Xenopulsator

Turning Biology into Voltage

Qamra Jema Khan, Shun Yan Mimi Poon,
Thomas Gurry, and John Sy

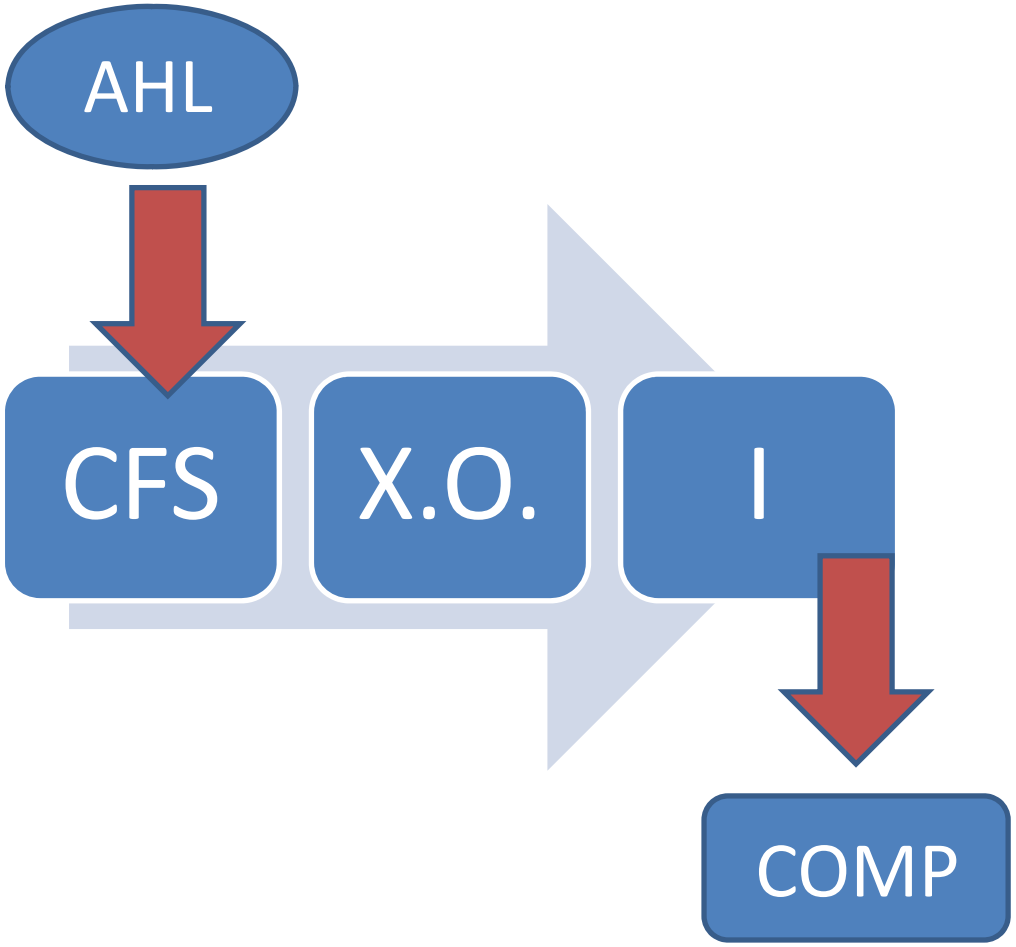
The Project

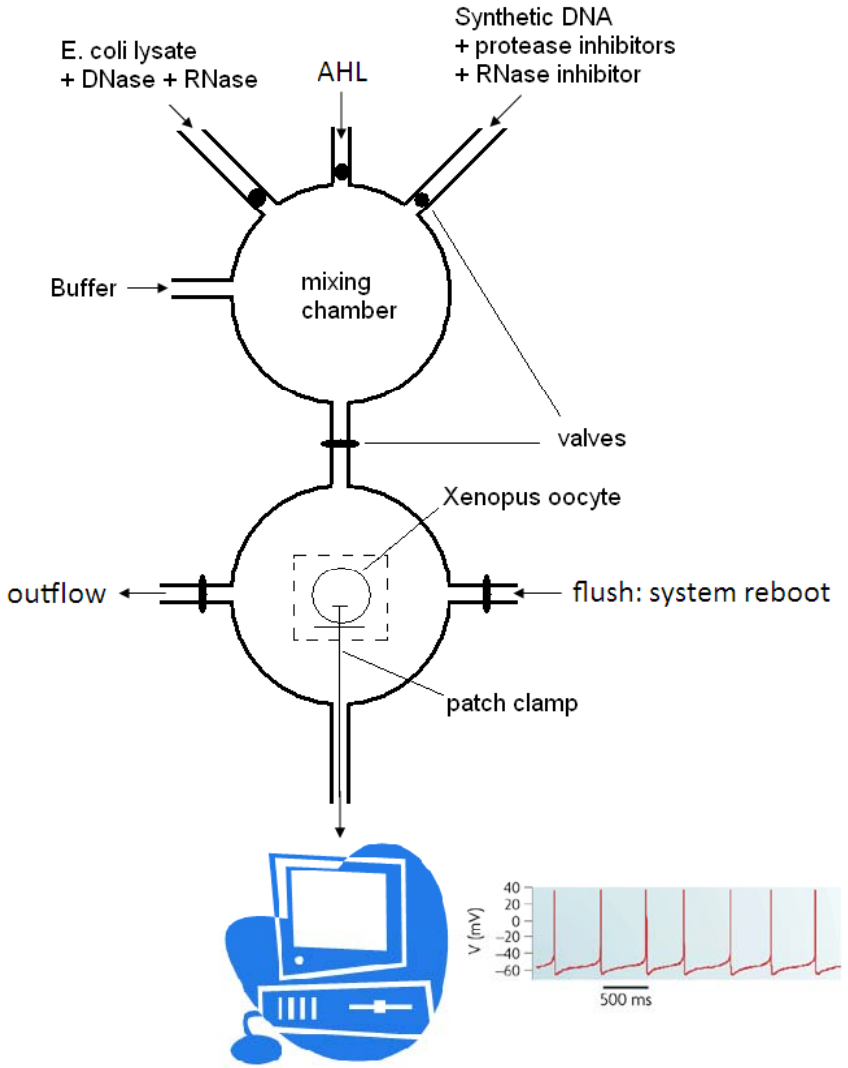
- Introduction
- System Design (The Biochemistry)
  - Cell Free System
  - Xenopus Oocyte
- Modelling (The Engineering)
  - The Model
  - Sensitivity Analysis
- Conclusion

Design Specifications

- Input from ***Biological*** System
  - Universal & Reliable way of communicating between biological systems
- Output to ***Electrical*** System
  - Measurable Quantity with Low Noise
- Small & Diffusible molecule between components

Architecture/Implementation





Acetylcholine Synthesis

Aim:

To produce acetylcholine (ACh) upon the presence of AHL

AHL: signalling molecules found in *Vibrio fischeri*

ACh: chemical that causes action potential in neuronal synapses in eukaryotes.

Chemical conversion, therefore needs chemical precursors on the first place!!

E. coli cell extract

Naturally existing molecules in *E. coli*

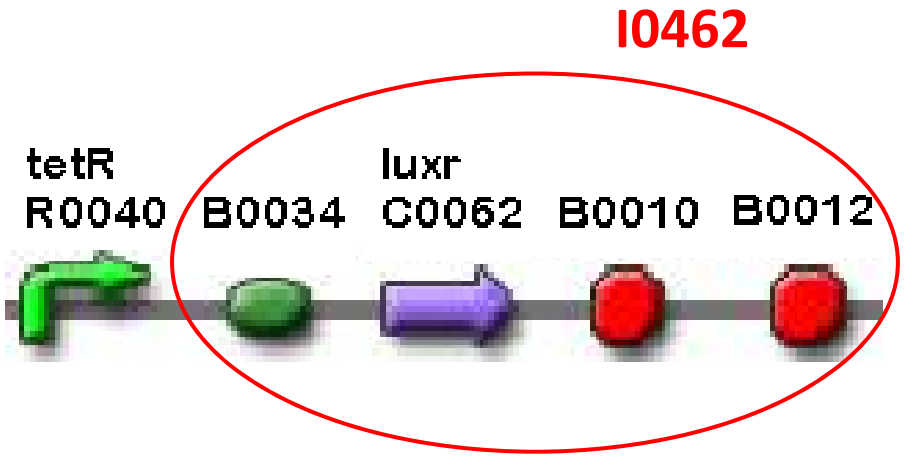
- phosphatidyl ethanolamine (PtdEA)
- S-adenosyl-L-methionine (SAM)
- Acetyl CoA

Required for ACh synthesis

Main construction steps

1. AHL detection device
2. AHL-dependent Acetylcholine biosynthesis device

AHL Detection Device



Parts

- Promoter tetR (R0040): constitutively ON
- Ribosome Binding Site RBS (B0034)
- *luxr* gene (C0062): encodes LuxR protein, binds to Lux promoter in complex with AHL
- 2 x terminating sequences B0010 & B0012

MIT registry: characterised **I0462**

= luxR protein generator

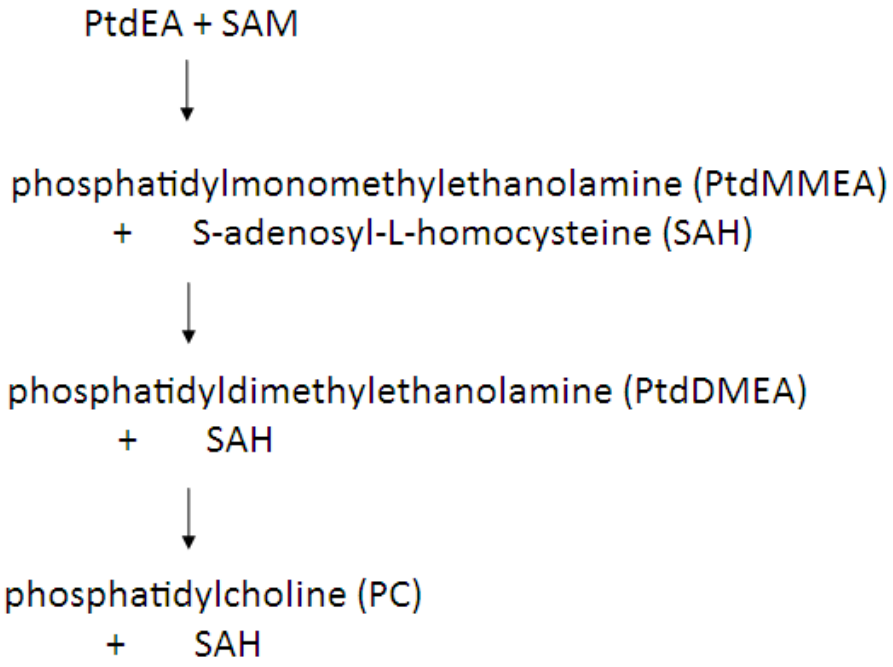
= RBS + *luxr* + TS + TS

Acetylcholine (ACh) biosynthetic pathway

Enzymes involved:

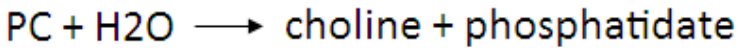
1. **Phosphatidylethanolamine N-methyltransferase (PEMT) EC 2.1.1.17**

   - sequential methylation



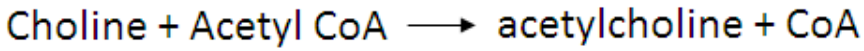
2. **Phospholipase D4 (PLD4) EC 3.1.4.4**

   - phosphate removal

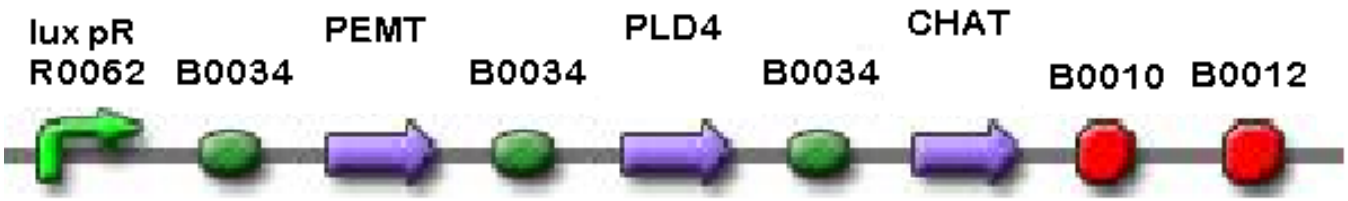


3. **Choline Acetyltransferase (CHAT) EC 2.3.1.6**

   - acetyl transfer to choline



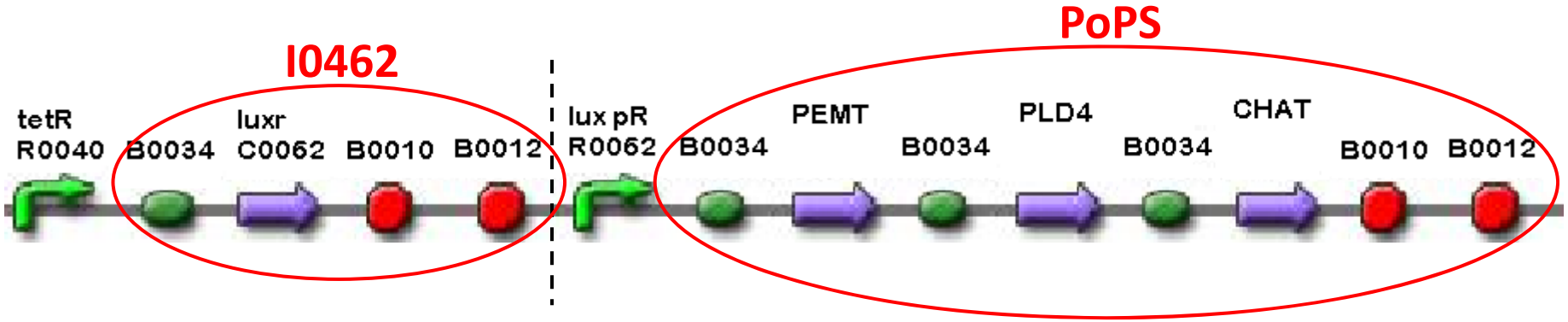
AHL-dependent ACh Biosynthesis Device



Parts

- Promoter lux pR (R0062): induced by binding of AHL/LuxR complex
- 4 x Ribosome Binding Sites RBS (B0034)
- Gene encodes PEMT
- Gene encodes PLD4
- Gene encodes CHAT
- 2 x terminating sequences B0010 & B0012

Synthetic DNA Plasmid



- total size: 2.7kbp (small plasmid)
  - cost effective and high efficiency for PCR

MIT registry: characterised **F2620** =

takes AHL as the device input and a PoPS as the output from a LuxR-regulated operator

Xenopus Laevis Oocytes

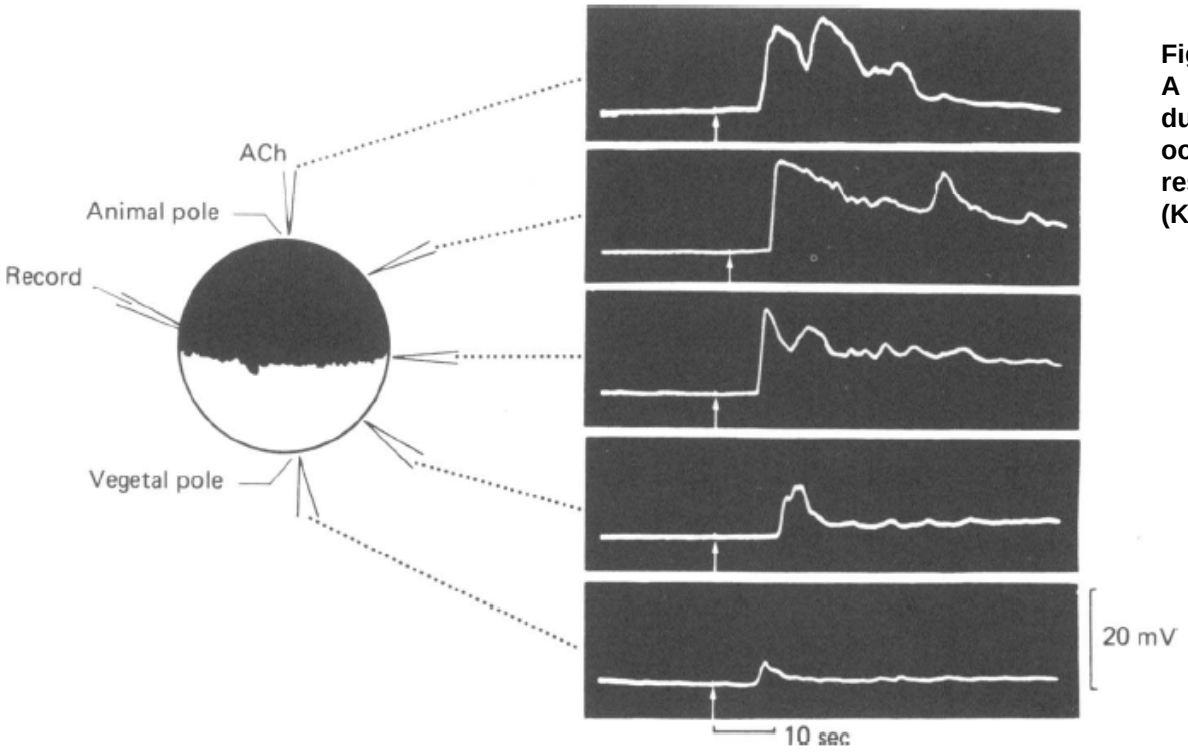


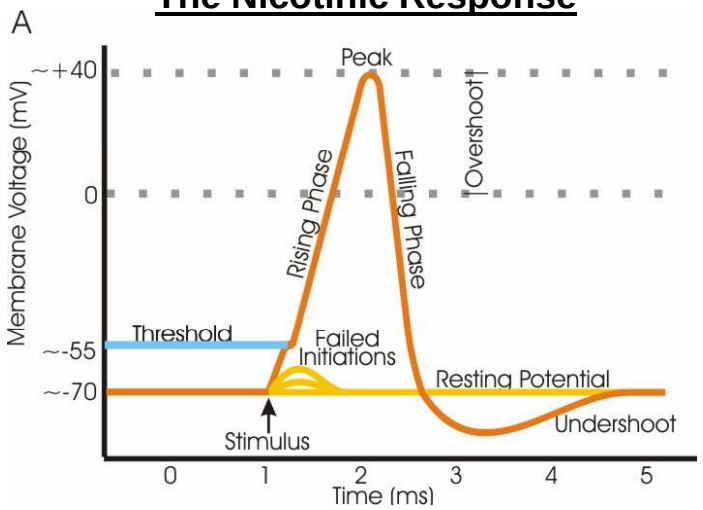
Figure 1: Regional difference in ACh sensitivity. A constant pulse of ACh (1 ×10-6 A, 100 msec duration) was applied to various regions of the oocyte surface and the corresponding responses were recorded on an oscilloscope. (Kusano et al, 1981)

- Commonly used in electrophysiological experiments.
- Are quite large ≈ 1.0 mm.
- Stage 4 to 5 oocytes normally used.
- These oocytes are usually covered with a layer of follicle cells which should be removed.
- Different parts of the oocyte have different sensitivities to acetylcholine.

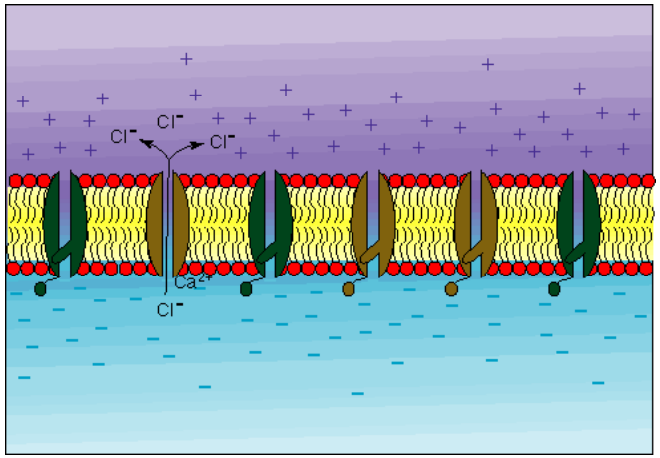
Transduction of a Membrane Potential

Resting potential → Depolarisation → Hyperpolarization

The Nicotinic Response



The Muscarinic Response



- Acetylcholine stimulates the muscarinic receptors in the membrane.
- This causes the muscarinic receptors to secrete second messenger inositol triphosphate (IPT3).
- This causes Ca to be released from the endoplasmic recticulum. Intracellular Ca causes the opening of the Cl channels.
- The efflux of Cl causes the depolarisation of the membrane.

Xenopus Oocyte Nicotinic Receptors

Experiments have been carried out on human nicotinic acetylcholine receptors (hnAChR) expressed in *Xenopus* oocytes. This involved cloning the α7 receptor from cDNA libraries prepared from human brain. The results were as follows:

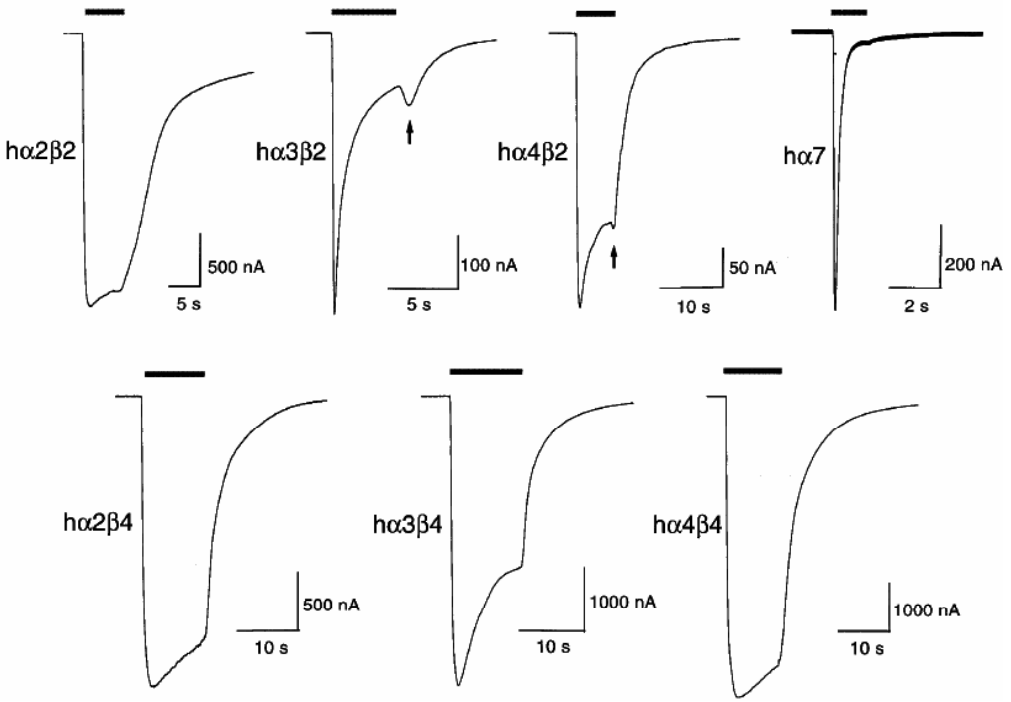


Figure 2 Representative traces showing the current responses to maximally effective concentrations of ACh in oocytes injected with mRNA encoding various human nicotinic receptors. Data shown in figures 1 to 3 were obtained from oocytes voltage-clamped at -60 mV. Of the β2-containing receptors, hα3βb2 receptors showed the fastest decay kinetics to ACh application. Similarly, hα3β4 receptors showed more apparent desensitization than did hα2β4 or hα4β4 receptors (bottom row). Currents recorded from hα7 nAChRs decayed very rapidly (upper right panel). Note the transient inward current observed in hα3β2- and hα4β2-injected oocytes upon removal of agonist (arrows). Maximally effective concentrations of ACh for the oocytes shown here were 300 mM for hα2β4 and hα4β4 receptors, 1 mM for hα2β2, hα3β4, hα4β2 and hα7 receptors and 3 mM for hα3β2 receptors.[i]

[i] Source: Pharmacological characterization of recombinant human neuronal nicotinic acetylcholine receptors h alpha 2 beta 2, h alpha 2 beta 4, h alpha 3 beta 2, h alpha 3 beta 4, h alpha 4 beta 2, h alpha 4 beta 4 and h alpha 7 expressed in Xenopus oocytes.

Xenopus Oocyte Muscarinic Receptors

•Research shows that Xenopus oocytes have ‘native’ ACh receptors in their surface membranes. These receptors are muscarinic (i.e. they are more sensitive to muscarine than nicotine. Because they are muscarinic, a small delay of half a second to a minute occured before they were triggered by ACh.)

•Cl ions act as the major current carrier in these oocytes, as the most common response observed was depolarisation in the presence of ACh.

•2 types of muscarinic receptors M1 and M3.

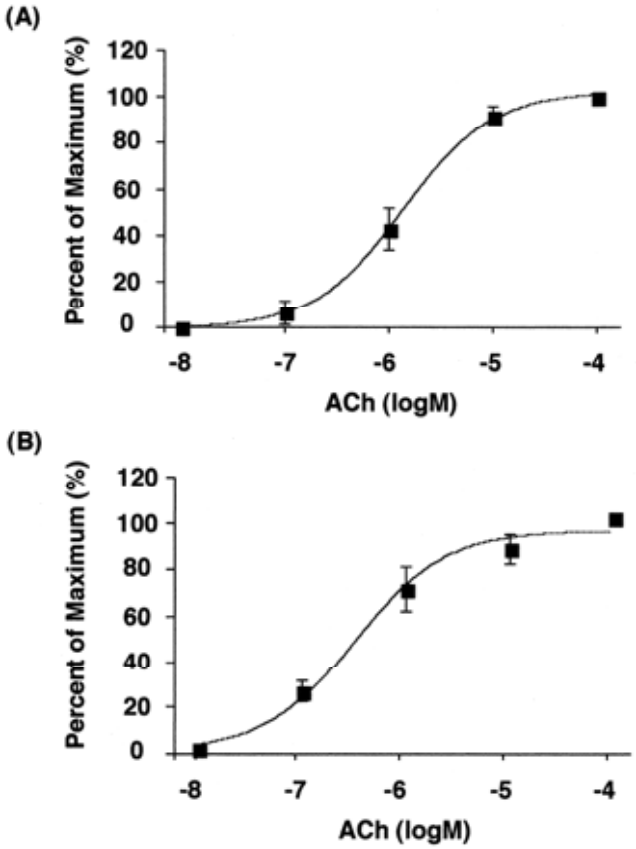


Figure 2:Concentration-response curve for acetylcholine (ACh) activation of a Ca2+ -activated Cl– current in *Xenopus* oocytes expressing M1 (A) or M3 receptors (B). Oocytes were voltage-clamped at –70 mV. ACh (10 nM–100 µM) was applied for 20 s, and the peak current was measured. Values are the mean ± sem for 10 oocytes. In some cases, the error bars are smaller than the symbols.[i]

[i] Source: The Effects of the Tramadol Metabolite O-Desmethyl Tramadol on Muscarinic Receptor-Induced Responses in *Xenopus* Oocytes Expressing Cloned M1 or M3 Receptors (2005)

Receptor Challenges

- Receptor desensitisation:
  - Nicotinic Receptors: Desensitisation of receptors occurs because prolonged or repeat exposure to a stimulus often results in decreased responsiveness of that receptor for a stimulus.
  - Muscarinic Receptors: This occurs because if G protein coupled receptors are exposed to their ligand for a long period of time, they will be desensitised. Therefore test solutions should be washed out as soon as the peak response is achieved.

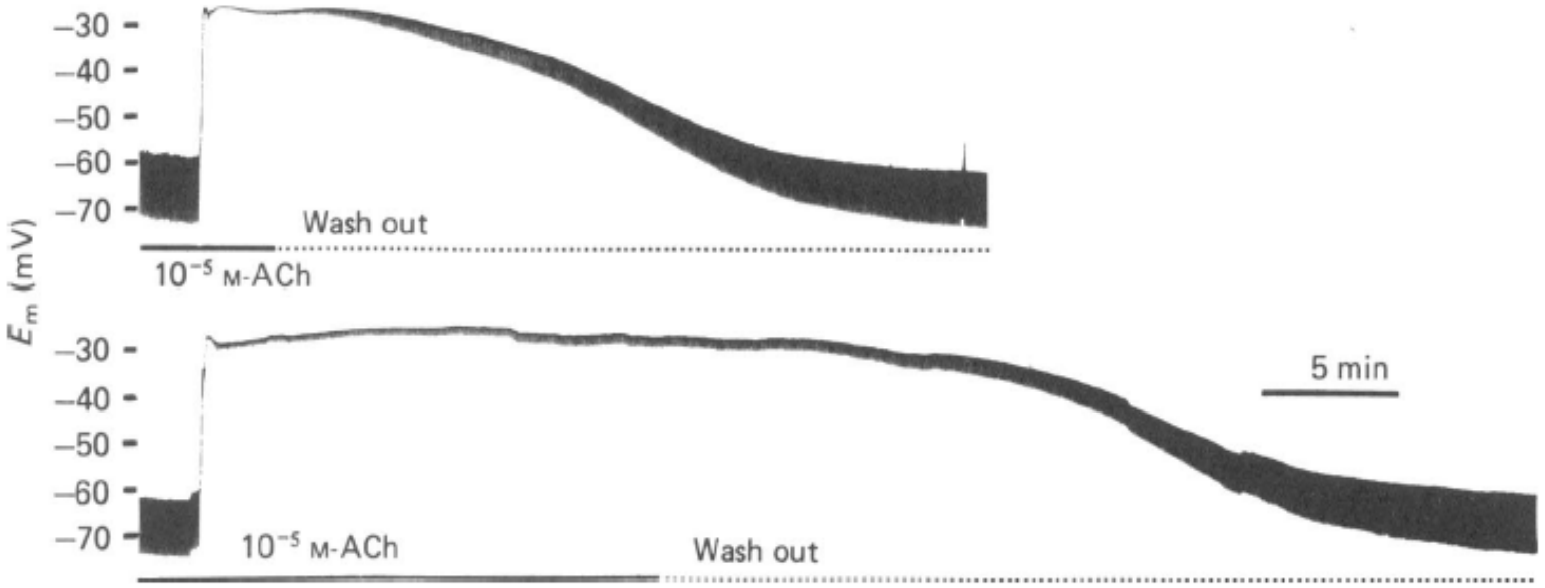
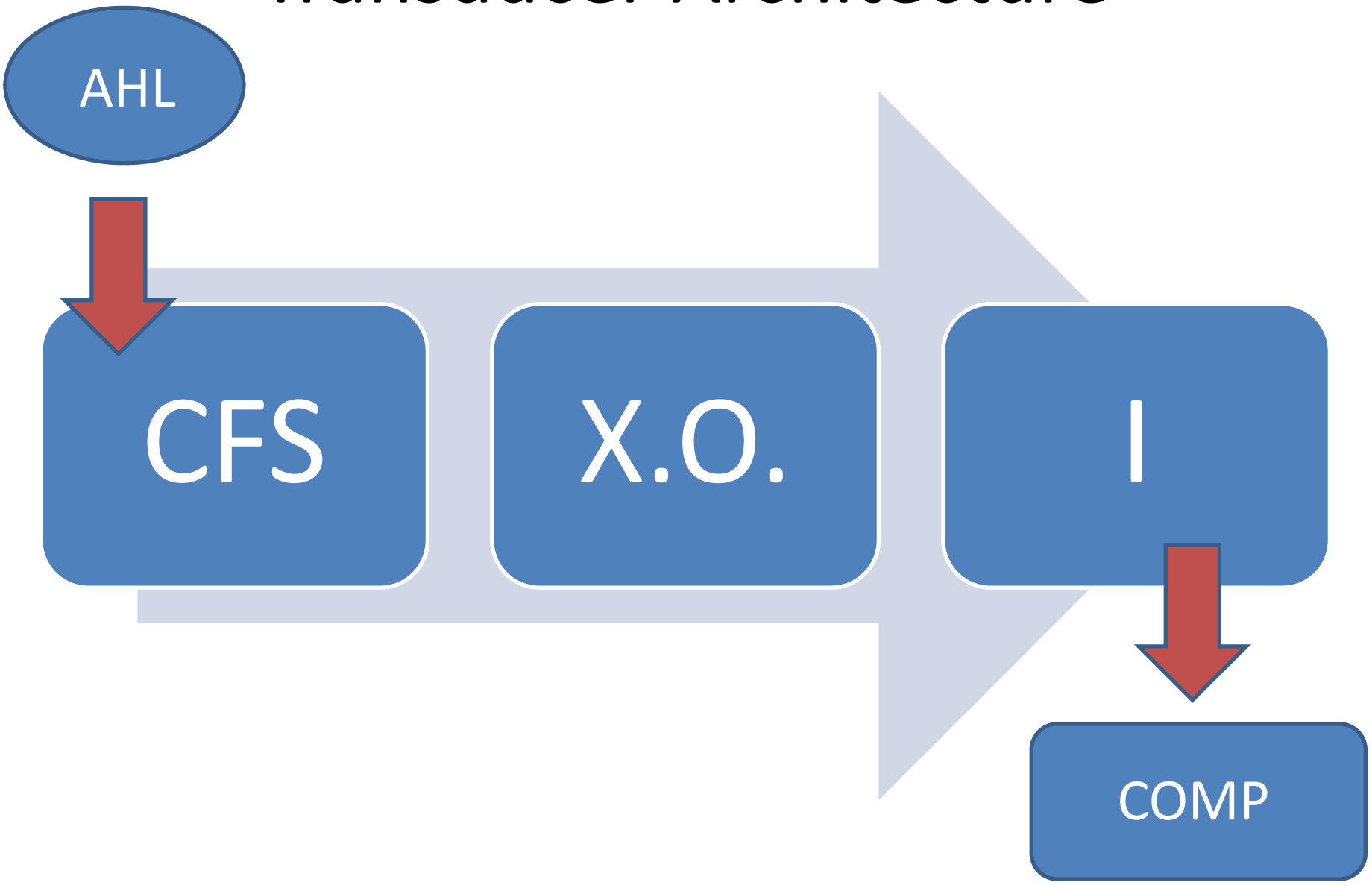
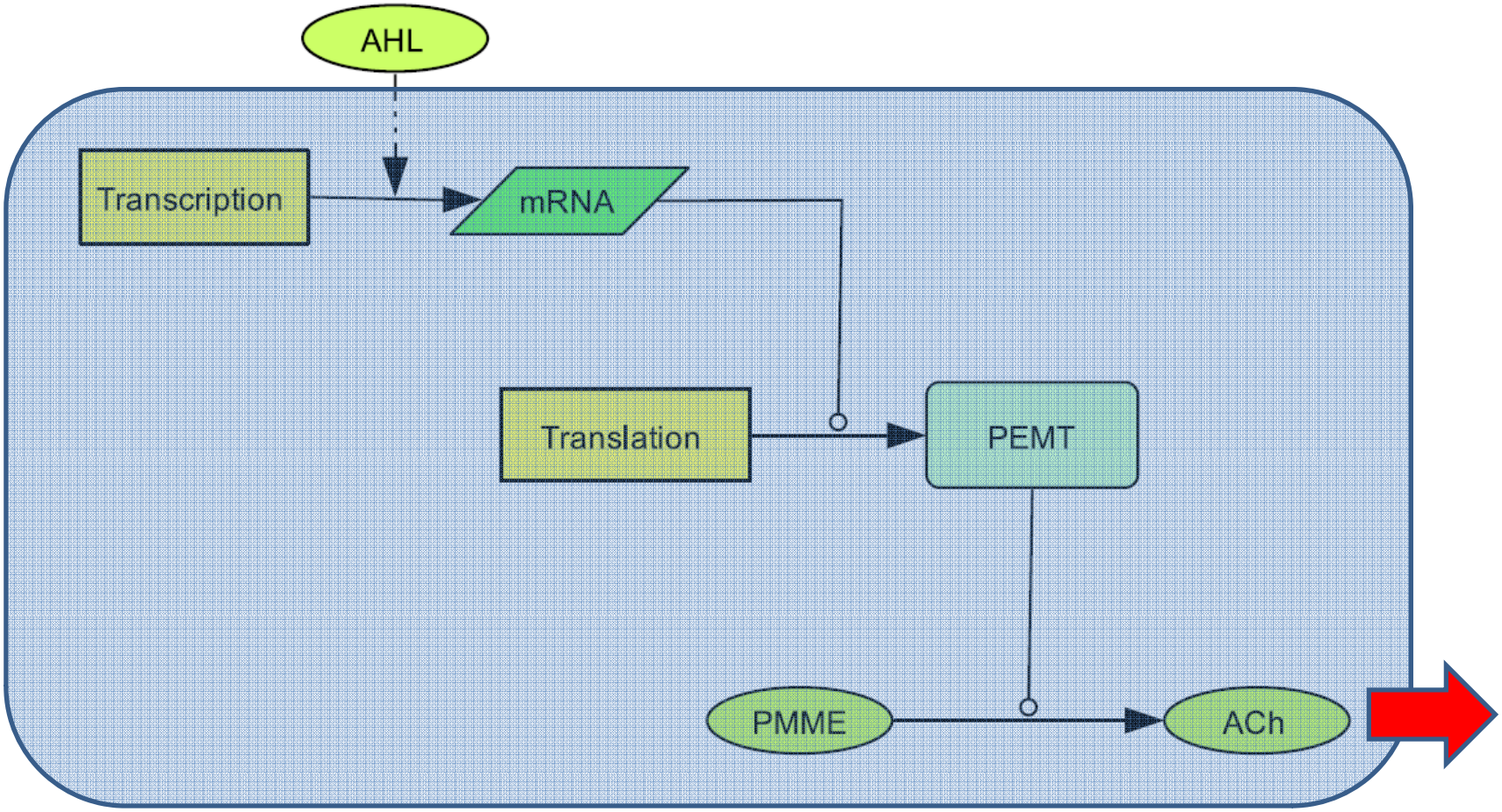


Figure 5 Concentration-response curve for acetylcholine (ACh) activation of a Ca2+-activated Cl– current in *Xenopus* oocytes expressing M1 (A) or M3 receptors (B). Oocytes were voltage-clamped at –70 mV. ACh (10 nM–100 µM) was applied for 20 s, and the peak current was measured. Values are the mean ± sem for 10 oocytes. In some cases, the error bars are smaller than the symbols.Source: The Effects of the Tramadol Metabolite O-Desmethyl Tramadol on Muscarinic Receptor-Induced Responses in *Xenopus* Oocytes Expressing Cloned M1 or M3 Receptors (2005)

Transducer Architecture



Cell Free System



Cell Free System

$$\frac{d[mRNA]}{dt} = \frac{k_1[AHL]^n}{K_{m1}^n + [AHL]^n} - d_1[mRNA]$$

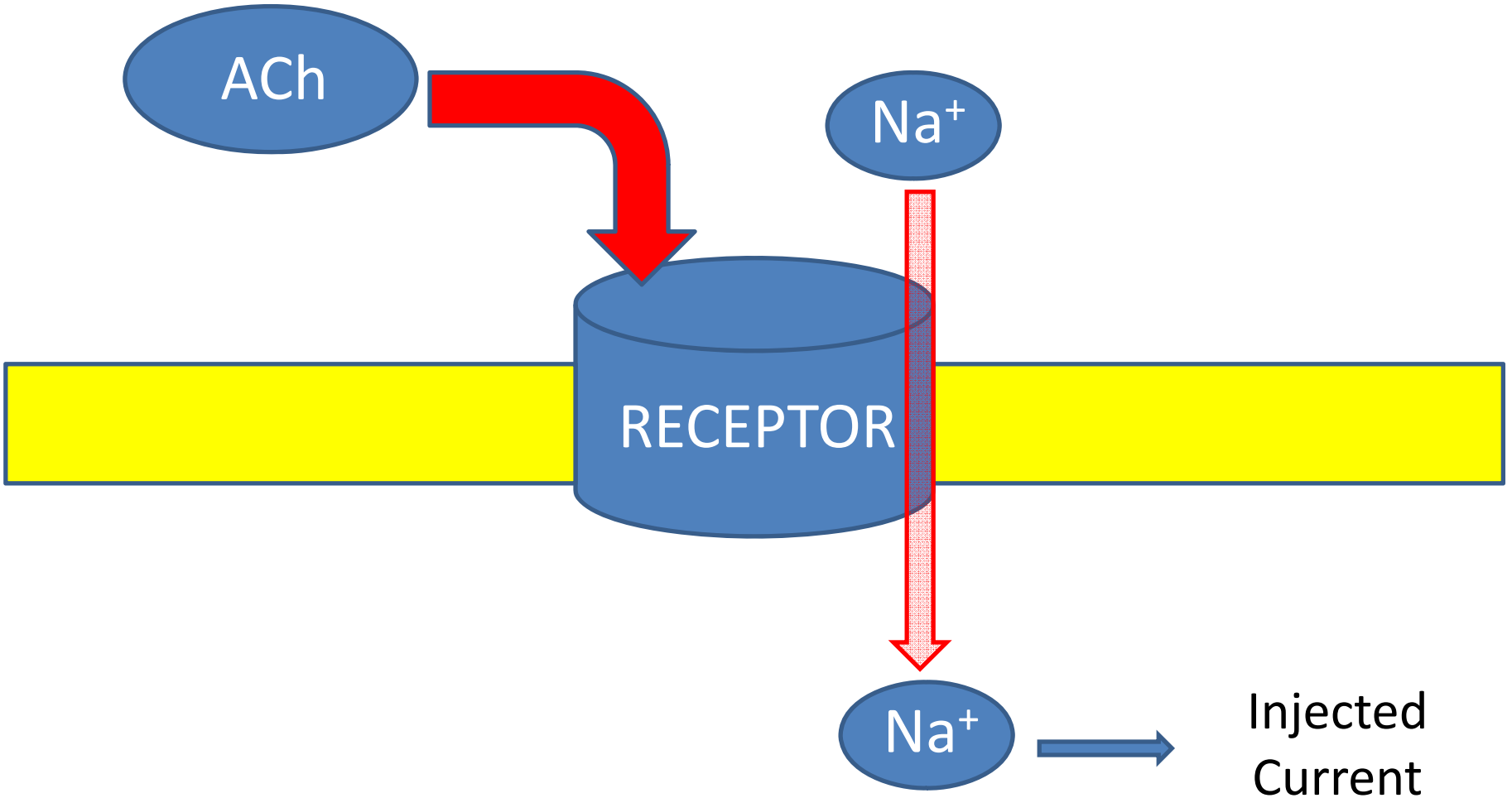
$$\frac{d[PEMT]}{dt} = k_2[mRNA] - d_2[PEMT]$$

$$\frac{d[ACh]}{dt} = \frac{k_3[PEMT][PMME]}{K_{m2} + [PMME]} - k_4[ACh]$$

- Assume Steady State Concentrations:

$$[ACh]^* = \frac{k_1 k_2 k_3}{k_4 d_1 d_2} \frac{[AHL]^n}{K_{m1}^n + [AHL]^n} \frac{[PMME]}{K_{m2} + [PMME]}$$

Xenopus Oocyte ACh Receptors



Xenopus Oocyte ACh Receptor

$$I_{nAChR} = N \frac{I_{max}[ACh]^n}{[ACh]^n + EC_{50}^n}$$

Voltage Gated Ion Channels

- Wilson Neocortical Neuron Model
  - Membrane Capacitance
  - Voltage Gated Ion Channels
    - Potassium (K^+)
    - Sodium (Na^+)
  - Leaky Membrane
  - Calcium Dependence
- Adapted from Hodgkin-Huxley, Simplified

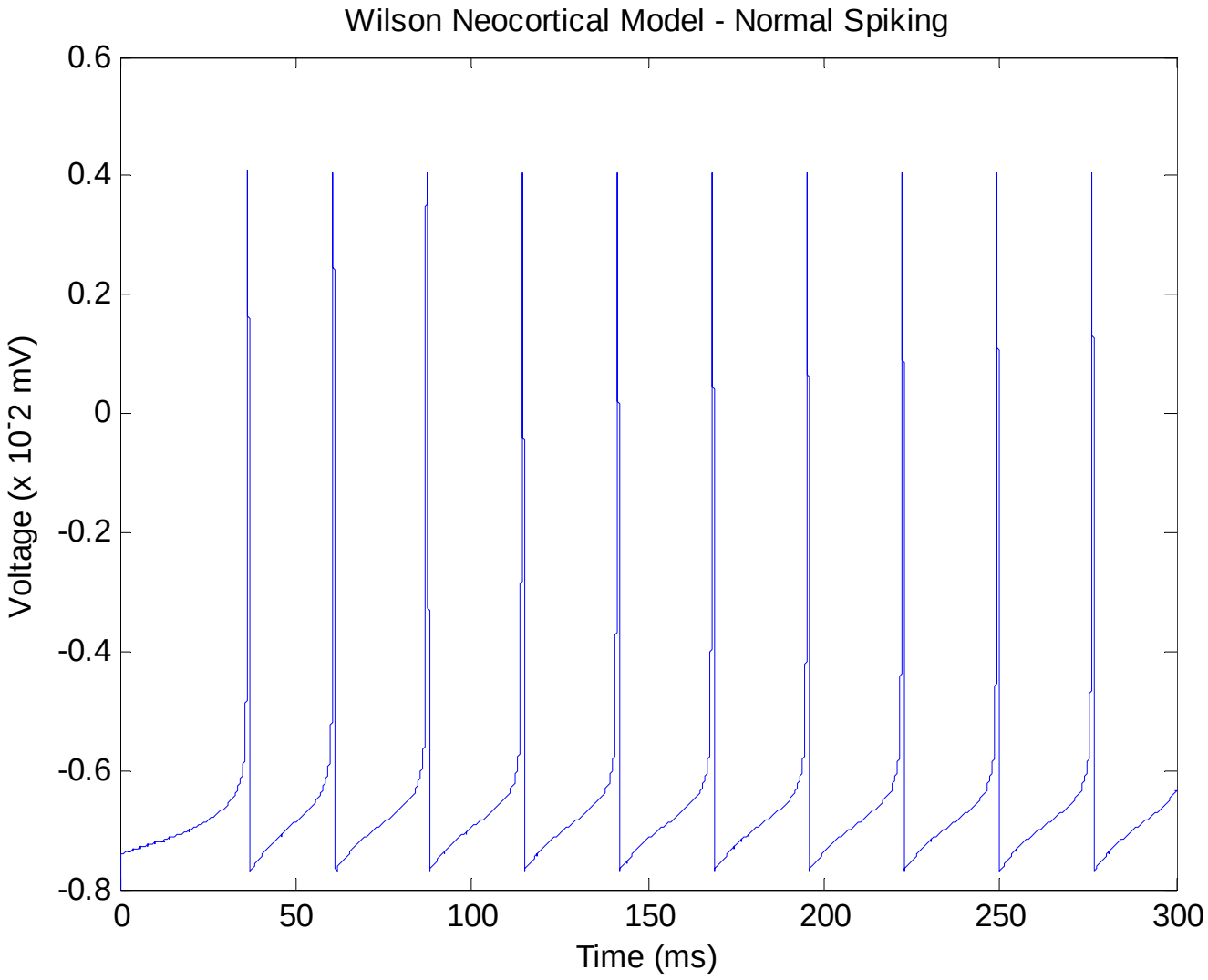
Voltage Gated Ion Channels

$$\frac{dV}{dt} = -m_\infty[V](V - 0.5) - 26R(V + 0.95) - g_T T(V - 1.2) - g_H H(V + 0.95) + I_{nAChR}$$
$$\frac{dR}{dt} = \frac{1}{\tau_R}(-R + R_\infty[V])$$
$$\frac{dT}{dt} = \frac{1}{14}(-T + T_\infty[V])$$
$$\frac{dH}{dt} = \frac{1}{45}(-H + 3T)$$

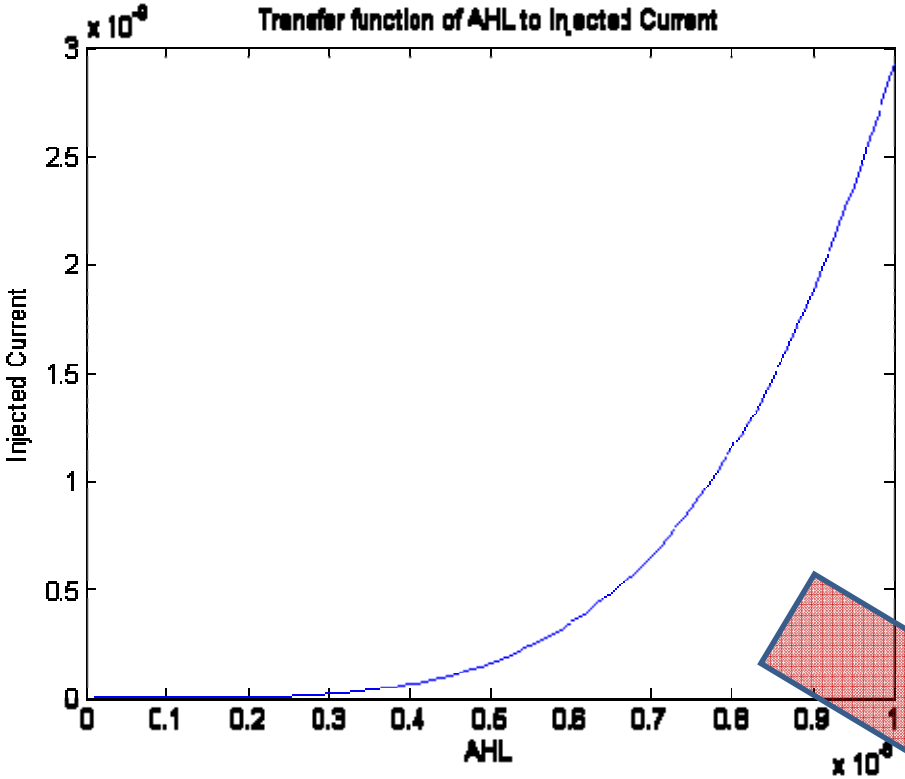
$$m_\infty[V] = 17.8 + 47.6V + 33.8V^2$$
$$R_\infty[V] = 1.24 + 3.7V + 3.2V^2$$
$$T_\infty[V] = 8(V + 0.725)^2$$

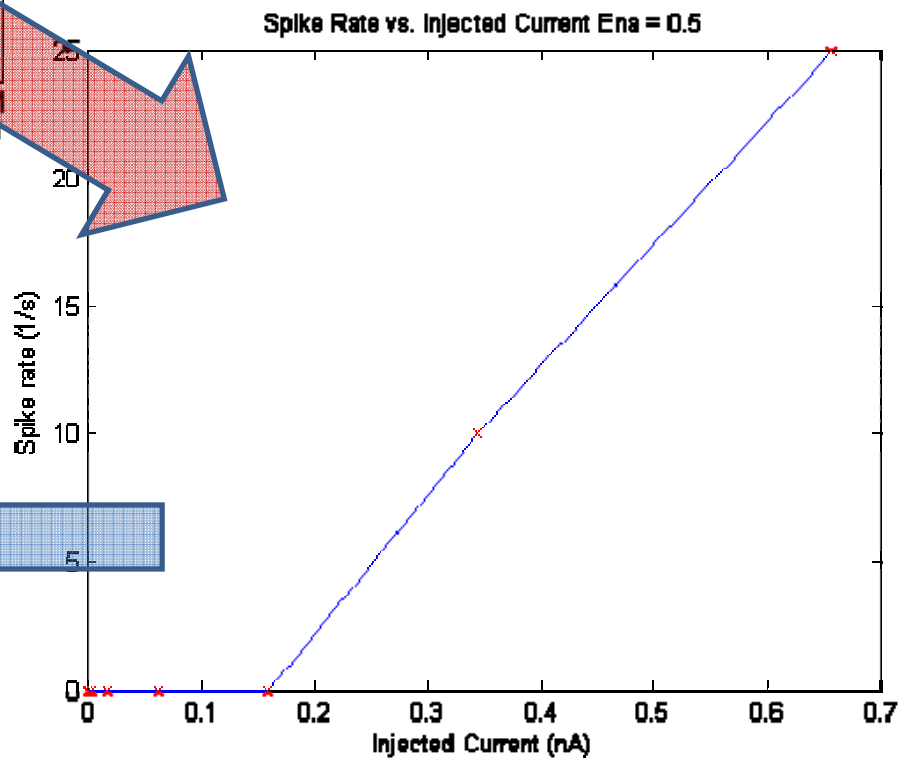
Values obtained from experimental results

Voltage Gated Ion Channels



Information Flow



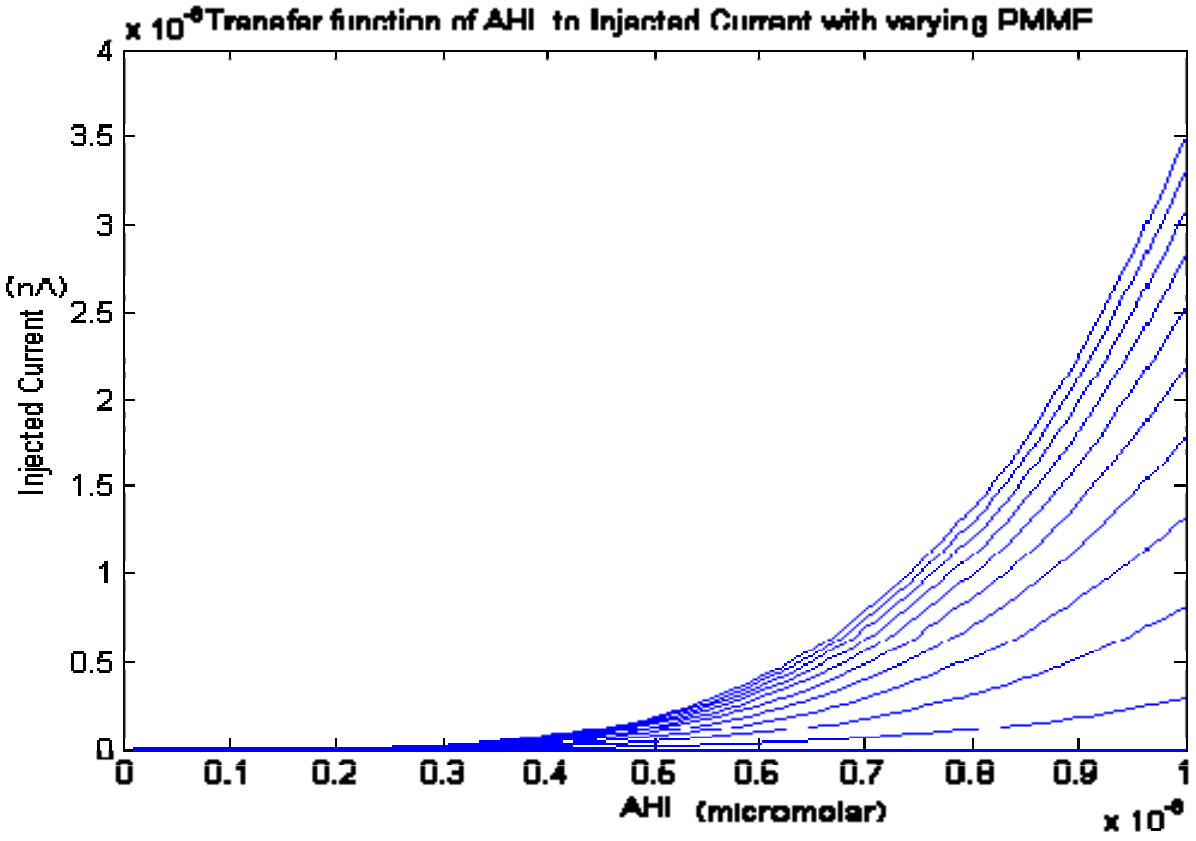


System Sensitivity

- Want a robust yet **sensitive** device with dynamic range
- **Difficulty**: synthesis of ACh in timely fashion
- **Solutions:**
  - strong RBS and promoter (no available **strong** AHL-dependent promoter in registry!)
  - increase oocyte sensitivity to ACh

Sensitivity Analysis

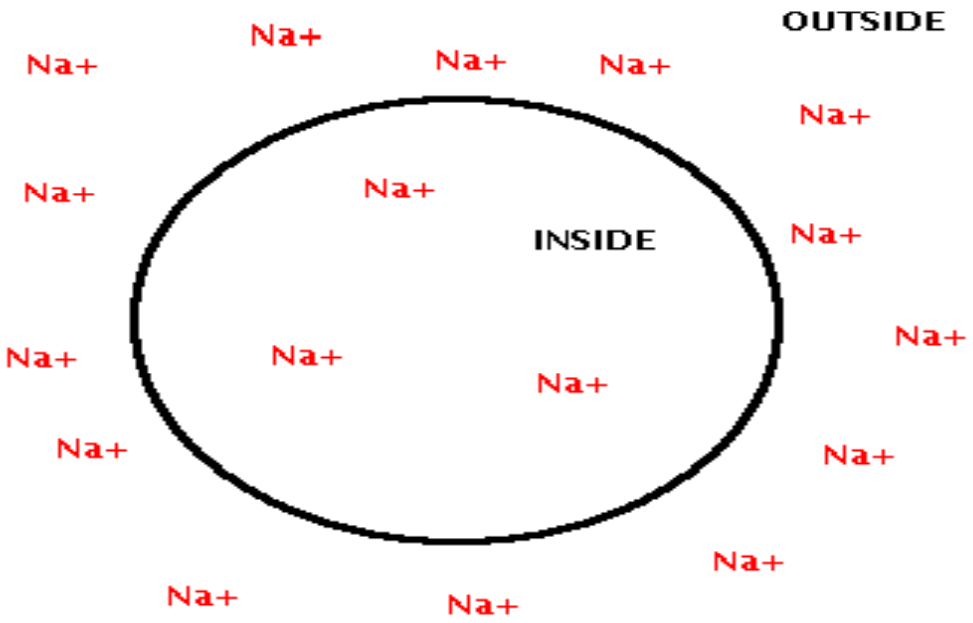
- Increase [PMME] synthesis



Sensitivity Analysis

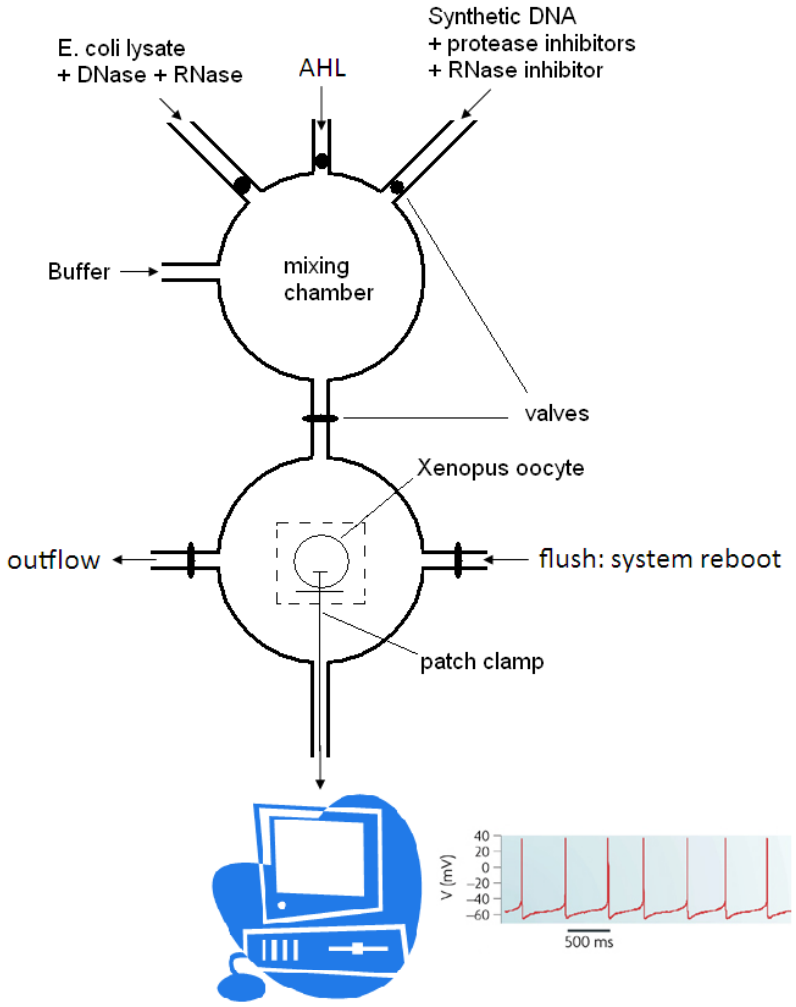
- Oocyte fires an action potential if V_m reaches the **firing threshold V_f**
- To increase sensitivity, bring resting oocyte **V_{eq}** closer to **V_f**
- Do this by tweaking the resting membrane potential relative to each ion, **E_{ion}**

Sensitivity Analysis



$$E_{ion} = \frac{RT}{zF} \ln\left(\frac{[ion]_{outside}}{[ion]_{inside}}\right)$$

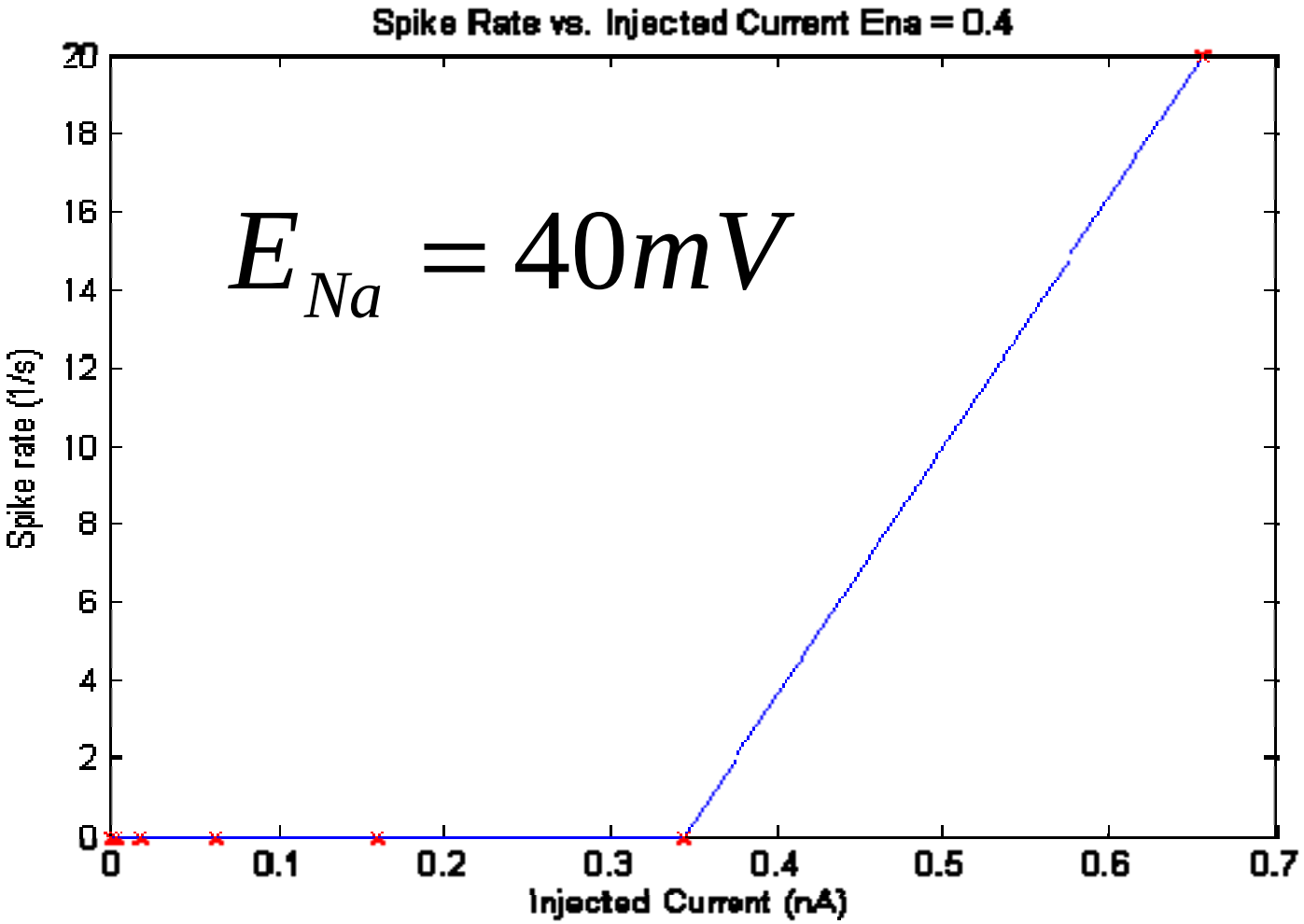
Sensitivity Analysis



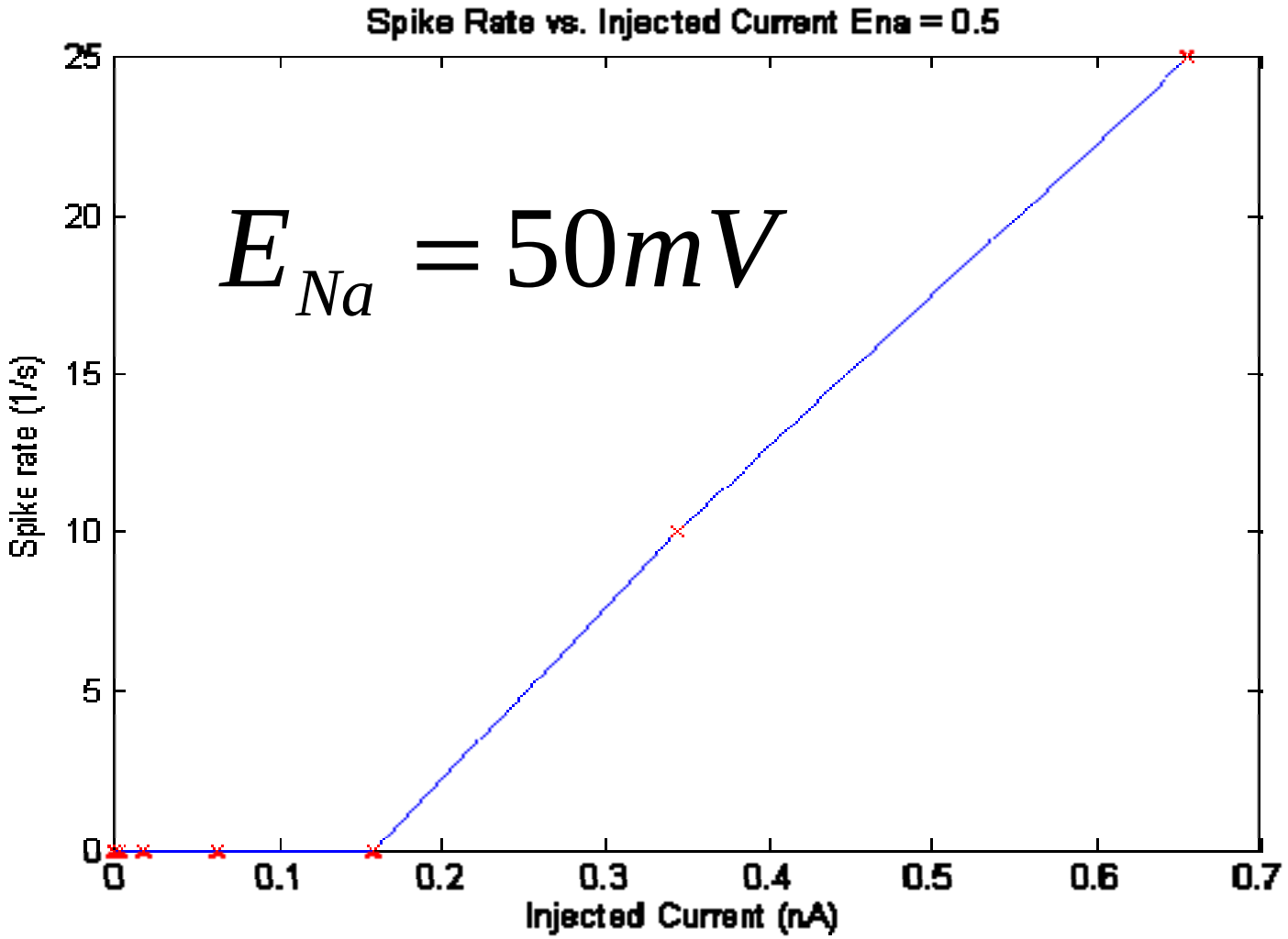
Sensitivity Analysis

$$\frac{dV}{dt} = \sum_{ions} -\gamma_{ion}(V - E_{Na}) + I_{nAChR}$$

Sensitivity Analysis



Sensitivity Analysis



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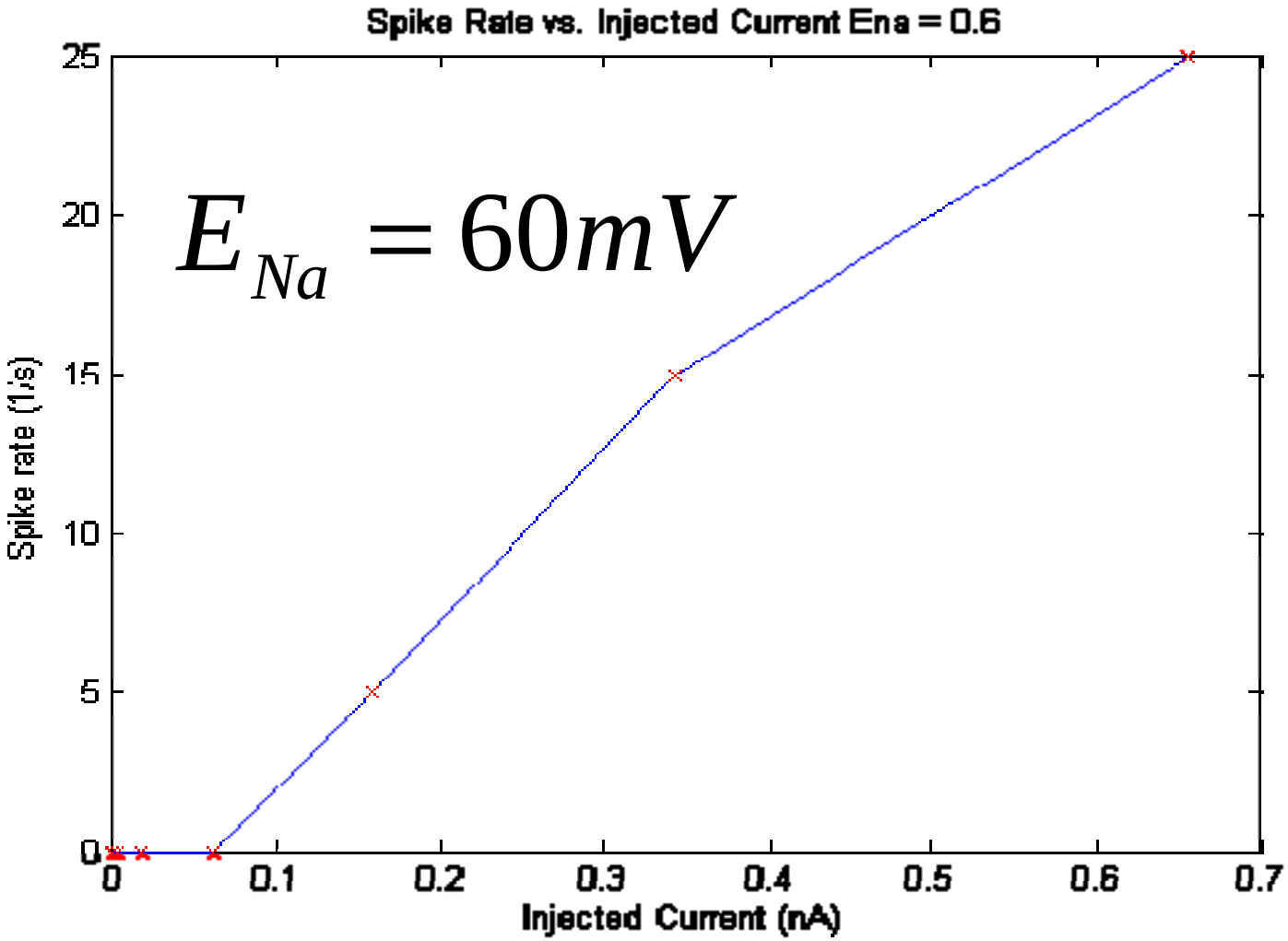

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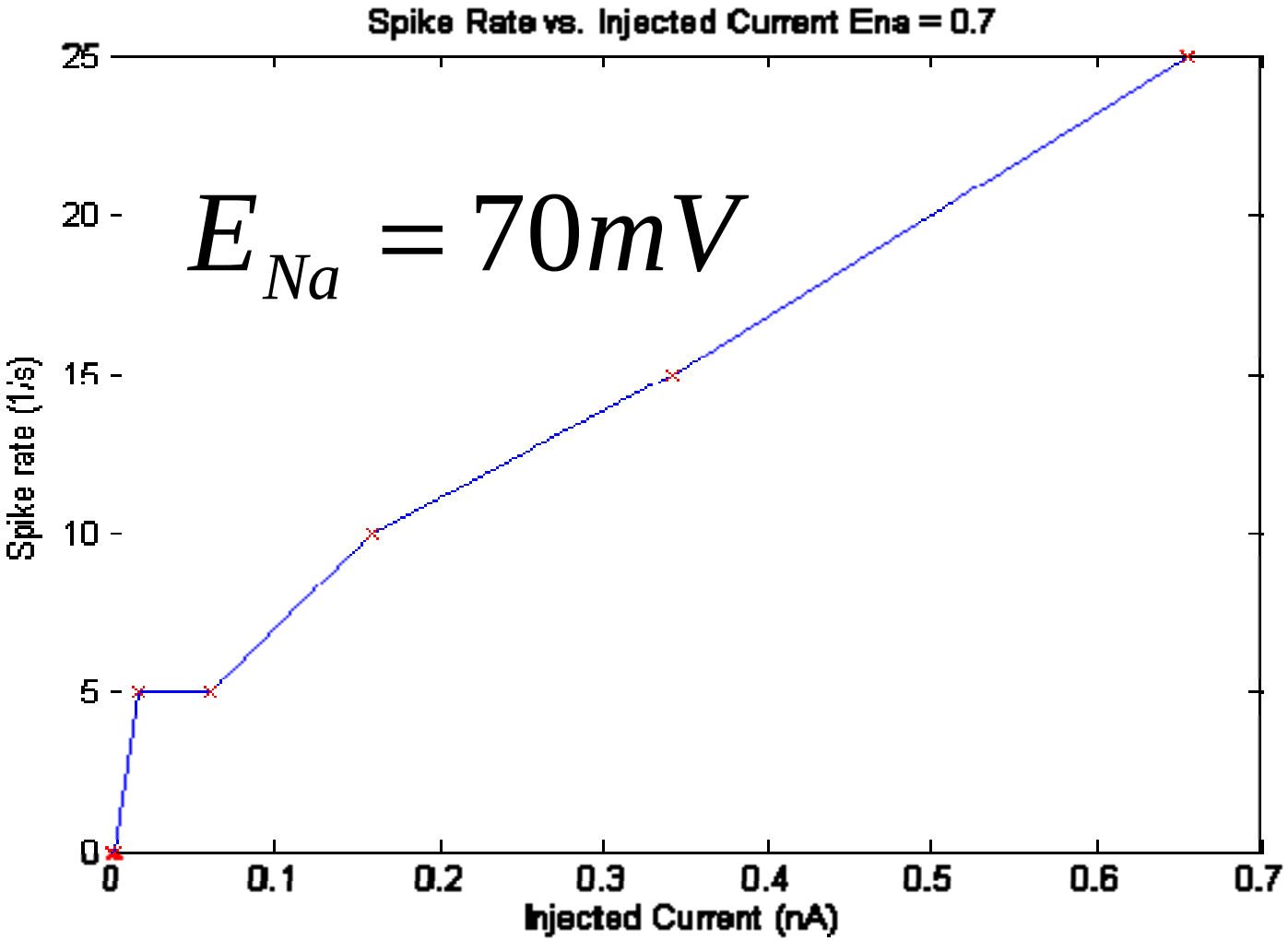

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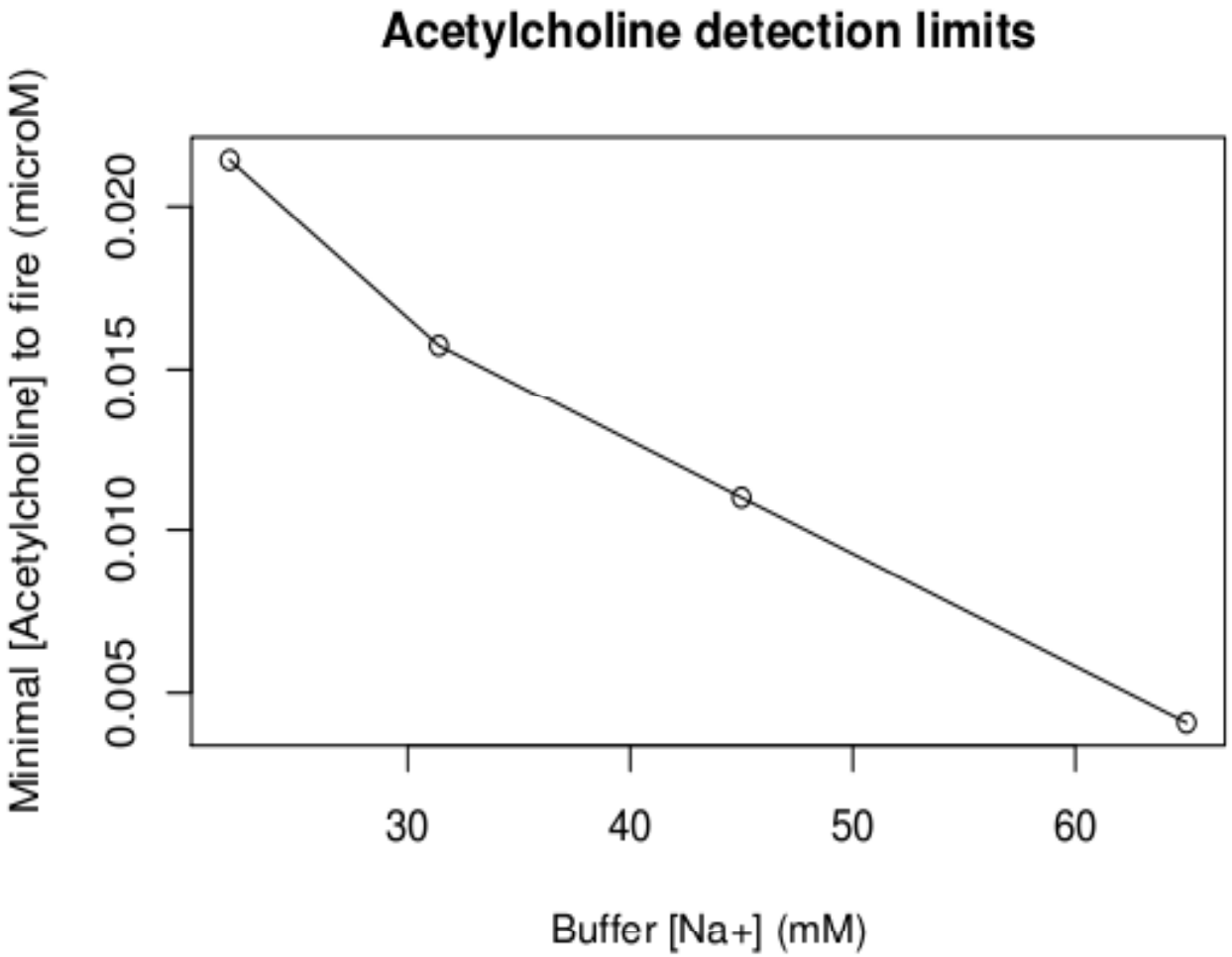

Conclusion

- Reusability (change the promoter e.g. iron, arabinose)
- Tunable sensitivity and dynamic range
- Can implement greater signal complexity
  - Different inputs coupled to different neurotransmitters
  - Action potential waveform – Ca^{2+} channels
- Applications

Acknowledgements

- To those who will read our report…Thanks!

AND

- Mimi for her wonderful Paint skills,
- Qamra’s muscarinic receptors,
- Thomas’ afternoons,
- And John’s colorful descriptions