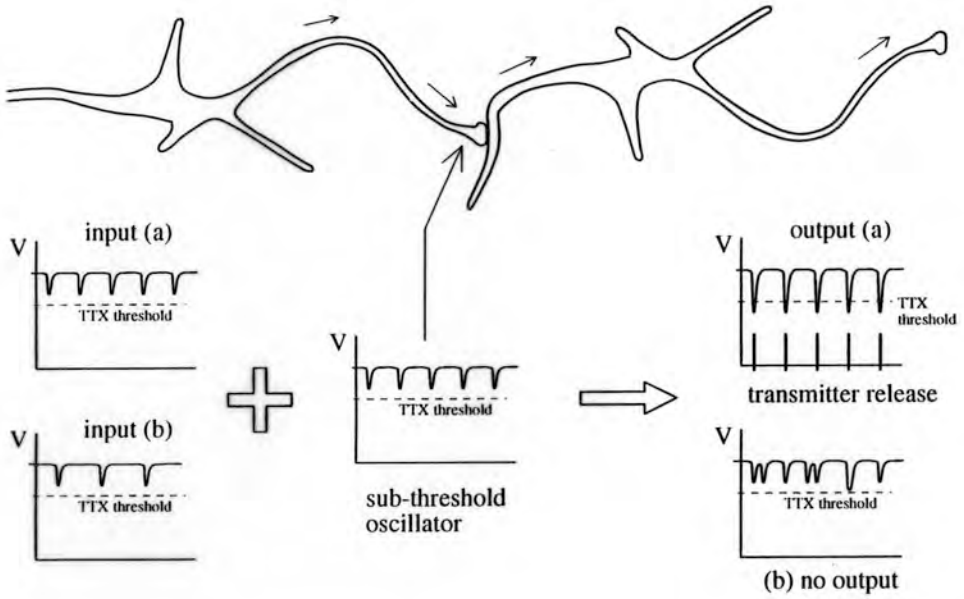


FIGURE 9. Frequency discrimination at a pre-synaptic terminal via a sub-threshold oscillator

AIM

TO MAKE AN ELECTRONIC-BIOLOGICAL INTERFACE FROM A CO-OPERATIVE, HIGH GAIN, ION PORE IN A SiN_x MEMBRANE IN A SILICON SUBSTRATE.

APPROACH

1) ESTABLISH MOLECULAR STRUCTURE OF ION PORE

2) FABRICATE HARDWARE OF SENSOR

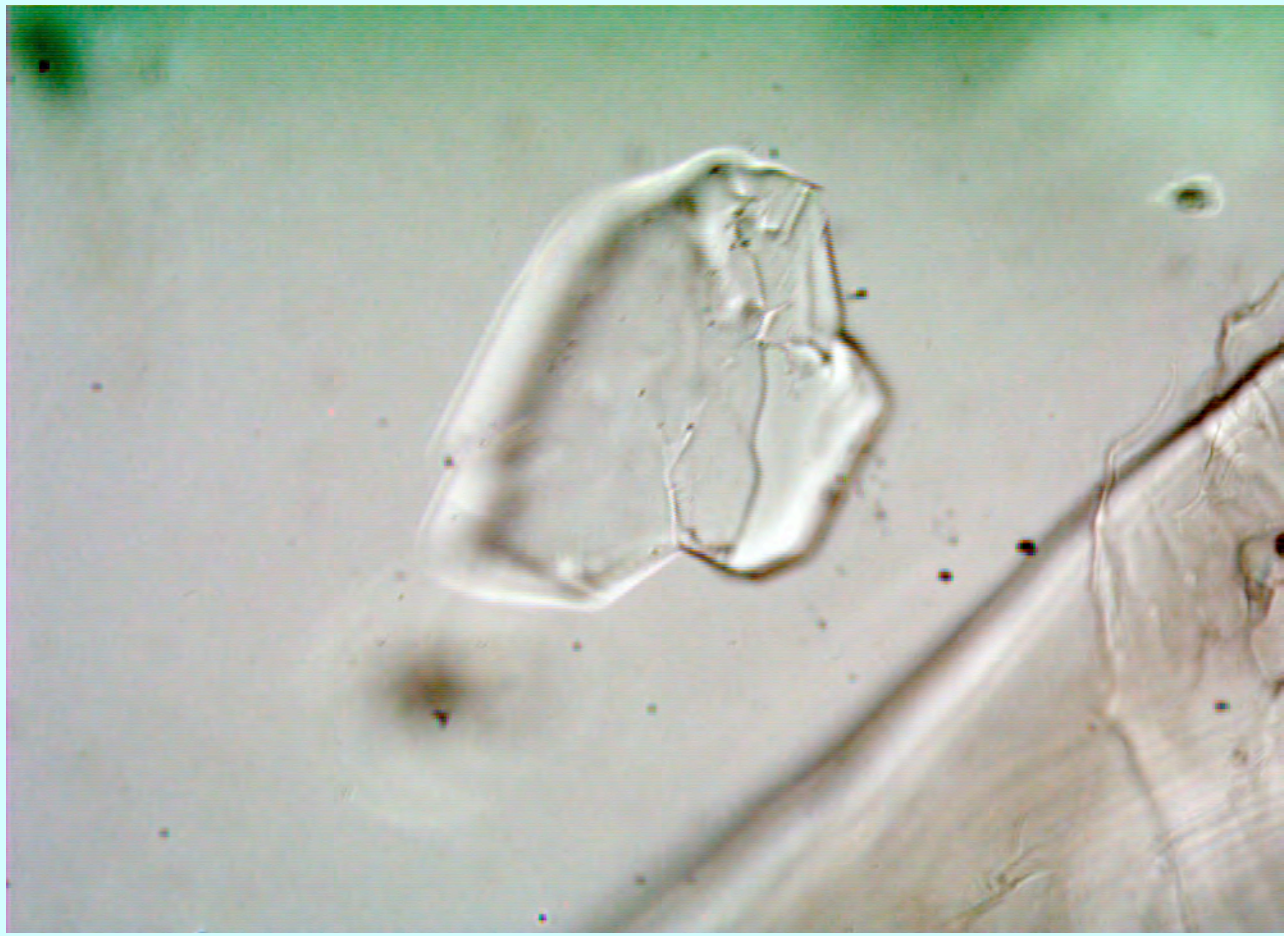
3) MODIFY SEQUENCE OF ION PORE TO MAKE IT MULTI-LIGAND SENSITIVE

STRUCTURE

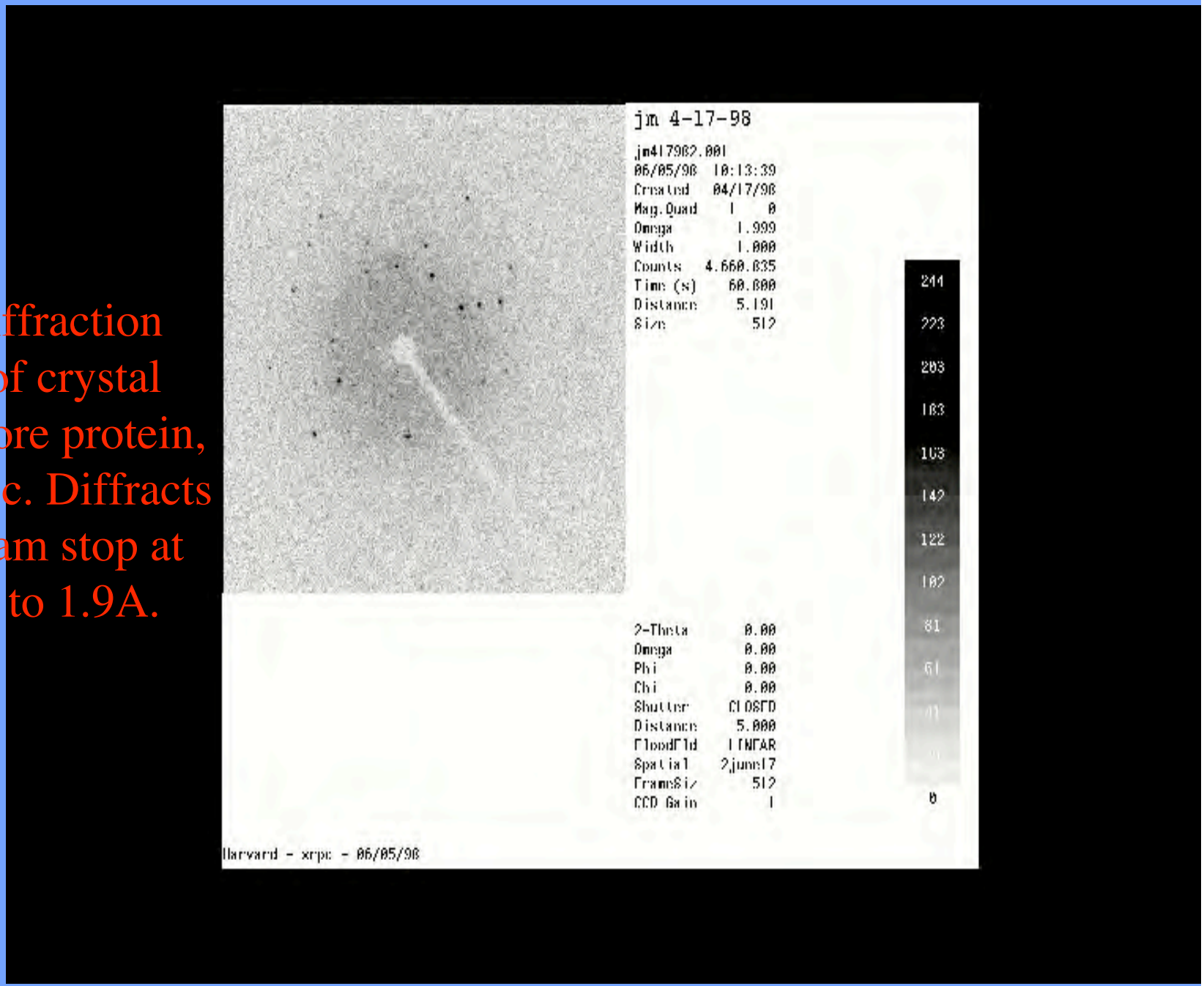
CRYSTALS OF SUBUNIT C GROWN IN ORGANIC SOLVENTS

CRYSTAL AND DIFFRACTION PATTERN NEXT 2 SLIDES

Crystal of subunit c grown in organic solvent. Nucleation promoted via dialysing in silicon polymer. The cluster is subdivided to produce single diffracting crystals. Amino acid analysis and Edman sequencing confirm that it is subunit c.



X-ray diffraction pattern of crystal of ion pore protein, Subunit c. Diffracts from beam stop at 28A out to 1.9A.

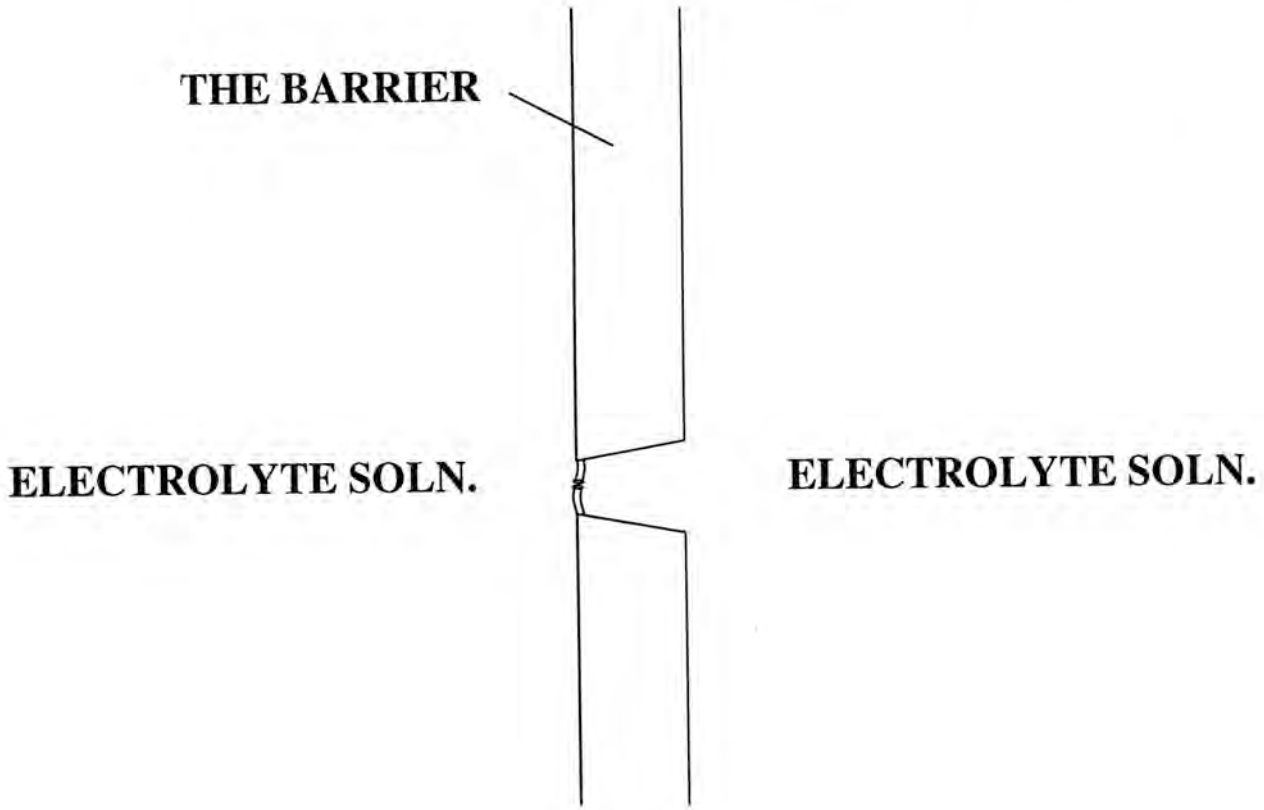


SENSOR HARDWARE

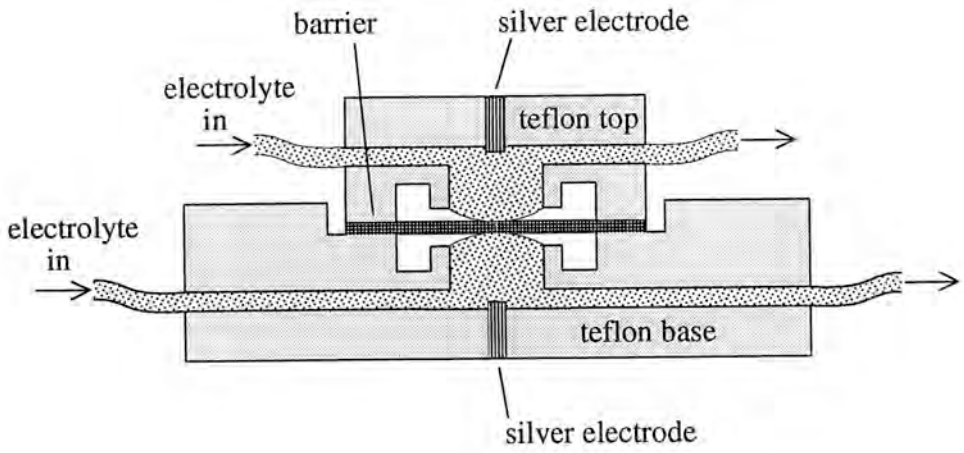
Concept of pore in barrier of silicon nitride
Chamber to test pore in SiNx membrane
SEM of nm holes in SiNx membrane
Multiple holes on silicon chip
Current trace of functioning pore in barrier

NEXT 5 SLIDES

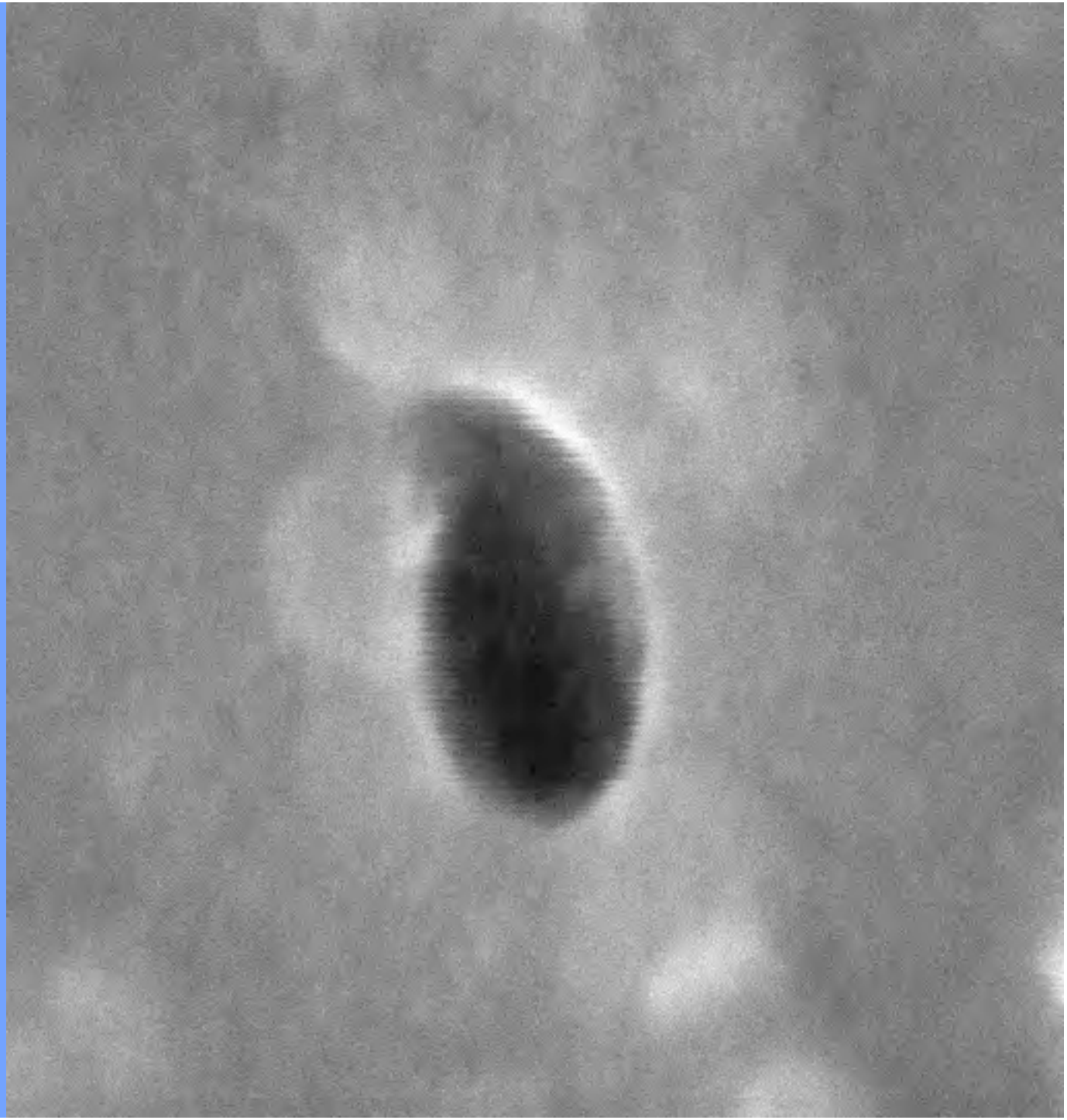
THE ESSENTIAL COMPONENT OF THE BIOLOGICAL-ELECTRONIC INTERFACE IS:



Test Chamber for Barriers



SEM of a nanofabricated 130 x 180 nm hole in a 250nm thick SiNx membrane. The hole was patterned by direct-write electron beam lithography and reactive ion etching.

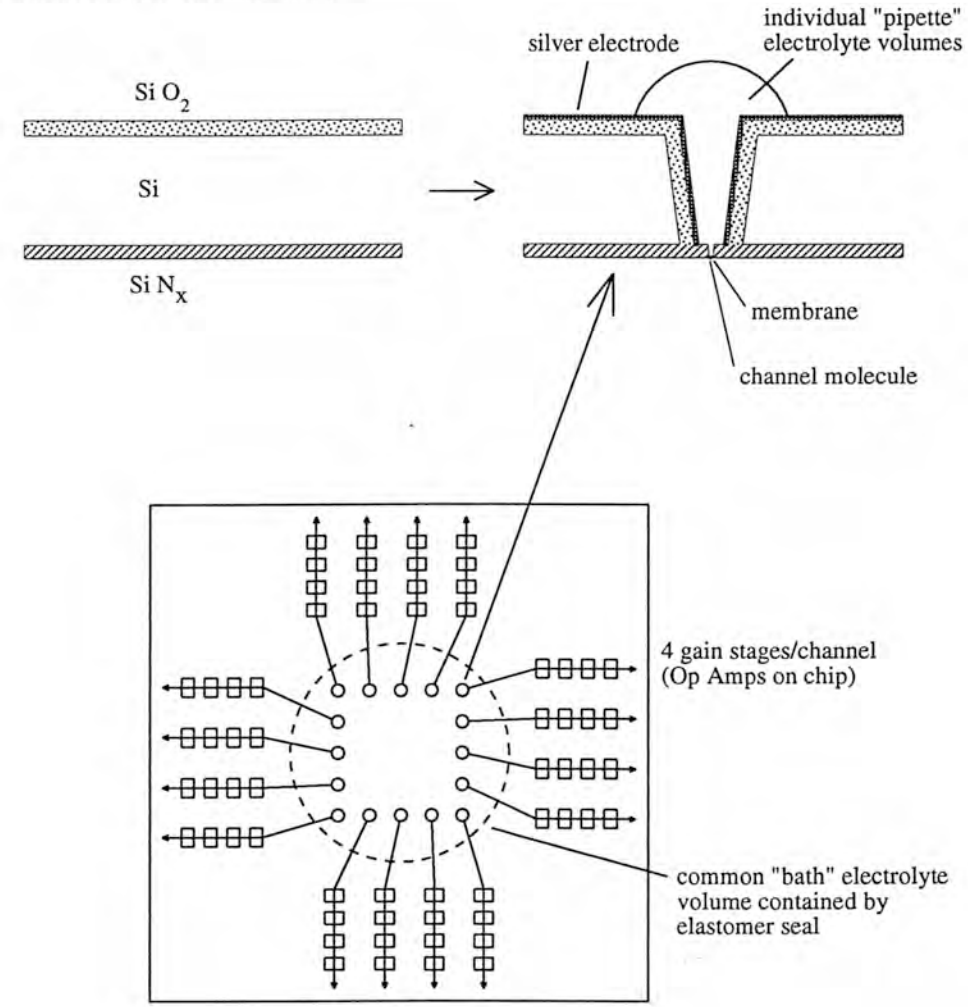


Concept for Sensor Chip

statistical sampling of 16 ion channels

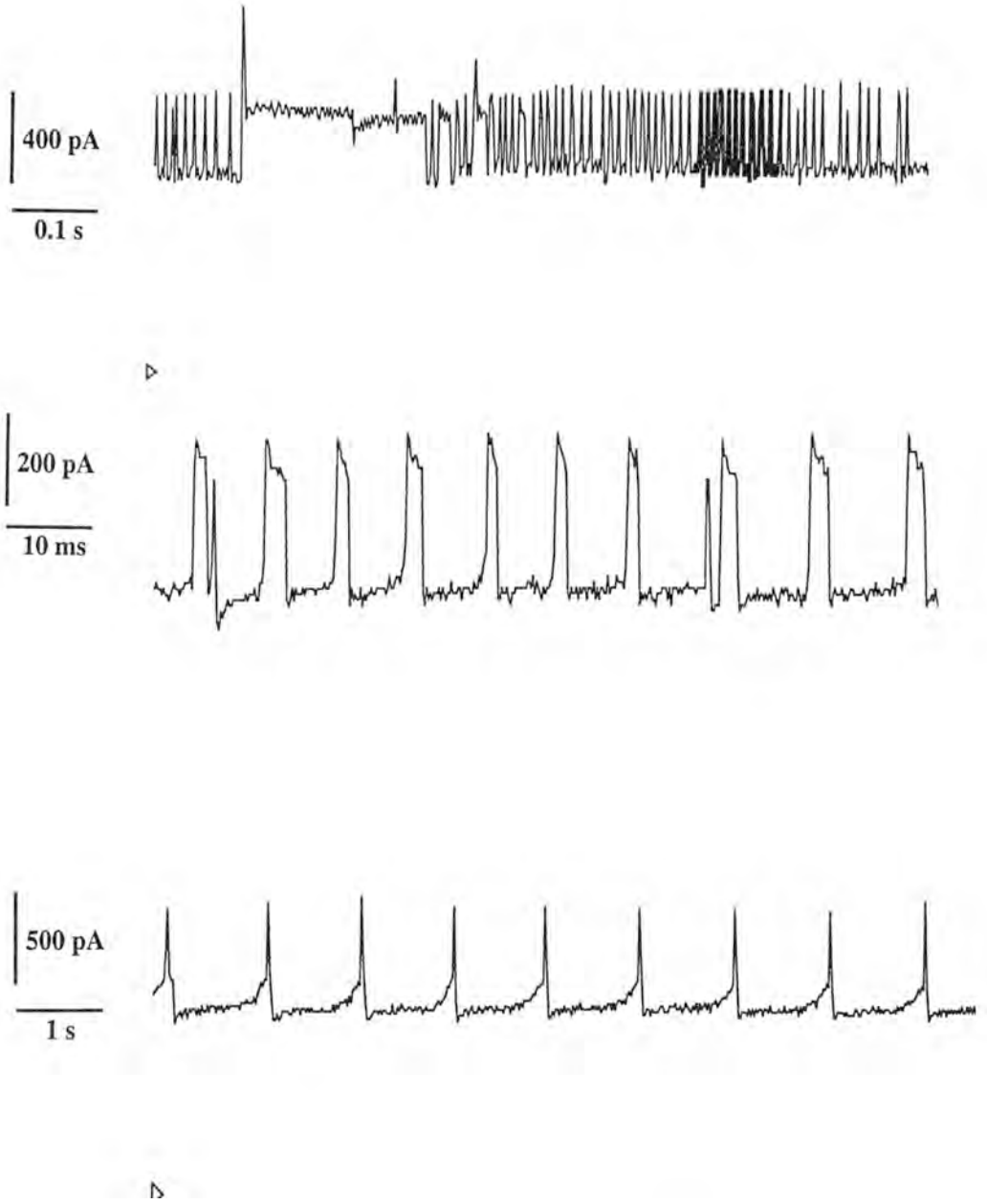
standard silicon technology

microholes made lithographically



ION PORE IN BARRIER

Ion pore functions with same oscillating current in barrier 1μm holes as in classic patch clamp assay



EXPERIMENTAL APPROACH TO MAKE PORE MULTI-LIGAND SENSITIVE

ESTABLISH SHORT AMINO ACID SEQUENCES TO ADD TO SUBUNIT C, THE PORE PROTEIN, TO MAKE IT BIND A VARIETY OF LIGANDS

MODIFY CODE OF THE 2 SUBUNIT C GENES (PI AND P2) SUCH THAT MODIFIED SUBUNIT C, SENSITIVE TO NEW LIGANDS IS MADE IN CELL LINE EXPRESSION SYSTEMS

CHECK THAT THE MODIFIED PORE PROTEINS SENSE SPECIFIC LIGANDS AND PRODUCE CLEAR SPECIFIC SIGNALS

INSERT THE MODIFIED SUBUNIT C PROTEINS
IN SENSOR CHIP

SUMMARY

The crystallization of the pore will indicate new ways to solve other membrane protein structures. Most hazardous chemicals dock with membrane proteins. Protecting personnel from chemical hazards requires a knowledge of the structure of as many membrane proteins as possible.

The ion pore when incorporated into a silicon chip will function as a sensor.

The high gain and fast response time of the electronic-biological sensor will greatly aid personnel encountering hazardous chemicals.

The protein in the sensor can be monitored for hours-days and the data used to understand brain function and disease states associated with over-accumulation of the pore protein (Batten disease) a common neurodegenerative disease.

Relevant references for the sensor work

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•McGeoch J. E. M., McGeoch M. W., Carter D. J. D., Shuman R. F. and Guidotti G. A biological to electronic interface with pores of ATP synthase subunit c in a silicon nitride barrier. *Med. Biol. Eng. Comput*., **38**, 113-120 (2000).

•McGeoch J. E. M., McGeoch M. W., Mao R. and Guidotti G. Opposing actions of cGMP and calcium on the conductance of the F_0 subunit c pore. *Biochem. Biophys.Res. Comm* **274**, 835-840 (2000).

•McGeoch J. E. M and Guidotti G. Batten disease and the control of the F_0 subunit c pore by cGMP and calcium. *Eur J Paed Neurol,* **5**, suppl A, 147-150 (2001).