A Model for assessing ATP demands of sustained high frequency firing

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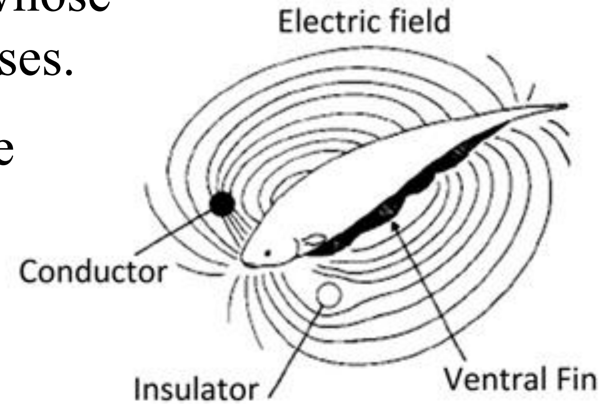
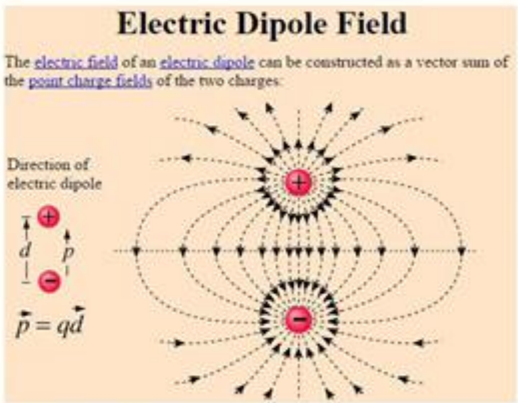
ELECTRIC **O**RGAN **D**ISCHARGE (**EOD**)

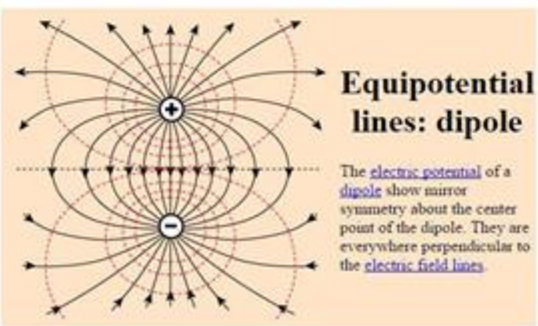
oscillating dipole
produces electric fields whose distortions the fish senses.

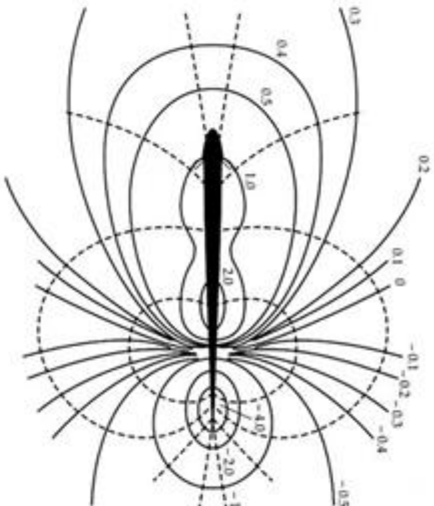
Eigenmannia....

<u>Focus</u>? – e-discharge mechanisms (not sensing)

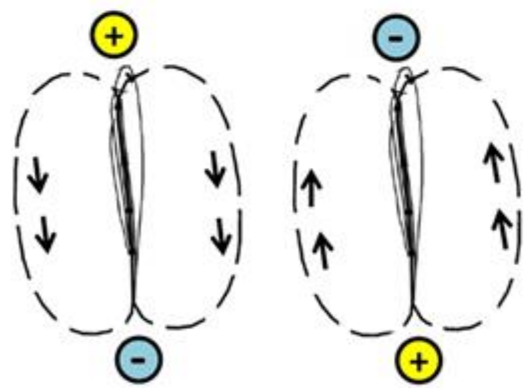








Solid- equipotential
Dashed – field lines



Currents flow same pattern as the flipping electric fields (Ohm's law --i.e., current flow varies with "voltage drop")

Electric eel (tetanic contracture of prey)

Stuns the fish before eating it

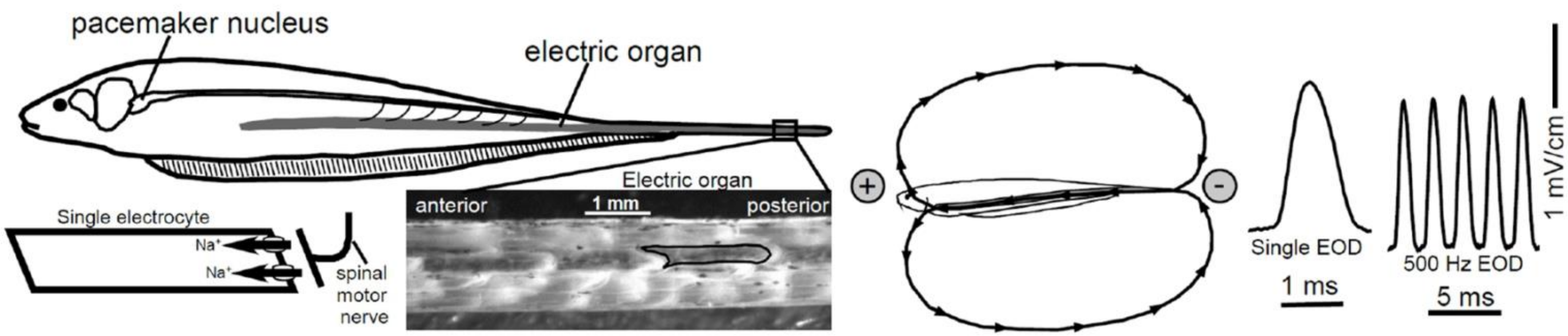
But our interest is in weakly electric fish which produce electric organ discharges continuously throughout their lifetime.

Eigenmannia -- *a weakly electric fish*

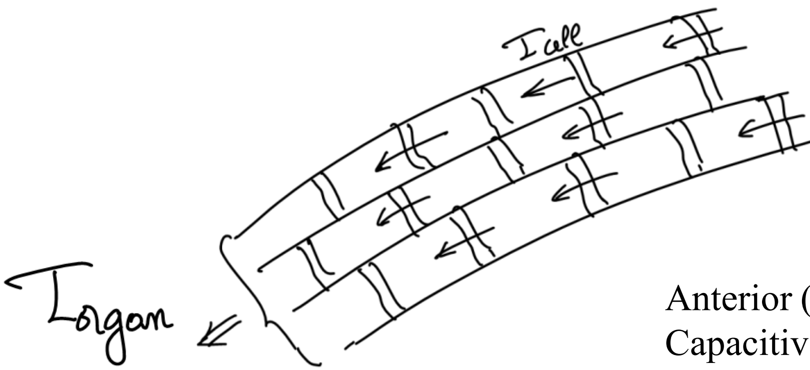
electric organ -- thousands of massive (~1 mm) electrocytes

derived from fused "muscle" cells (syncytium ~30,000 nuclei)

– the EOD requires synaptic input from the Central Nervous System pacemaker



Posterior (P): Navs and Kvs generating APs



Anterior (A):
Capacitive role

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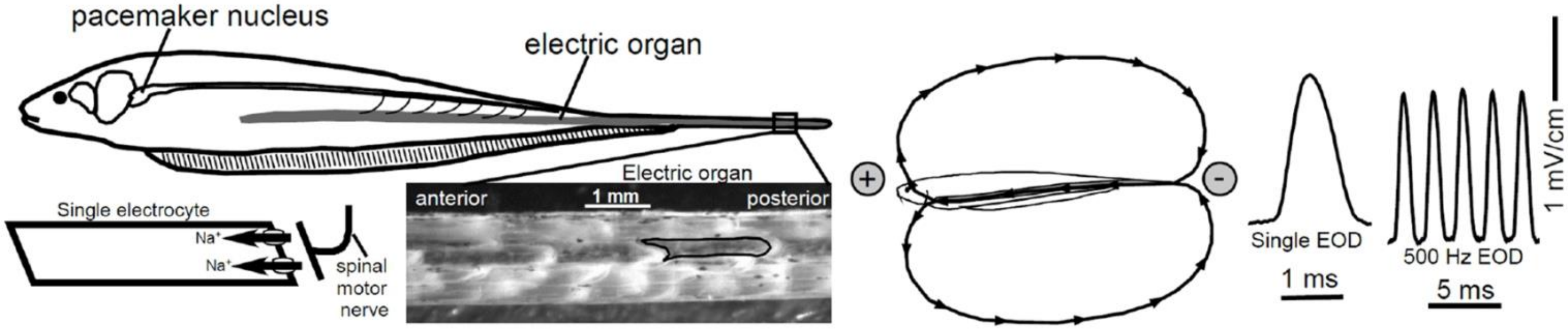
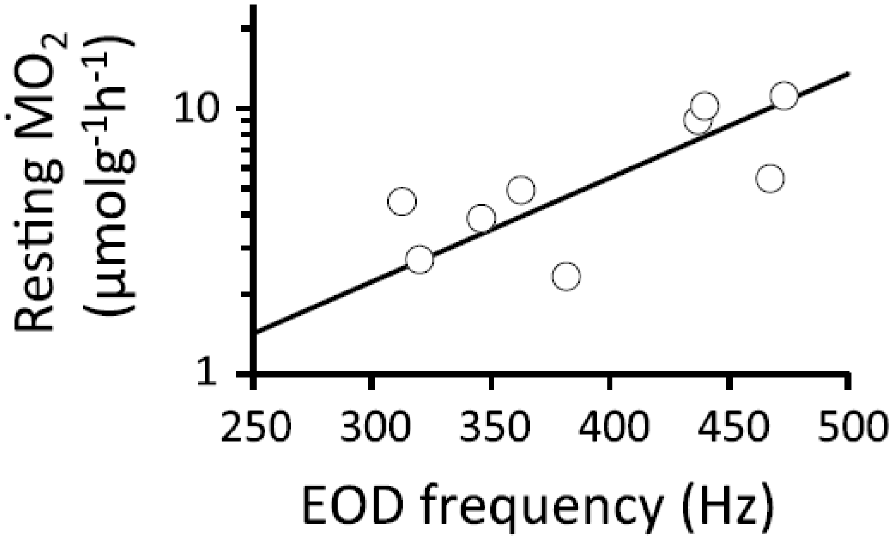


Lewis, Gilmour, Moorhead, Perry, Markham 2014
J Neurosci 34:197
Action potential energetics at the organismal level reveal a trade-off in efficiency at high firing rates.

- *Eigenmannia* EOD – synchronous **tonic APs – *never stop!***
- major fraction of **whole animal metabolism**.
- *EOD + electric sensing*: navigate, communicate, locate prey.
- Jamming Avoidance Response (**JAR**) – CNS-controlled ΔAP frequency when a conspecific using a similar freq is nearby (average JAR: Δ ~10 Hz – easily measurable)

- whole fish respirometry (flasks) - exploit JARs to estimate ATP consumed per AP – HOW? First, measure background O_2 consumption, then "fake" a conspecific. This elicits a sustained change in AP frequency (EODs monitored throughout).

Lewis et al., 2014



Their major expt'l result:

ATP/Hz is *NOT* fixed

EODs cost grows exponentially with freq
--- the respirometry data
summarized by the ATP/Hz slope
(*semi-log plot*)

tenor → → soprano

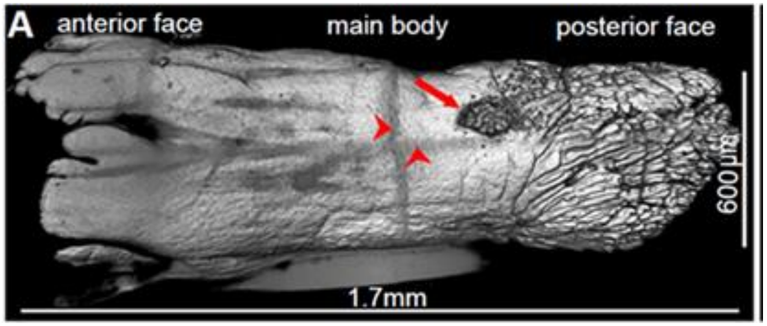
MOREOVER

– the ***incremental*** costs in ATP/Hz
determined by eliciting JARs
in 6 fish with very different baseline
EOD frequencies concurs.

SO –

tenor = cheap, soprano = "expo"-expensive

(remember … *semi-log plot*)

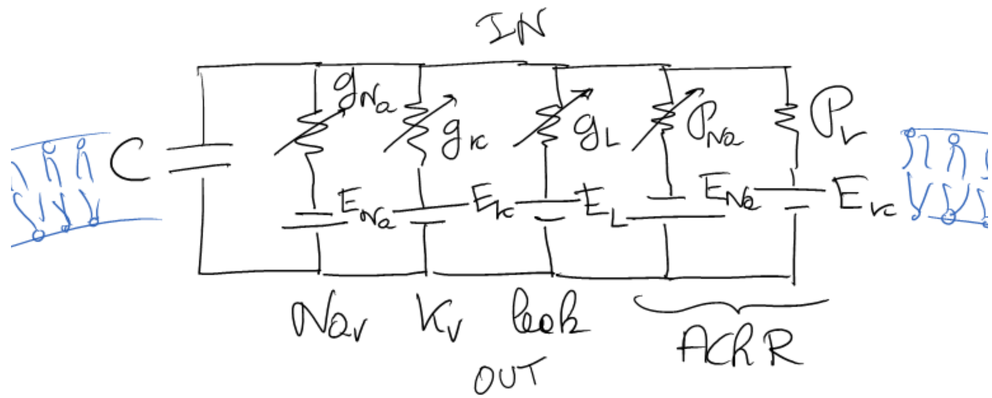


Our aims: design the generation of action potentials (APs) by electrocytes and explain the frequency dependence of the energy requirements

We designed a model consistent with available experimental data. Today we will focus on the posterior end which drives the electric organ discharge.

A Hodgkin Huxley type model which calculates the changes in membrane voltage as ions flow in and out of the electrocyte's posterior face.

$$C\frac{dV_m}{dt} = -I_{Na} - I_K - I_{leak} - I_{Na}^{AChR} - I_K^{AChR}$$



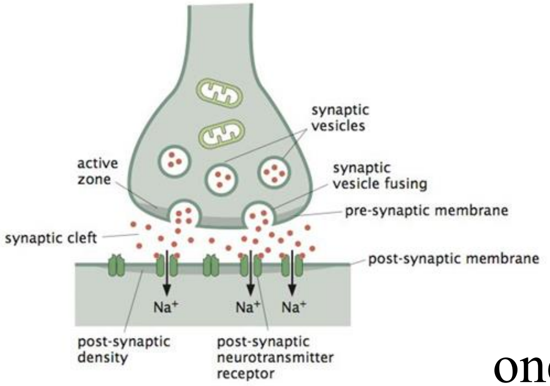
Each current has its own kinetics either voltage driven (Nav, Kv) or ligand driven (AChR)

$$I_{Na} = g_{Na}(V_m - E_{Na}),\quad g_{Na} = \bar{g}_{Na} m^3 h \quad \frac{dm}{dt} = \alpha_m(1-m) - \beta_m m$$

$$I_K = g_K(V_m - E_K),\quad g_K = \bar{g}_K n^4 \quad \frac{dn}{dt} = \alpha_n(1-n) - \beta_n n \quad \frac{dh}{dt} = \alpha_h(1-h) - \beta_h h$$

Leak current

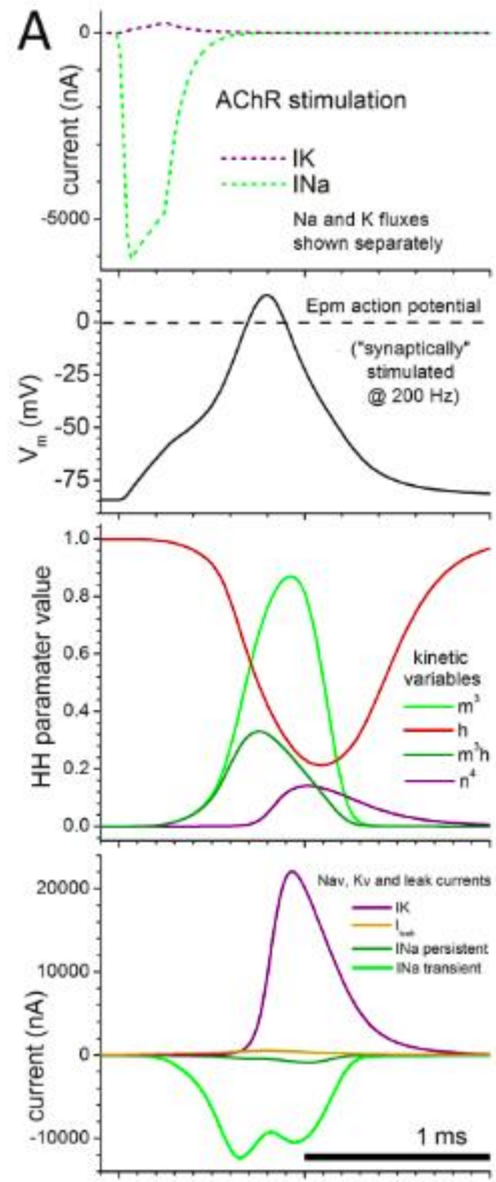
$$I_{leak} = g_{leak}(V_m - E_{leak})$$

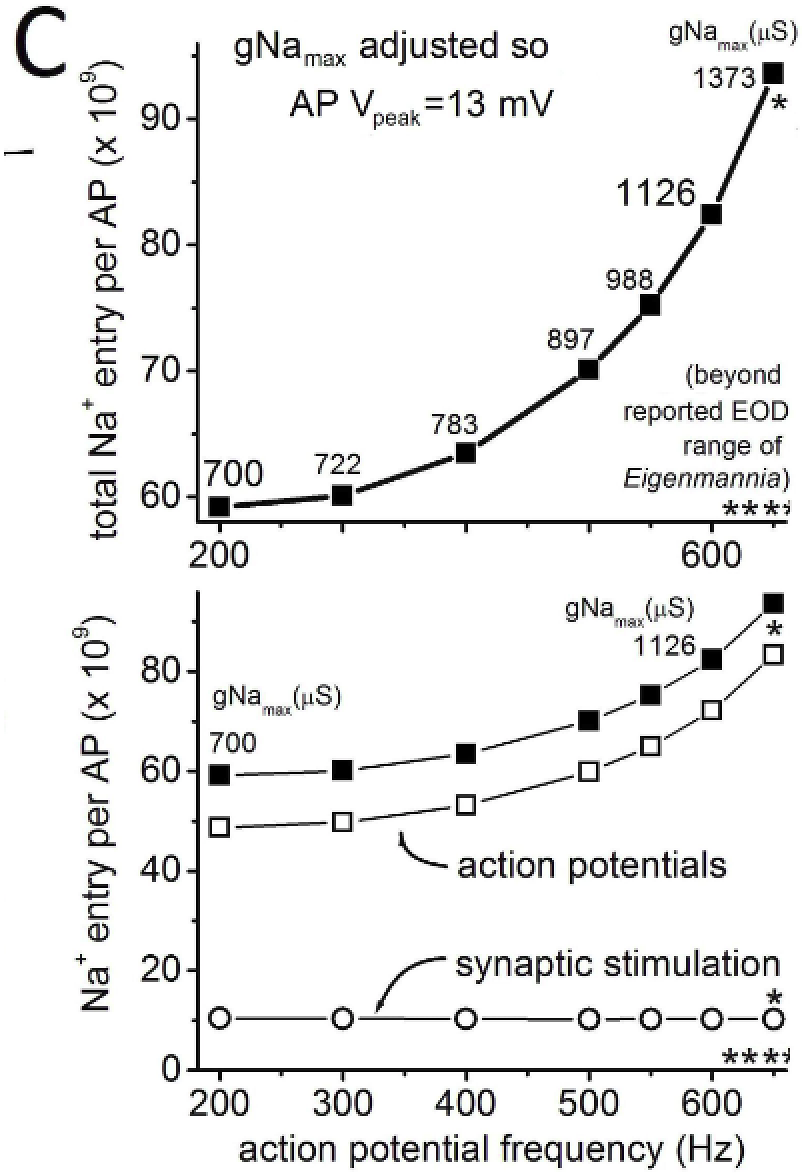


one action potential

the kinetic variables

the ion currents from voltage gated Nav and Kv channels





Increase in ATP measured as a Na entry* demand not exponential less than a factor 2 over the relevant range of frequencies.

It is difficult to conceive that the observed exponential increase would arise from the generation of AP

So where does it come from?

* Na^+ has to be pumped out which requires ATP

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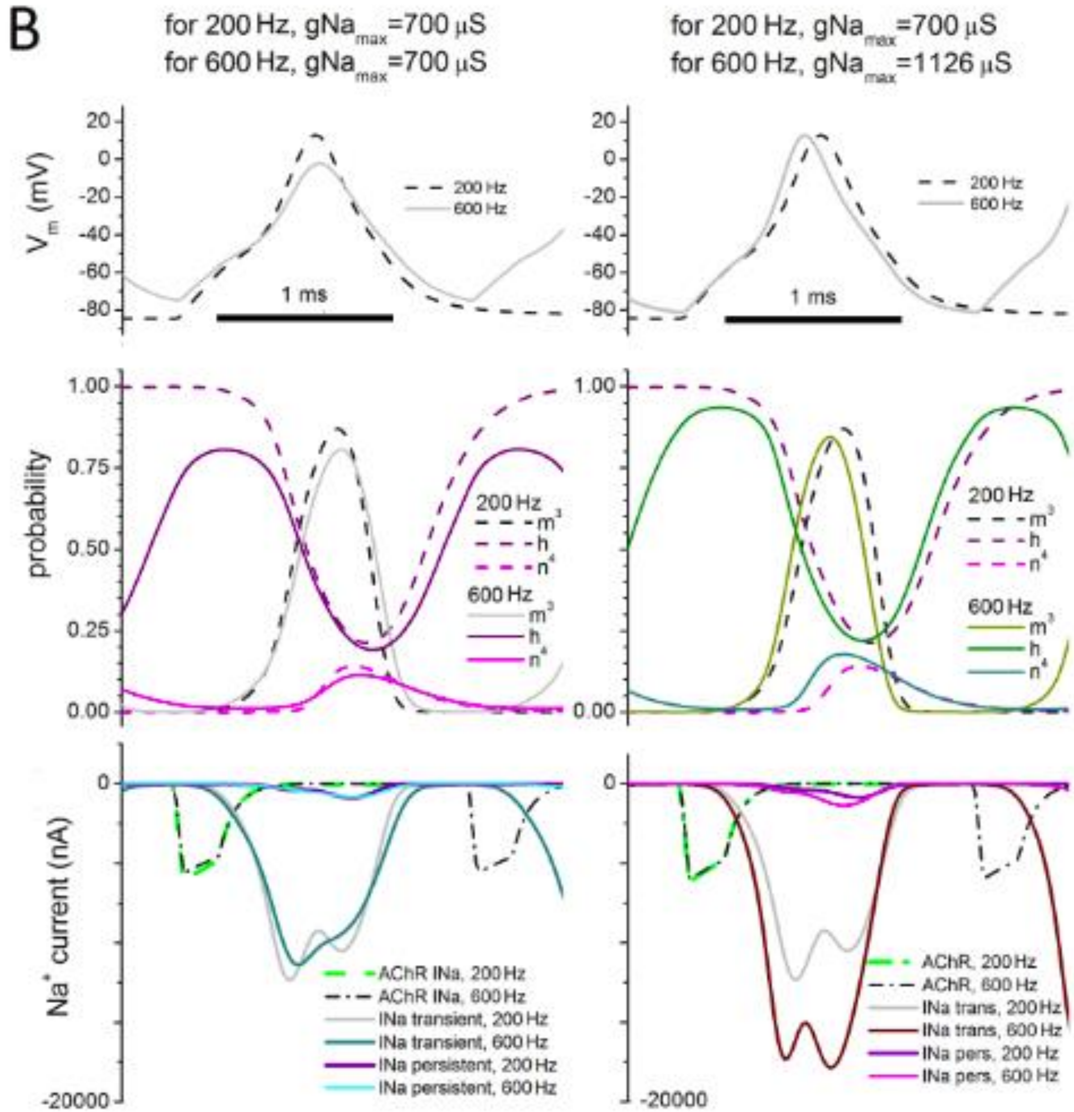
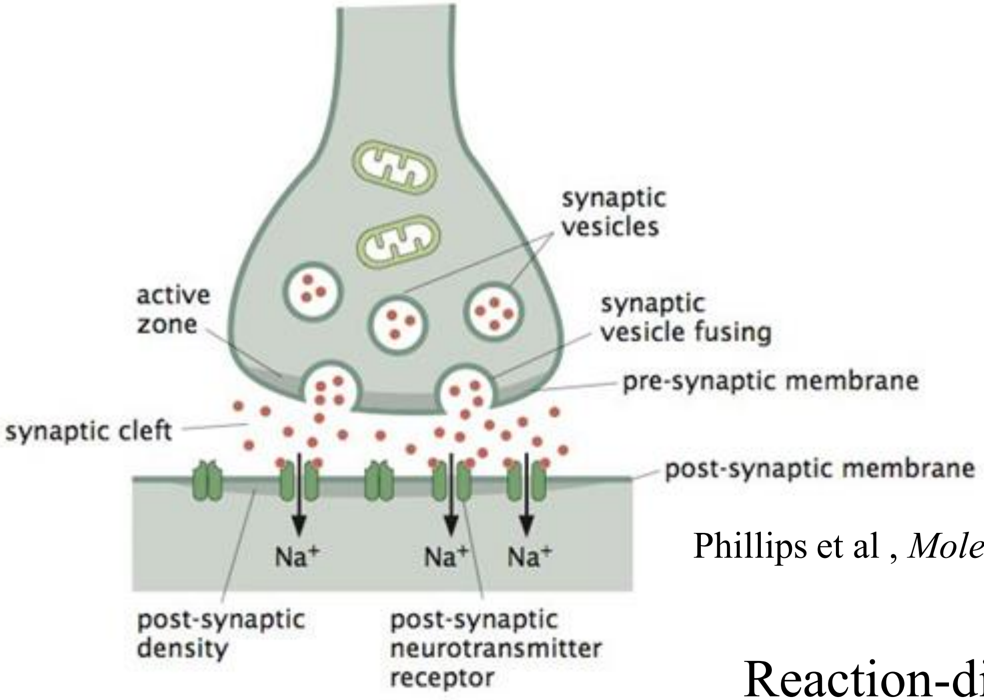
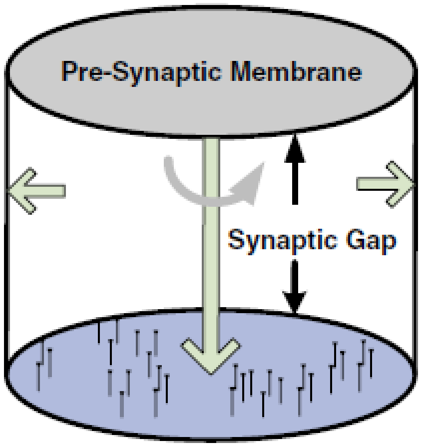
The increased demand of ATP based on Na^+ entry is a result of overlap of action potentials





The high measured increase in cost has then to arise from increased demands related to generating high frequency stimulus

Phillips et al , *Molecular Biol. of the Cell*

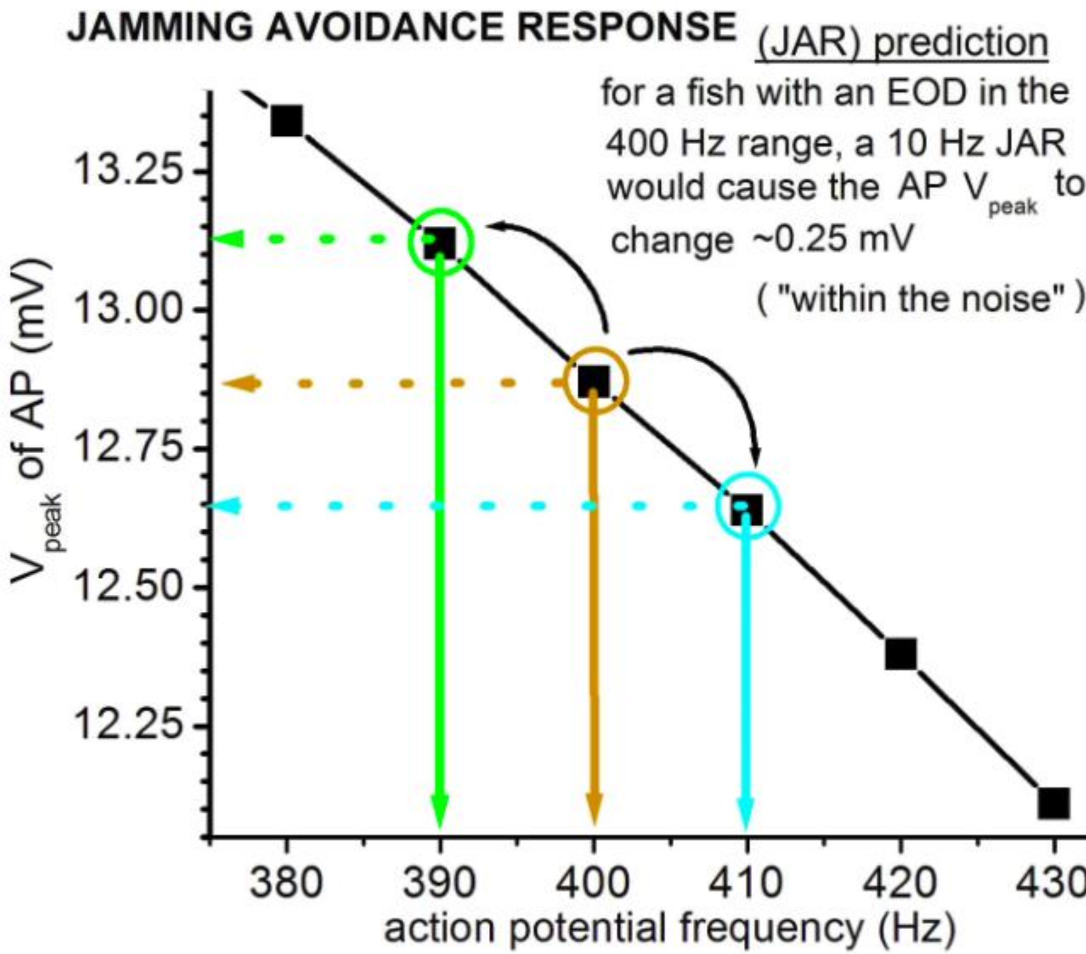


Reaction-diffusion process where ACh released by vesicles diffuse across the gap partly intercepted by acetylcholinesterase. But others reach the posterior end of the electrocyte attaching to AChR leading to stimulating current initiating the action potential.

Khaliq et al, J. Neurosci. 2011

Fish shift their frequency when close to a conspecific.

We show that this could be accomplished without the need to adjust their ion conductances



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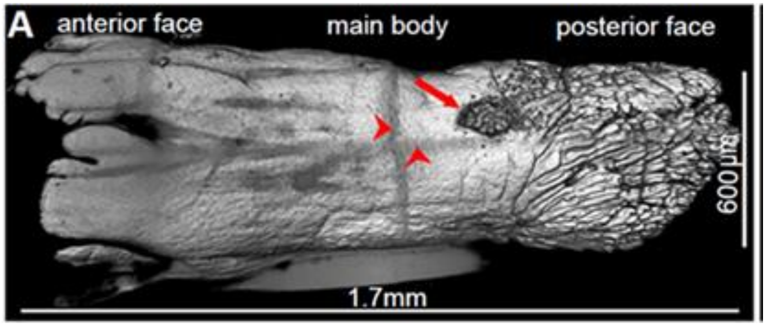
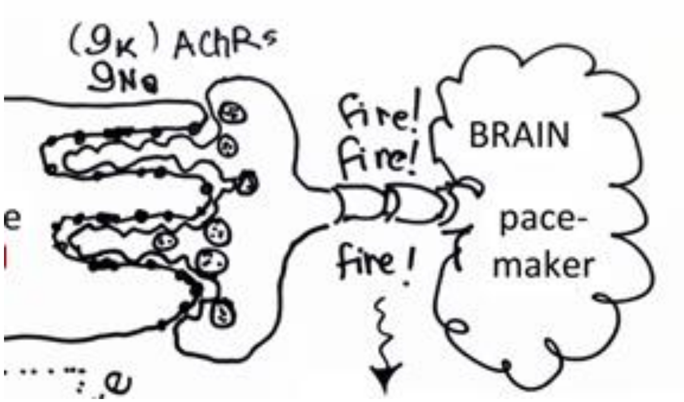
What is next?

- Whole electrocyte model



- Modeling the synaptic transmission: how the synapse is designed to permit firing frequencies up to 1000Hz or periods of the order of 1 ms



Summary

- Many electric fish are capable of producing oscillating electric dipoles generated by columns of electrocytes (derived from muscle cells)
- The action potentials (APs) are similar to those of neurons (Nav, Kv, and Na/K pumps) but are generated at very high frequencies (200-500Hz in *Eigenmannia*, but in other fish up to a 1000Hz)
- The ATP cost of generating electric organ discharges increases as a power law with increased frequency contrary to the observed exponential increase indicating that the cost of providing the brain stimuli leading to the synaptic transmissions is high.
- Still need the operation of the full electric organ and that of synaptic transmission

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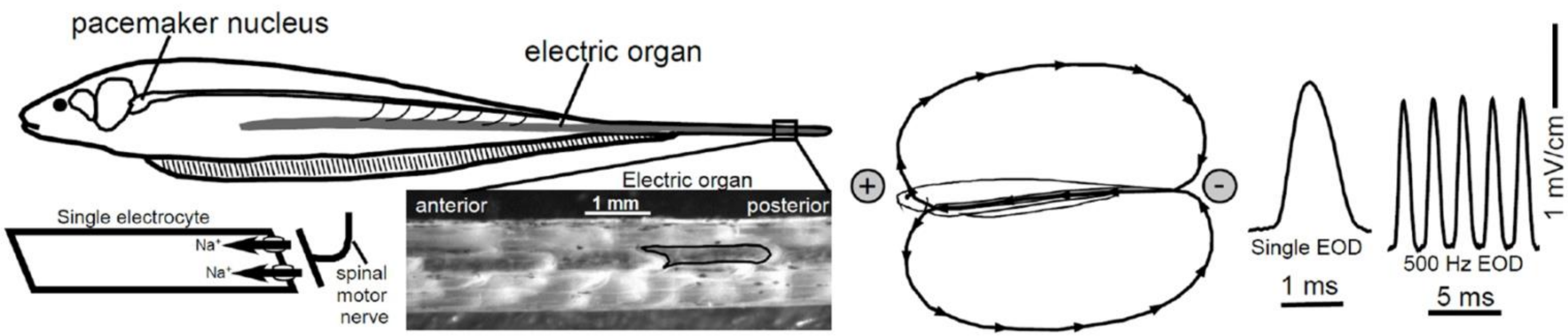
– the EOD requires synaptic input from the CNS pacemaker



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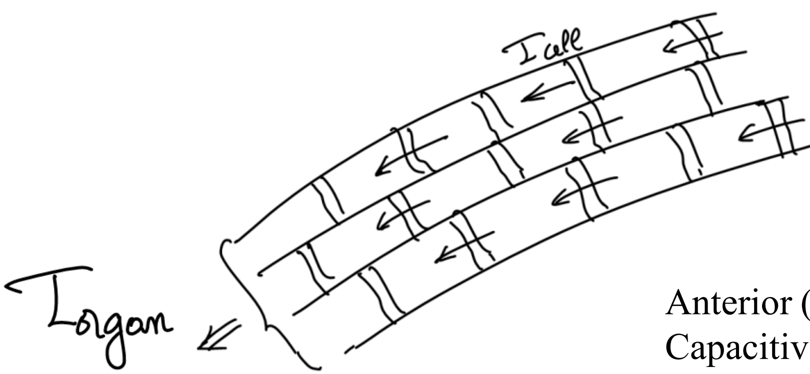

Anterior (A):
Capacitive role

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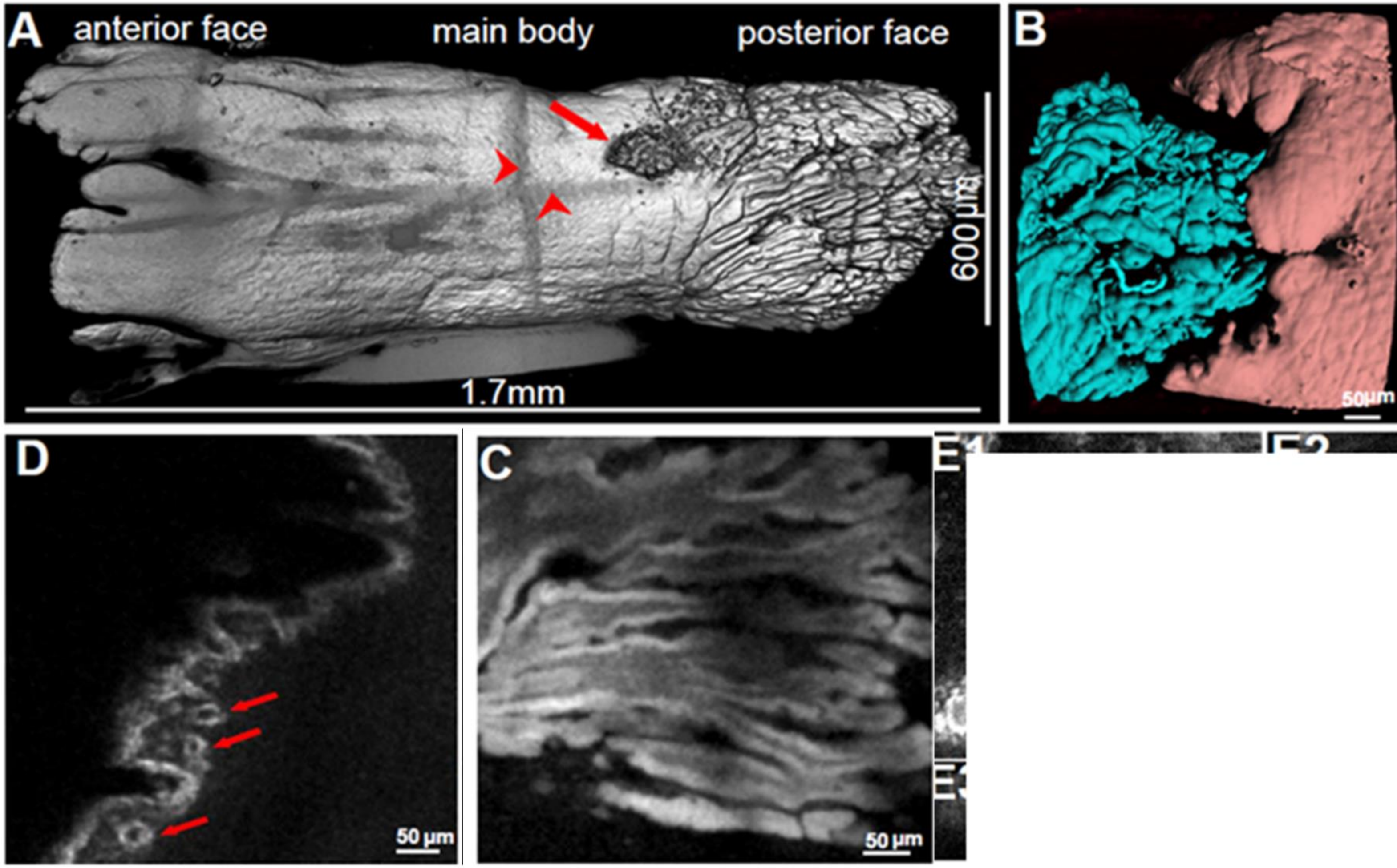
Ban, Smith, Markham Figure 2 2014



AP generation in a single electrocyte

Anterior (A):
Capacitive role

Posterior (P): Navs and Kvs generating APs

V_A =potential at anterior end wrt to outside

V_P =potential at posterior end wrt to outside

$C_A \approx$ 18nF with invag. 7.5 x larger

$C_P \approx$ 48nF

Equivalent circuit

R_{gap}= gap resistance

R_{cyt}= cytoplasma resis.

$R_{cell}= R_{gap}+R_{cyt}$

R_{ext}= external load

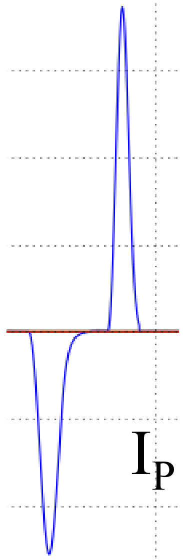
$V_A= V_2-V_1$

$V_P= V_3-V_4$

A full EOD cycle as seen from one electrocyte

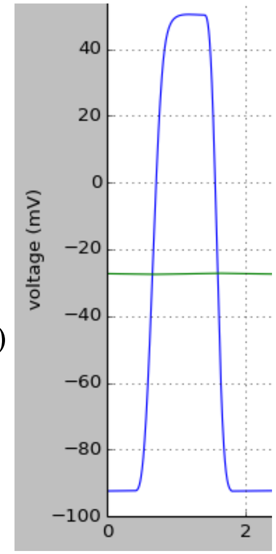
(in the steady state)

1.- Influx of Na^+ at P (current $I_P < 0$), outflow of K^+ ($I_P > 0$)



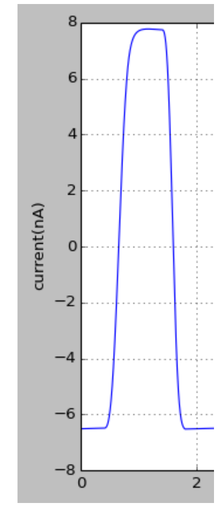
I_P

V_P (blue)



500Hz spike shown

V_A (green)

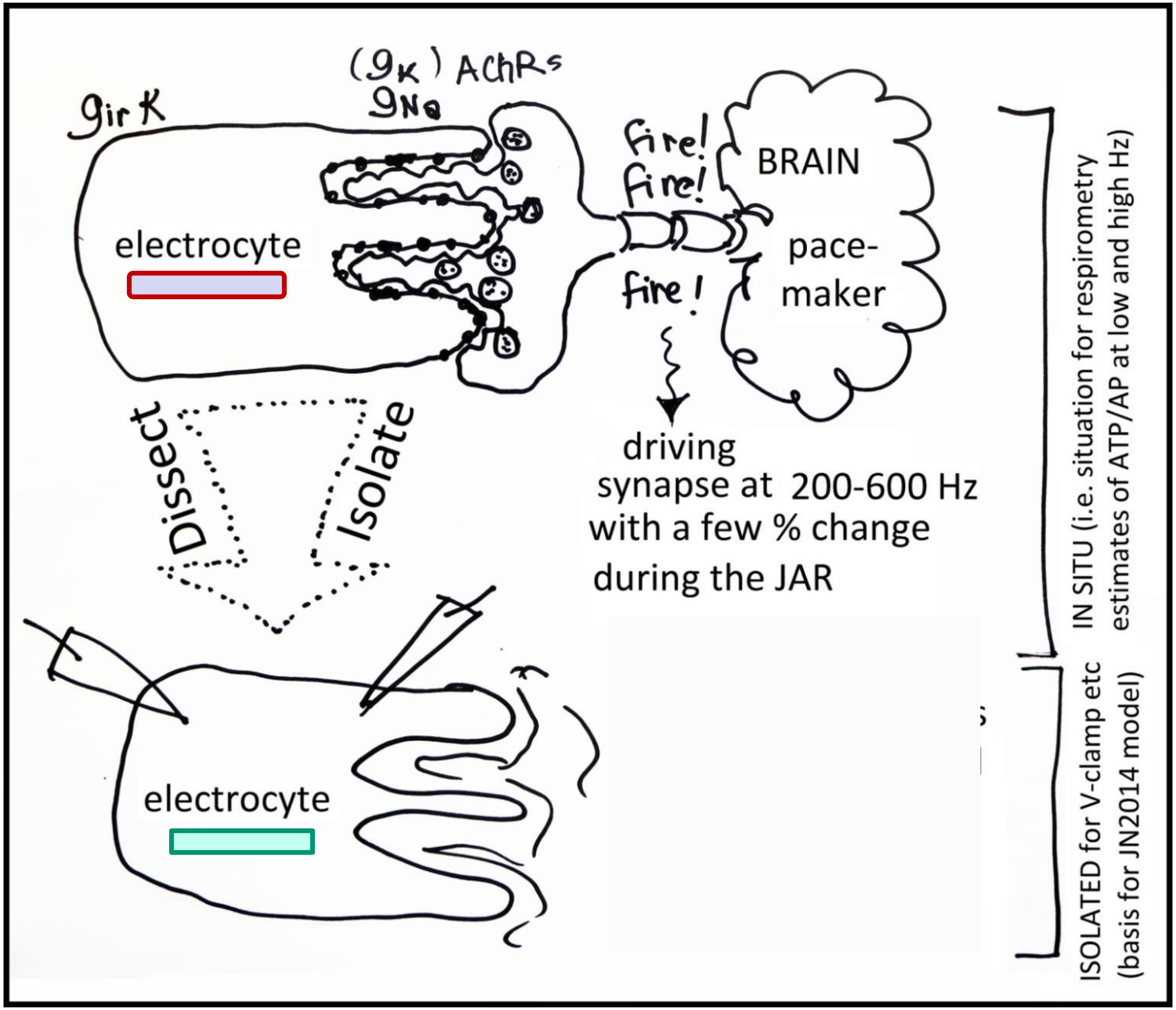


I_E

2.- an AP is generated at P. V_A stays nearly constant: successive APs have charged up the capacitive membranes at A to V_A.

3.- When $V_A > V_P$ current I_E is head negative, then as $V_A < V_P$ it becomes head positive

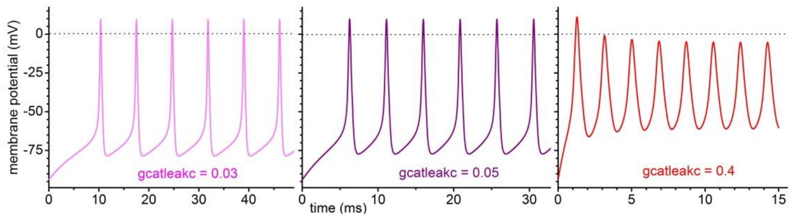
$$I_{cell} = \frac{V_P - V_A}{R_{cyt} + R_{gap} + \frac{R_{ext}}{n}}$$

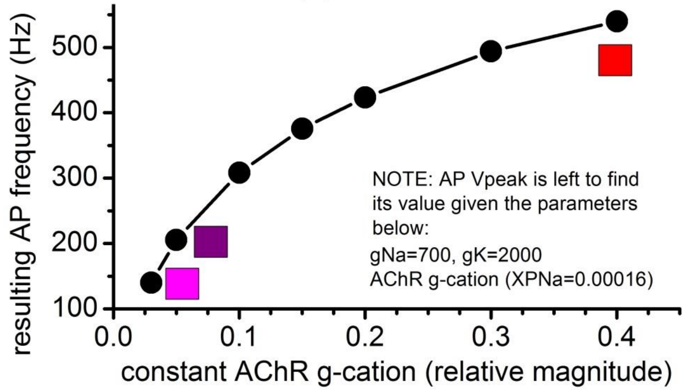

(gK) AChRs
gNa
girK
electrocyte
Fire!
Fire!
fire!
BRAIN
pace-
maker
driving
synapse at 200-600 Hz
with a few % change
during the JAR
Dissect
Isolate
electrocyte
IN SITU (i.e. situation for respirometry
estimates of ATP/AP at low and high Hz)
ISOLATED for V-clamp etc
(basis for JN2014 model)

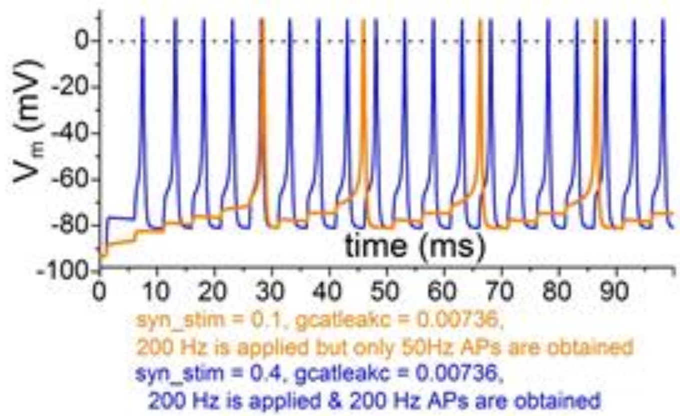
Proposed model for posterior generation of action potentials

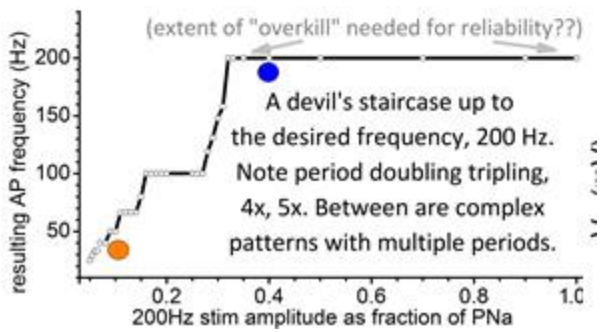
Constant activation of AChRs would elicit high frequency APs over a wide Hz range(as shown), depending on [ACh].
BUT any small Δ[ACh] would cause ΔHz & thus, failed communication /sensing.

To ensure firing at "desired" Hz, in spite of [ACh] vagaries, fish likely mixes subthreshold [ACh] + pulsatile ACh. Here (idealized case, i.e. no [ACh] noise) desired Hz (blue) is achieved once pulsatile component is sufficiently large.









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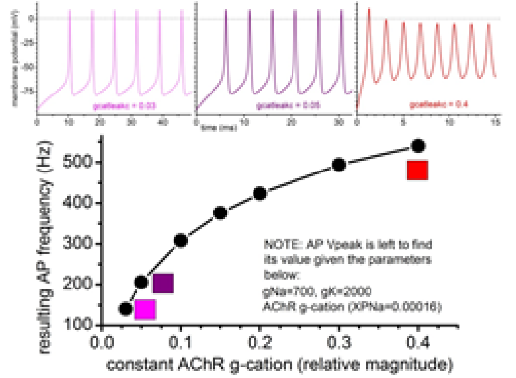
- Still need the operation of the full electric organ and that of synaptic transmission

Epm g_{cation} clamp (to mimic AChR activation) – fixed, pulsatile, mixed



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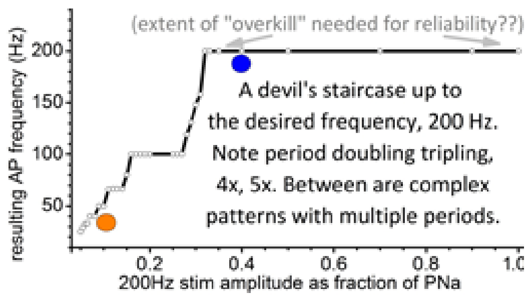

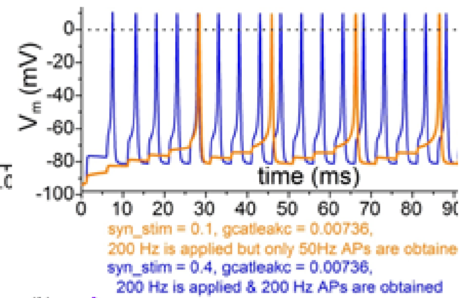

In the face of the chosen subthreshold AChR activation & AChR "noise", a 0.3x stim @ 200 Hz is too weak, but a 0.42 stim yields a solid AP output at the desired 200 Hz with only a tiny "wobble" at the foot of the APs.