Electrophysiology methods for neural bifurcation analysis

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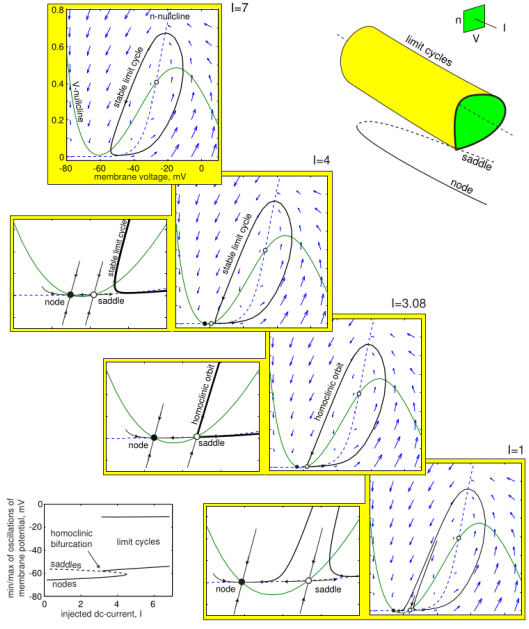
Presentation plan

- 30 second overview of my project
- Overview of my mircrofluidics questions
- Review of potential solutions

About my project

- Hodgin and Huxley provided a model of neural dynamics
- It turns out we can explain all observations from classical neuroscience using dynamical systems theory
- These explanations typically focus on the bifurcations a neuron can exhibit
- Bifurcations are analysed from models; I'm wanting to do a bifurcation analysis on a real, living neuron
- Goal: consider a single neuron, and experimentally find bifurcations in its dynamics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n-nullcline
V-nullcline
stable limit cycle
0.8
0.6
0.4
0.2
0
-80
-60
-40
-20
0
membrane voltage, mV
n
V
I
limit cycles
saddle
node
I=4
stable limit cycle
node
saddle
I=3.08
homoclinic orbit
node
saddle
I=1
node
saddle
min/max of oscillations of membrane potential, mV
homoclinic bifurcation
limit cycles
saddles
nodes
injected dc-current, I

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The big question

The dynamics of any given neuron typically change when current is injected into that neuron. I'm wanting to observe and control these changes experimentally. I need an experimental setup that would allow me to...

- Apply a current into a neuron

QUESTION: what would be an appropriate experimental setup to achieve this?

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- Observe that neuron's membrane potential

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- Apply a current into a neuron
- Observe that neuron's membrane potential
- Keep the neuron alive as long as possible

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- Apply a current into a neuron
- Observe that neuron's membrane potential
- Keep the neuron alive as long as possible
- (If it were possible, also measure each ion channel's average conductance)

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Bath MEA

- Idea:
  - Use the current microfluidics device, or a minor modification of it
- Strengths:
  - Builds on existing work and expertise
- Weaknesses:
  - Can't isolate the dynamics of an individual neuron
  - Can't give a specific neuron a current input
  - Can't measure membrane potentials

Glass pipette patch clamp

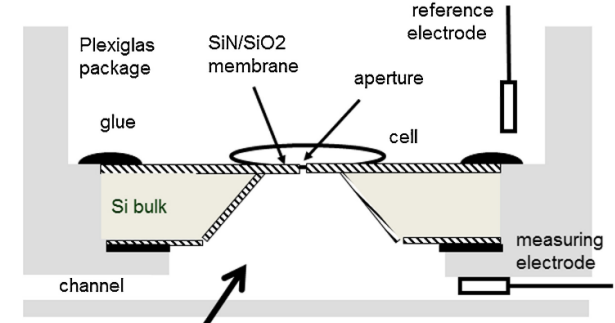
- Idea:
  - Use the classical glass pipette method for a whole-cell patch clamp
  - Measure membrane potential and inject current using the electrode
- Strengths:
  - Allows for studying the dynamics of individual neurons
  - Easy to inject current, and to measure membrane potential
- Weaknesses:
  - Patch clamping can be difficult
  - Neuron might not survive as long since we can't control nutrients and waste as easily

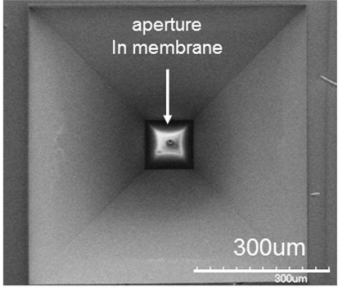
Off-the-shelf automated patch clamp

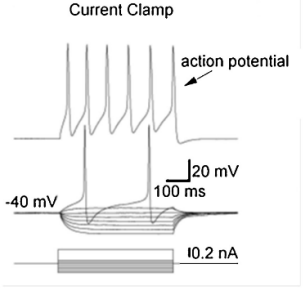
- Idea:
  - Same as before, but use an automated machine to do the patch clamping
- Strengths:
  - Allows for studying the dynamics of individual neurons
  - Easy to inject current, and to measure membrane potential
  - Much easier than manual patch clamping, no training required
  - Allows constant perfusion for providing nutrients and removing waste
- Weaknesses:
  - More expensive than DIY methods (unless we can borrow a machine from somewhere)
  - Might be hard to interface with a custom CBC control system

DIY microfluidic patch clamper

- Idea:
  - Combine the MEA and patch clamping methods
  - Build a planar patch clamping microfluidics device in-house
- Strengths:
  - Allows for studying the dynamics of individual neurons
  - Easy to inject current, and to measure membrane potential
  - Much easier than manual patch clamping, no training required
  - Allows constant perfusion for providing nutrients and removing waste
  - Cheaper and more customisable than buying a machine
- Weaknesses:
  - Need to design another microfluidics device


reference
electrode
Plexiglas
package
SiN/SiO2
membrane
aperture
glue
cell
Si bulk
measuring
electrode
channel


aperture
In membrane
300um
300um


Current Clamp
action potential
20 mV
100 ms
-40 mV
0.2 nA

Microfluidics fluorescence chip

- Idea:
  - Use an existing microfluidics chip, built for GFP imaging
  - Use calcium imaging to observe a neuron's behaviour
- Strengths:
  - Recently developed proteins allow the observations of individual action potentials
  - Might be able to estimate membrane potential from calcium imaging
  - Would allow the use of off-the-shelf chips, with no further developments
- Weaknesses:
  - No obvious way to inject current into the neuron, so any control inputs would have to be pharmacological
  - Probably can't investigate the dynamics of single isolated cells, only networks

The big question (again)

Which of these methods would best allow me to...

- Apply a current into a neuron
- Observe that neuron's membrane potential
- Keep the neuron alive as long as possible
- (If it were possible, also measure each ion channel's average conductance)