A Model for assessing ATP demands of sustained high frequency firing

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ELECTRIC ORGAN DISCHARGE (EOD) oscillating dipole produces electric fields whose distortions the fish senses.

Focus? – e-discharge mechanisms (not sensing)
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) electroytes
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
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 $\Delta$AP frequency when a conspecific using a similar freq is nearby (average JAR: $\Delta \sim 10$ 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 summarised by the ATP/Hz slope
  
  *(semi-log plot)*

**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.

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

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

\[ I_{Na} = g_{Na}(V_m - E_{Na}) \]
\[ I_{K} = g_{K}(V_m - E_{K}) \]
\[ I_{leak} = g_{leak}(V_m - E_{leak}) \]

Leak current

\[ \frac{dg}{dt} = \alpha_n (1-n) - \beta_n n \]
\[ \frac{dg}{dt} = \alpha_x (1-x) - \beta_x x \]

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

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.


Phillips et al, Molecular Biol. of the Cell
Fish shift their frequency when close to a conspecific.

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

Anterior (A):
Capacitive role

Posterior (P): Navs and Kvs generating APs
AP generation in a single electrocyte

Anterior (A): Capacitive role

Posterior (P): Navs and Kvs generating APs

\[ V_A = \text{potential at anterior end wrt to outside} \]
\[ V_P = \text{potential at posterior end wrt to outside} \]

\[ C_A \approx 18\text{nF with invag. 7.5 x larger} \]
\[ C_P \approx 48\text{nF} \]

Equivalent circuit

\[ V_A = V_2 - V_1 \]
\[ V_P = V_3 - V_4 \]

\[ R_{gap} = \text{gap resistance} \]
\[ R_{cyt} = \text{cytoplasm resis.} \]
\[ R_{cell} = R_{gap} + R_{cyt} \]
\[ R_{ext} = \text{external load} \]
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\(^+\) (\(I_P > 0\))

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

\[
I_{\text{cell}} = \frac{V_P - V_A}{R_{\text{cyt}} + R_{\text{gap}} + \frac{R_{\text{ext}}}{n}}
\]
IN SITU (i.e., situation for respirometry)
estimates of ATP/ADP at low and high Hz

ISOLATED for V-clamp etc
(basis for JN2014 model)

driving synapse at 200-600 Hz
with a few % change
during the JAR
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 ∆[ACh] would cause ∆Hz & 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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To ensure firing at “desired” Hz, in spite of [ACh] vagaries, fish like mixes subthreshold [ACh] + pulsatile ACh. Here (idealized case, i.e. no [ACh] noise) desired Hz (blue) is achieved once pulsatile component is sufficiently large.

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.