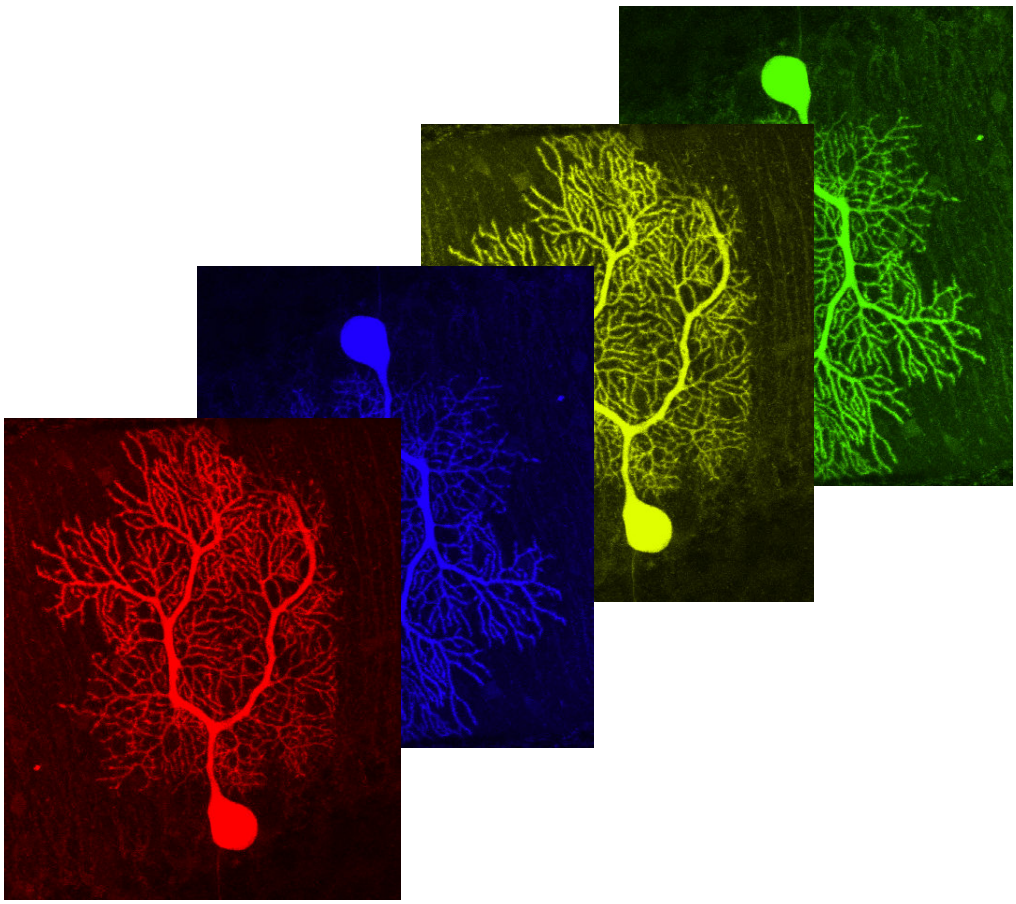
ION CHANNELS 2019

CSHL - Ion Channels in Synaptic & Neural Circuit Physiology Course - 2019

Contents



Ion Channels in Synaptic & Neural Circuit Physiology

Course Outline

The primary goal of the course is to investigate, through lectures and laboratory work, the properties of ion channels that allow neurons to carry out their unique physiological functions. Particular focus will be on specific voltage- and ligand-gated ion channels, their role in neuronal behavior, synaptic transmission and neuronal integration. Engineering of ion channels for use as optogenetic tools and *in vivo* recordings will also be addressed. The research interests of guest lecturers reflect these areas of emphasis.

The laboratory component of the course introduces students to electrophysiological approaches for the study of ion channels in recombinant expression systems and in their native environments. Hands-on exercises include analysis of ion currents using patch-clamp recordings in heterologous expression systems, in primary neuronal cultures and in acute brain slice preparations. Different recording configurations will be used to examine macroscopic or unitary current activity. The advantages and disadvantages of different methodologies, preparations and recording techniques will be considered in relation to the specific scientific questions asked.

The teaching staff will consist of Instructors, Teaching Assistants (TAs) and Guest Lecturers (see following page). Some, but not all, elements will involve pre-prepared course material; each topic will be introduced in lectures and then investigated in the lab. Each lab exercise will be preceded by a session in which the appropriate experimental design, the specific methodology and the eventual data analysis will be discussed.

Introduction to Labs

Welcome to the Ion Channels & Synaptic Transmission Lab!

The selected labs go hand in hand with the topics covered in the morning lectures. They will focus on fundamental aspects of electrophysiological recordings, ion channel biophysics in primary cultures from Dorsal Root Ganglia (DRG) and synaptic transmission and integration in acute slices from the mouse hippocampus, cortex and cerebellum. In the last week of the course, you will perform *in vivo* patch-clamp recordings.

We will guide you through the lab practicals in a short briefing after lunch. You will then go to the lab, collect and analyze the data and present/discuss them in the evening. Handouts on specific topics are also included in the **Appendix**.

During the Labs there might be some changes or protocol variation but this is something inherent to biological sciences.

If there is anything that you would like us to cover in more detail, please let us know: we will try to do our best. Scientific discussions with Instructors, TAs and speakers are strongly encouraged and required!

ION CHANNELS 2019

		Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
		3rd	4th	5th	6th	7th	8th	9th
Week 1	Speaker		**Nelson SPRUSTON**	**Instructors**	**Bruce BEAN**	**Christine BEETON**	**Fernanda LAEZZA**	
	9:00 AM 10:30 AM		Fundamentals of Neurophysiology	**Patch-clamp Theory:** Components of a Rig / Amplifier & Digitizer	Overview of ion channels	Potassium channels	Sodium channels	**FREE**
	10:40 AM 12:30 AM	**Arrival at CSH laboratory**	Principles of Bioelectricity	**Patch-clamp Practice:** Rs Compensation / Bridge balance	Speaker Research Talk	Speaker Research Talk	Speaker Research Talk	
	Lunch							
	1:00 PM		Lab Briefing	Lab Briefing	Lab Briefing	Lab Briefing	Lab Briefing	
	1:30 PM 5:00 PM		**Lab #1** Get acquainted with the Rig	**Lab #2** Rig set up **Dr. J Witkowski: Ethics lecture**	**Lab #3** Series resistance compensation (Model cell)	**Lab #4** Potassium currents (DRGs)	**Lab #5** Sodium currents (DRGs)	**FREE**
	Dinner							
	7:00 PM	**Social: Welcome - Cheese & Wine** Beckman Building 2nd Floor		Chalk Talk / Analysis	Chalk Talk / Analysis	Chalk Talk / Analysis	Chalk Talk / Analysis	
	8:00 PM Late		**Lab #1** Rig Set up	**Lab #2** Rig set up 2	**Lab #3** Series resistance compensation (DRG neuron)	**Lab #4** Potassium currents (DRGs)	**Lab #5** Sodium currents (DRGs)	**FREE**
Week 2		10th	11th	12th	13th	14th	15th	16th
	Speaker	**Paul KAMMERMEIER**	**Andrew PLESTED**	**Sonia GASPARINI**	**Mathew XU-FRIEDMAN**	**Linda OVRESTREET-WALDICHE**	**Jeremiah COHEN**	
	9:00 AM 10:30 AM	Calcium channels	Single channels / Unitary currents	*Ih* - HCN channels	Synaptic excitation EPSCs	Synaptic inhibition IPSCs	*in vivo/in vitro* extracellular recordings	**FREE**
	10:45 AM 12:30 AM	Speaker Research Talk	Speaker Research Talk	Speaker Research Talk	Speaker Research Talk	Speaker Research Talk	Speaker Research Talk	
	Lunch							
	1:00 PM	Lab Briefing	Lab Briefing	Lab Briefing	Lab Briefing	Lab Briefing	Lab Briefing	
	1:30 PM 5:00 PM	**Lab #6** Calcium current (DRGs)	**Lab #7** Setting up a rig for single channel recording	**Lab #8** Ih recordings (Hippocampus CA1)	**Lab #9** mEPSCs (Cerebellum)	**Lab #10** mIPSCs (Cerebellum)	**Lab #11** Cortical Connectivity **Social: Course BBQ**	**Social: Sailing Trip** from 1:30 PM
	Dinner							
	7:00 PM	Chalk Talk / Analysis	Chalk Talk / Analysis	Chalk Talk / Analysis	Chalk Talk / Analysis	Chalk Talk / Analysis	Chalk Talk / Analysis	
	8:00 PM Late	**Lab #6** Calcium current (DRGs)	**Lab #7** Single channel recording of Glycine receptors (HEK cells)	**Lab #8** Ih recordings (Hippocampus CA1)	**Lab #9** mEPSCs (Cerebellum)	**Lab #10** mIPSCs (Cerebellum)	**Lab #11** Cortical Connectivity (Electrical stimulation)	**FREE**
Week 3		17th	18th	19th	20th	21st	22nd	23rd
	Speaker	**Jesper SJOSTROM**	**Tiago BRANCO**	**Ian DUGUID**	**McLean BOLTON**	**Josh DUDMAN**	**Jeff DIAMOND**	
	9:00 AM 10:30 AM	Synaptic plasticity	Synaptic integration	*in vivo* - intracellular recordings	ChR2 - biophysics	ChR2-Circuits interrogation	**Plenary lecture**	**LAB Clear up**
	10:45 AM 12:30 AM	Speaker Research Talk	Speaker Research Talk	Speaker Research Talk	Speaker Research Talk	Speaker Research Talk		
	Lunch							
	1:00 PM	Lab Briefing	Lab Briefing	Lab Briefing	Lab Briefing	Lab Briefing		
	1:30 PM 5:00 PM	**Lab #12** Synaptic plasticity - LTP/LTD (Hippocampus)	**Lab #13** Dendritic and double patch-clamp (Hippocampus)	**Lab #14** Free Lab: choose your Prep	**Lab #15** Recording photosensitive currents	**Lab #16** Circuit mapping using optogenetics tools	**FREE**	**LAB Clear up**
	Dinner							
	7:00 PM	Chalk Talk / Analysis	Chalk Talk / Analysis	Chalk Talk / Analysis	Chalk Talk / Analysis	Chalk Talk / Analysis		**Social: Course Banquet** from 6 PM
	8:00 PM Late	**Lab #12** Synaptic plasticity - LTP/LTD (Hippocampus)	**Lab #13** Dendritic and double patch-clamp (Hippocampus)	**Lab #14** Free Lab: choose your Prep	**Lab #15** Recording photosensitive currents	**Lab #16** Circuit mapping using optogenetics tools	**Student Presentation**	

From June 15th in vivo Labs

Chalk talk: 5 min presentation / Analysis: general aspects on how to conduct / Pratical aspects

Cold Spring Harbor Laboratory

Code of Conduct for all Participants in Cold Spring Harbor Courses

Cold Spring Harbor Laboratory is dedicated to pursuing its twin missions of research and education in the biological sciences. The Laboratory is committed to fostering a working environment that encourages and supports unfettered scientific inquiry and the free and open exchange of ideas that are the hallmarks of academic freedom.

Consistent with the Laboratory's missions, commitments and policies, the purpose of this Code is to set forth the Laboratory's expectations for the professional conduct of participants enrolled in the Laboratory's advanced courses program, including instructors, lecturers, assistants, trainees, vendors and aides. This Code is intended to be consistent with the policies the Laboratory has in place governing conduct by its own faculty, trainees, students and employees.

In attending, course participants agree to:

1. Treat fellow participants and Cold Spring Harbor staff with respect, civility and fairness, and without bias based on race, color, religion, sex, national origin, citizenship status, sexual orientation, gender identity or expression, age, disability, marital status, veteran status, genetic information, or any other criteria prohibited under applicable federal, state or local law.

2. Use all Cold Spring Harbor Laboratory facilities, equipment, computers, supplies and resources responsibly and appropriately, as you would at your home institution.

3. Abide by the alcohol policy (see below).

Similarly, course participants agree to refrain from:

1. Discrimination in violation of Laboratory policy based on age, gender, race, ethnicity, national origin, religion, disability, or sexual orientation.

2. Behavior that is disrespectful of others and unprofessional interpersonal behavior that interferes with the working and learning environment.

3. Unwanted physical contact with others or threats of such contact.

4. Sexual harassment or harassment based on age, gender, race, ethnicity, national origin, religion, disability or sexual orientation.

5. Loss of civility that interferes with the working and learning environment (for example shouting, personal attacks or insults, throwing objects or other displays of temper).

6. Misappropriation of Laboratory property or excessive use of resources for personal use.

Breaches or Violations of the Code of Conduct

Course leaders (instructors) are responsible for maintaining a learning environment in accordance with the principles and expectations outlined in the Code of Conduct. Course leaders (instructors), either jointly or individually, are the primary individuals to resolve breaches of the Code of Conduct that may arise during their course, and should aim to resolve conflicts and counsel course participants in a non-threatening, constructive and private manner as appropriate.

In cases where the course leadership fail to or are unable to resolve the conflict or breach of the Code of Conduct, or are themselves considered to be in breach or violation, said conduct should be reported to program leadership in person or by email:

Dr. David Stewart Grace Auditorium, Office 204; 516 367 8801; stewart@cshl.edu

Dr. Charla Lambert Hershey Laboratory, Office 214; 516 367 5058 ; clambert@cshl.edu

The Laboratory will take action as needed to resolve the matter, up to and including immediate expulsion of the offending participant(s) from the meeting, dismissal from the Laboratory, and exclusion from future academic events offered by CSHL.

Course Alcohol Policy

1. No consumable alcohol should be stored or consumed in course laboratories, prep rooms, cold rooms or scientific refrigerators.

3. Consumption of alcohol is incompatible with the operation of scientific or laboratory equipment, and operation of such equipment while under the influence of alcohol is strictly forbidden.

4. Moderate consumption of wine and beer at evening seminars, informal discussions and gatherings is acceptable. Course leaders should ensure that adequate non-alcoholic beverages are available whenever alcohol is served.

5. No provision of alcohol by vendors is permitted.

6. Course participants consuming alcohol are expected to drink only in moderation at all times during the course.

7. Excessive promotion of a drinking culture with any course is not acceptable or tolerated by the Laboratory. No course participant should feel or be made to feel pressured or obliged to consume alcohol at any course-related event or activity.

CSHL Beach Policy

1. Use of the beach is restricted to CSHL staff and guests - groups of 10 or more must have approval from CSHL Security (8870).
2. Beach and picnic area are closed from midnight to 6am.
3. Open fires are prohibited by New York State law.
4. Contact CSHL Security for use of kayaks and sailboats (8870).
5. Swim at your own risk.

August 2017

ION CHANNELS 2019

Instructors

Annalisa Scimemi
SUNY Albany, USA
ascimemi@albany.edu

Christoph Schmidt-Hieber
Institut Pasteur, Paris, France
christoph.schmidt-hieber@pasteur.fr

Nicolas Wanaverbecq
INT, Aix Marseille Université, Marseille, France
nicolas.wanaverbecq@univ-amu.fr

Angelika Lampert
Aachen University, Aachen, Germany
alampert@ukaachen.de

Jan Gründemann
University of Basel, Basel, Switzerland
jan.grundemann@unibas.ch

TAs

Raya Atlanta Bott
Aachen University, Aachen, Germany

Ruy Gomez-Ocadiz
Institut Pasteur, France

John McCauley
SUNY Albany, USA

Maurice Petroccione
SUNY Albany, USA

Course elements

A mix of lectures and laboratory instruction with an emphasis on interactive discussion and hands-on work at the rig.

Morning lectures
Lectures by Instructors and Guest speakers - didactic / historical elements plus a description of recent research.

After-lunch/dinner briefings
Short sessions in which theoretical and practical considerations are addressed before lab work.

Laboratory work
In the afternoons and evenings, recordings will be made to investigate topics covered in that day's lecture.

Student talks and data analysis
Short talks about ongoing work in your home lab and short sessions aimed at describing different analysis methods.

Social activities

Welcome 'Cheese & Wine' - June 3 at 7 pm

Course BBQ - June 15 at 4 pm

Sailing trip - June 16 at 1:30 pm

Course Banquet - June 23 at 6 pm

Full details will be provided on arrival

Information

Any concerns or questions should be addressed to:

Rachel Lopez or **Vivian Vuong** (Course Registrars)
Cold Spring Harbor Laboratory
Meetings & Courses Program
Phone: (516) 367-8346
Fax: (516) 367-8845
http://meetings.cshl.edu/travel.html

ION CHANNELS 2019

Guest lecturers

Nelson SPRUSTON
HHMI Janelia Research Campus
sprustonn@janelia.hhmi.org

Bruce BEAN
Harvard Medical School
bruce_bean@hms.harvard.edu

Christine BEETON
Baylor College of Medicine
beeton@bcm.edu

Fernanda LAEZZA
University of Texas Medical Branch
felaezza@utmb.edu

Paul KAMMERMEIER
University of Rochester Medical Center
Paul_Kammermeier@urmc.rochester.edu

Andrew PLESTED
FMP, Berlin
plested@fmp-berlin.de

Sonia GASPARINI
LSUHSC
sgaspa1@lsuhsc.edu

Matthew XU-FRIEDMAN
SUNY Buffalo
mx@buffalo.edu

Linda OVERSREET-WADICHE
University of Alabama at Birminghan
lwadiche@uab.edu

Jeremiah COHEN
Johns Hopkins University
jeremiah.cohen@jhmi.edu

Jesper Sjostrom
McGill University
jesper.sjostrom@mcgill.ca

Tiago BRANCO
Sainsbury Wellcome Centre
t.branco@ucl.ac.uk

Ian DUGUID
University of Edinburgh
ian.duguid@ed.ac.uk

McLean BOLTON
Max Plank Florida Institute
McLean.Bolton@mpfi.org

Josh DUDMAN
HHMI JaneliaResearch Campus
dudmanj@janelia.hhmi.org

Keynote Speaker

Jeffrey DIAMOND
NIH/NINDS
diamondj@ninds.nih.gov

Lab #1 – Get acquainted with the rig and acquisition software

Build an electrophysiology rig and get acquainted with its different components

1- Objectives

Today we will help you get acquainted with your rig, acquisition software and set-up all the components necessary to carry out electrophysiological experiments for the rest of the course.

2- Objectives

1. Set-up electronic equipment
2. Set-up solution exchange system
3. Reduce electrical noise in your system
    - Ground the set up to get a noise signal <2-5 pA
    - Faraday cage
    - Electrically isolate amplifier
    - Keep monitor away from amplifier
    - Keep set-up clean of salt and rust

More details and helpful tips can be found in the ***CSHL Protocol named*** "*Single-Channel Recording of Ligand-Gated Ion Channels*" by A. Plested

3- Checklist

- ☐ Electronic equipment
    - Amplifier, headstage
        - Reference wire
        - Electrode holder: silver wire, suction tubing
    - A/D converter, oscilloscope, computer
    - Manipulator
    - Video camera, monitor, microscope and IR illumination (Khöler)
- ☐ Reduce noise
    - TEST: plug in model cell (BATH mode) → zero and offset apply a 10 mV pulse

  What is the resistance? Use Ohm's Law V = IR.
    - Connect all metal devices to reference
        - Use low resistance wiring with good shielding
    - Shield noise source
        - Wrap in foil and ground with alligator clips. All cables need to be connected to a signal point that will be connected to the amplifier ground plug
- ☐ Extra (time permitting): test with a glass pipette and vacuum grease on a coverslip.

4- One step forward

With the **MODEL CELL**:

1. Get used to interact with your oscilloscope and understand what the traces mean.
2. Generate and test protocols for Labs 2 & 3 to familiarize yourself with the acquisition software.

5- NOTES:

Lab #2 – Compensating series resistance to accurately record currents (Part I)

Series resistance compensation with model cells and real neurons

1- Objectives

You will learn the sequential steps necessary to obtain whole-cell recordings and compensate for series resistance. You will learn to assess recording parameter and measure the intrinsic properties of an excitable cell. This training will be carry out first with a Model cell and on DRG neurons.

DRGs consist of neuronal cell bodies of primary afferent fibers. These fibers mediate transmission of a wide variety of sensory stimuli (proprioception, light touch, itch, nociception and thermoception). DRG neurons are heterogeneous in size (small, medium, large) and function. They can be isolated and recorded from acutely or after days in culture. In the days of the culture, DRG neurons are round with no or little processes and therefore offer optimal conditions with little space clamp issues to record accurately ionic currents.

2- Procedure

Finalize Rig set up and practice 'Seal Test' procedure and Rs compensation.

With the model cell, go through the steps necessary to form a whole-cell recording configuration:

1. In BATH mode: Offset adjustment
2. Get voltage/current conversion and gain factors and measure 'pipette' resistance
3. Switch to PATCH mode: Electrode capacitance compensation
4. Switch to CELL mode: Whole cell capacitance, series resistance compensation

3- Working with a neuron

With DRG neurons in **Voltage-clamp** (VC) mode:

1. Repeat procedure described above using a patch-pipette
2. Measure pipette resistance and compensate pipette capacitance
3. Establish a cell-attached configuration on a neuron with a holding potential (V_{hold}) of -60 mV
4. Measure seal resistance. What is the holding current at this potential?
5. Go in whole-cell mode. What does the trace you observe represent?
6. Measure access/series resistance, cell capacitance and input resistance

With DRG neurons in **Current-clamp** (CC) mode:

1. Measure resting membrane potential (RMP) from DRG neurons, note the size of the neuron (small, medium or large)
2. generate action potentials (APs) in DRG neurons by applying a current step protocol (see below). Record.

4- Methods

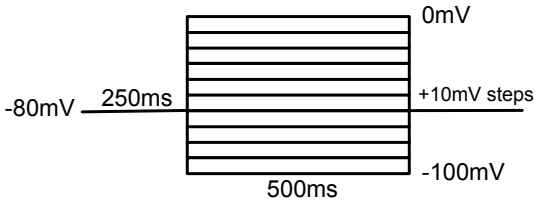
a) Recording solutions

Extracellular solution	in mM
NaCl	140
KCl	4
$CaCl_2$	2
$MgCl_2$	2
HEPES	10
D-glucose	10
pH, adjusted with NaOH	7.4
Osmolarity ($mOsm.Kg^{-1}$)	~300

Intracellular solution	in mM
K-gluconate	130
$MgCl_2$	1
$CaCl_2$	1
HEPES	10
EGTA	10
Mg-ATP	4
Na_2-GTP	0.3
pH, adjusted with KOH	7.2
Osmolarity ($mOsm.Kg^{-1}$)	~290

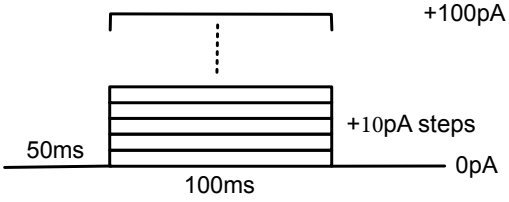
b) Recording protocols

Voltage steps: voltage-clamp



Repeat 10 times, recovery time 5s

Current steps: Current-clamp



Repeat 5 times, recovery time 5s

5- Analysis & Questions

1. Get used to reading from the oscilloscope and calculating parameters
2. Compare the whole-cell capacitance, input resistance and access resistance with cell size
3. Resting membrane potential relative to the DRG neuron size.
4. Create a frequency vs current (FI) plot

6- Useful Information/References

1. DRG neurons remain healthy for about 1 hr out of the incubator
2. Perfusion is preferred, but not necessary and do not let cells dry out
3. Recording pipettes:
    - Type of glass: borosilicate, with filament, regular/standard glass thickness (1.5mm outer diameter, 0.86 mm inner diameter)
    - Make sure glass is clean, fire-polished, use positive pressure during seal procedure
    - Minimize capacitance from electrode with:
        - Minimum internal solution
        - Minimum fluid level in recording chamber
        - Apply Sylgard near tip: electrically insulate pipette and prevent capillary action of solution up electrode

7- NOTES:

NOTES:

Lab #3 – Compensating series resistance to accurately record currents (Part II)

Series resistance compensation with model cell and in neurons
& Recording action potentials in dorsal root ganglion neurons

1- Objectives

Just to make sure everything is mastered... The same again but better!

2- Procedure

With the model cell, go through the steps necessary to 'form' a whole-cell recording configuration:

1. In BATH mode: Offset adjustment
2. Get voltage/current conversion and gain factors and measure 'pipette' resistance
3. Switch to PATCH mode: Electrode capacitance compensation
4. Switch to CELL mode: measure access and membrane resistance, cell capacitance
5. Compensate whole cell capacitance and series resistance
6. Once you feel confident move on to record DRGs.

With DRG neurons in **Voltage-clamp** (VC) mode:

1. Repeat procedure described above using a patch-pipette
2. Measure pipette resistance and compensate pipette capacitance
3. Establish a cell-attach configuration on a neuron with V_{hold} = -60 mV
4. Measure seal resistance. What is the holding current at this potential?
5. Go in whole-cell mode. What does the trace you observe represent?
6. Measure access/series resistance, cell capacitance and input resistance

With DRG neurons in **Current-clamp** (CC) mode:

1. Measure resting membrane potential from DRG neurons, note the size of the neuron (small, medium or large)
2. Generate action potentials (APs) in DRG neurons by applying a current step protocol (see below). Record.

3- Methods

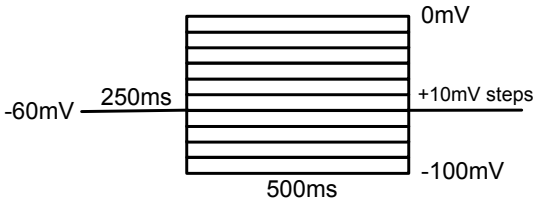
a) Recording solutions

Extracellular solution	in mM
NaCl	140
KCl	4
$CaCl_2$	2
$MgCl_2$	2
HEPES	10
D-glucose	10
pH, adjusted with NaOH	7.4
Osmolarity ($mOsm.Kg^{-1}$)	~300

Intracellular solution	in mM
K-gluconate	130
$MgCl_2$	1
$CaCl_2$	1
HEPES	10
EGTA	10
Mg-ATP	4
Na_2-GTP	0.3
pH, adjusted with KOH	7.2
Osmolarity ($mOsm.Kg^{-1}$)	~290

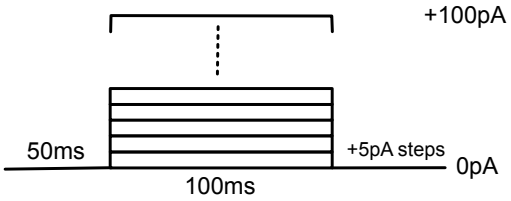
b) Recording protocols

Voltage steps: voltage-clamp



Repeat 10 times, recovery time 5s

Current steps: Current-clamp



Repeat 5 times, recovery time 5s

4- Analysis & Questions

1. Get used to reading from the oscilloscope and calculating parameters
2. Compare the whole-cell capacitance, input resistance and access resistance cell size
3. Resting membrane potential relative to the DRG neuron size
4. Create a frequency vs current (FI) curve

5- NOTES:

NOTES:

Lab #4 – K^+ currents in DRG neurons

1- Objectives

In VC mode, we can characterize ionic conductances, such as K^+ conductances, underlying the APs recorded in the first lab. DRG neurons express multiple K^+ channel types, including the delayed rectifier K^+ channel.
Delayed rectifier K^+ currents (I_K) are slowly inactivating, tetraethylammonium (TEA)-sensitive K^+ currents that contribute to the repolarization phase of the AP.
In this lab, we will characterize the activation and deactivation properties of this K^+ channel using different VC protocols and a solution designed to preferentially select this current.

2- Procedure

1. Obtain the I/V curve for I_K
2. Construct activation/inactivation curve for I_K
3. Determine the reversal potential of K^+

3- Methods

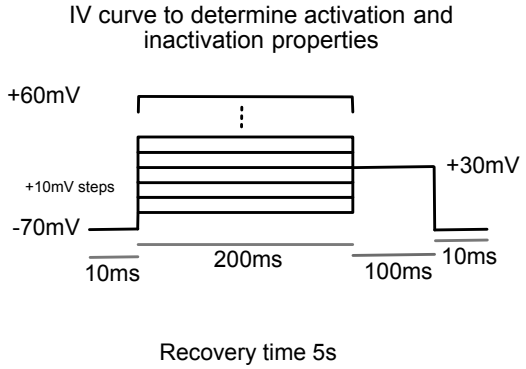
a) Recording solution

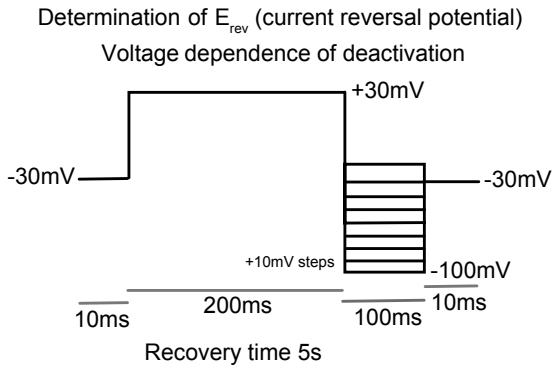
Extracellular solution	in mM
NaCl	150
KCl	2.5
$MgCl_2$	1
HEPES	10
D-glucose	10
TTX $^{\$}$	100 nM
4-aminopyridine (4-AP) $^{\#}$	5
$CdCl_2$ *	0.05
pH, adjusted with NaOH	7.4
Osmolarity ($mOsm.Kg^{-1}$)	~300
LIDOCAINE for TTX-resistant	

Intracellular solution	in mM
K-gluconate	130
$MgCl_2$	1
$CaCl_2$	1
HEPES	10
EGTA	10
Mg-ATP	4
Na_2-GTP	0.3
pH, adjusted with KOH	7.2
Osmolarity ($mOsm.Kg^{-1}$)	~290

Chemicals used to block specific channels (currents): $^{\$}$ voltage-gated Na^+ channels (inward currents), $^{\#}$ voltage-gated K^+ channels (outward currents) and * voltage-gated Ca^{2+} channels (inward currents). Note that addition of Cd^{2+} can make recordings (seal formation) more difficult.

b) Recording protocols





4- Analysis & Questions:

1. Plot the I/V curve for I_K
2. Plot the activation curve from the tail currents (current from steps down to -30 mV)
3. Why do we use tail currents instead of chord conductance from the I/V curve?
4. Plot the voltage dependence of deactivation from protocol 2
5. What is the reversal potential of K^+?
6. Why is it different from the calculated E_K (Nernst equation)?

5- Useful references

McCoy JG, Nimigean CM (2012) Structural correlates of selectivity and inactivation in potassium channels. ***Biochim Biophys Acta*** Feb;1818(2):272-85.

Armstrong CM (2003) Voltage-gated K channels. ***Sci STKE*** Jun 24;2003(188):re10.

6- NOTES:

NOTES:

Lab #5 – Na^+ currents in DRG neurons

1- Objectives

Voltage-dependent Na^+ channels are key elements for cell excitability and underlie the depolarizing phase of the AP. They produce regenerative currents and the channels inactivate. This current is one of the most difficult to record accurately because it is of large amplitude and with a fast onset. It is therefore crucial to conduct properly Rs and capacitance compensation.
Note: DRG neurons express both tetrodotoxin (TTX)-sensitive and TTX-resistant Na^+ channels. The expression of the TTX-resistant form is promoted in the presence of NGF in the culture medium. TTX-sensitive can be separated from the resistant ones using digital subtraction analysis.

2- Procedure

1. Perform a whole-cell configuration from DRG neurons and accurately compensate series resistance and membrane capacitance
2. Follow protocols below to record I_{Na} and analyze current properties.

3- Method

a) Recording solutions

Extracellular solution	in mM
*NaCl (or even lower)	60
$^{\$}$TEA-Cl	75
$CaCl_2$	1
$MgCl_2$	3
HEPES	10
D-glucose	10
$^{\#}CdCl_2$	0.1
pH, adjusted with TEA-OH	7.4
Osmolarity (mOsm.Kg^{-1})	~300

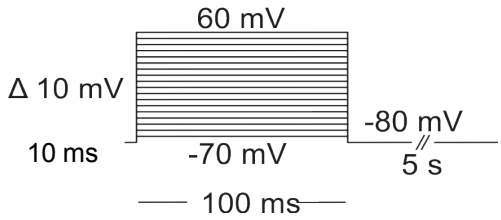
Intracellular solution	in mM
$^{\%}$CsF (Cesium-Fluoride)	125
NaCl	10
HEPES	10
EGTA	10
NaCl	10
pH, adjusted with TEA-OH	7.2
Osmolarity (mOsm.Kg^{-1})	~290

*: Extracellular Na^+ concentration was lowered to reduce I_{Na} peak amplitude and optimize voltage-clamp. $^{\$}$ and *: Used to block K^+ and Ca^{2+}currents, respectively to isolate Na + currents. $^{\%}$: Improve seal formation and therefore recording quality.

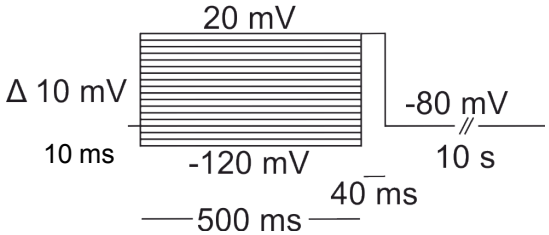
*** *Alternatively use a CsCl-Based intracellular solution*

b) Recording protocols

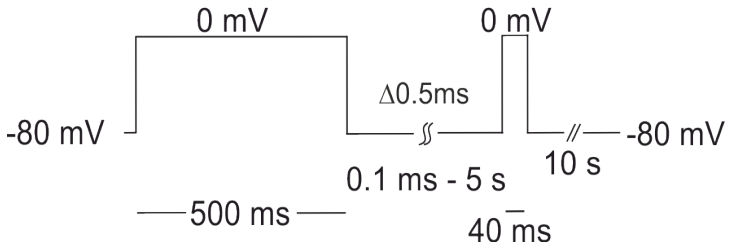
1. I/V curve for I_{Na}



2. Inactivation properties of voltage-gated Na^+ channels:
   - The voltage-dependence of steady-state fast inactivation



   - The time to recover from fast inactivation



Protocols 3x
1st: 0,1 to 10,1 ms with delta 1 ms
2nd: 20 to 100 ms with delta of 10 ms
3rd: 500 to 5000 ms with delta of 500 ms

4- Extra (time permitting)

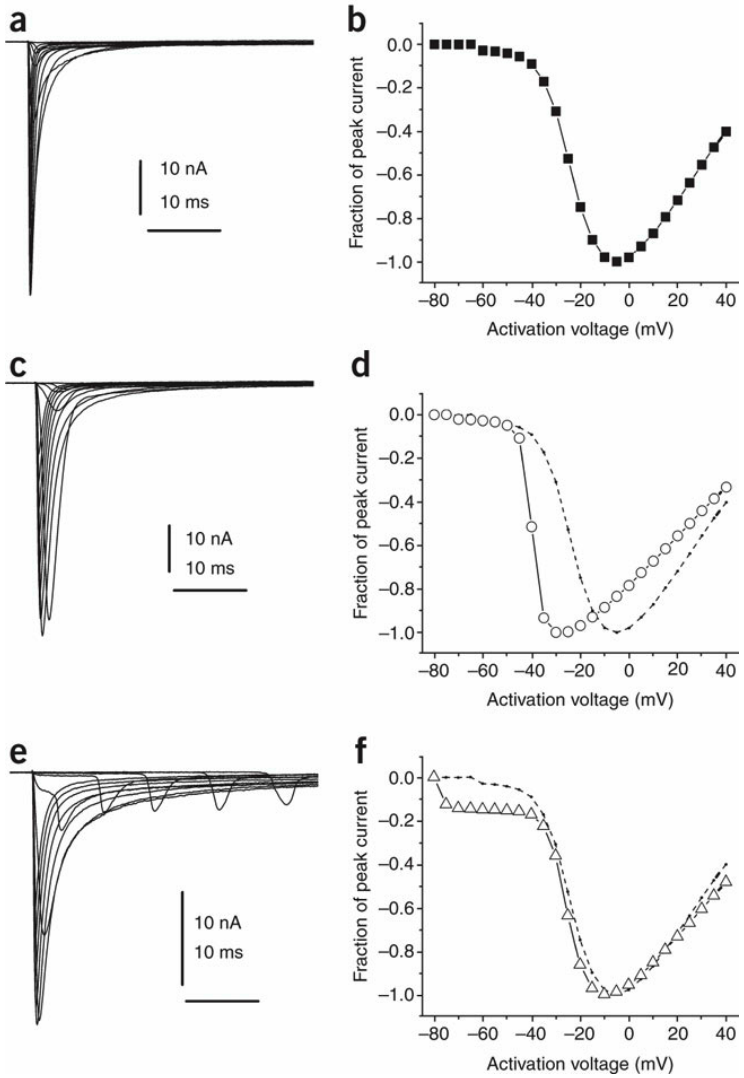
1. Turn off the series resistance compensation and run the I/V protocol; what happens?
2. If you see two types of Na^+ currents, apply 1μM TTX and run the protocols again

5- Analysis & Questions

1. Plot the I/V curve for peak I_{Na}. What is the reversal potential?
2. For the voltage-dependence of steady-state inactivation (h_∞ curve):
3. Test pulse peak I_{Na} as a fraction of the maximal inward current vs. pre-pulse voltage.
4. What proportion of Na^+ channels inactivate at resting membrane potential (~-60 mV)?
5. What do you notice about the kinetics during the inactivation test pulse?
6. For time to remove inactivation:
   Plot I_{Na} amplitude during the second pulse (as a fraction of I_{Na} amplitude during first pulse) vs. time after first test pulse.

6- Useful Information/References

1. Under special conditions, Na^+ can flow through other channels (e.g. Ca^{2+} channels).
2. The reversal potential is immune to series resistance errors, so it is not a good diagnostic of the quality of series resistance compensation.
3. Due to their rapid kinetics, Na^+ currents, particularly of large amplitude, can be difficult to voltage-clamp.
   Below are examples of Na^+ currents with good (**a**, **b**) and out of control (**c, d, e** and **f**) voltage-clamp recordings (from *Cummins et al., 2009*).



(**a**) Family of sodium current traces recorded from an adult DRG $Na_v1.8$-null neuron expressing recombinant $Na_v1.6$ channels showing adequate voltage control. (**b**) Peak current–voltage relationship from currents shown in panel **a**. Note that the current amplitude increases in a graded manner with voltage between -40 and -10 mV. (**c**) Family of sodium current traces recorded from an adult DRG $Na_v1.8$-null neuron expressing recombinant $Na_v1.6$ channels with inadequate voltage control. (**d**) Peak current–voltage from relationship from currents shown in panel **c**. Note that the current amplitude increases precipitously between -45 and -35 mV, indicating loss of voltage control. (**e**) Family of sodium current traces recorded from an adult DRG $Na_v1.8$-null neuron expressing recombinant $Na_v1.6$ channels illustrating inadequate space clamp. (**f**) Peak current–voltage from relationship from currents shown in panel (**e**). Note that at negative potentials (between -75 and -45 mV), sodium currents are activated with substantial delays, characteristic of the activation of sodium channels in neurites that are not adequately voltage-clamped. Dashed line in panels **d** and **f** shows the current–voltage data from panel **b** for comparison. All recordings were obtained in the presence of 300 nM TTX to block endogenous TTX-sensitive sodium channels.

Meents, J. E. and A. Lampert (2016) Studying sodium channel gating in heterologous expression systems. Neuromethods book series volume 113: ***Advanced Patch-Clamp Analysis for Neuroscientists***, pp 37-65.

Cummins TR, Rush AM, Estacion M, Dib-Hajj SD, Waxman SG (2009) Voltage-clamp and current-clamp recordings from mammalian DRG neurons. ***Nat Protoc.***;4(8):1103-12.

7- NOTES:

Lab #6 - Ca^{2+} channels in DRG neurons

1- Objectives

DRG neurons express a variety of voltage-gated Ca^{2+} channels, including low- (LVA) and high-voltage activated (HVA) channels. These two types of currents can be separated pharmacologically but also using specific activation protocol. LVA currents (so-called T-type currents) are transient currents activated at hyperpolarized membrane potential and inactivated above -60 mV.

In today's lab we will study the voltage-dependence of Ca^{2+} channels using tail currents and isolate LVA-Ca^{2+} currents if present. Note that not all DRG neurons express LVAs.

2- Procedure

1. Perform a whole-cell configuration from DRG neurons and accurately compensate series resistance and membrane capacitance
2. Set V_{hold} = -80 mV and follow the recording protocols to isolate I_{Ca} and analyze its properties

3- Method

a) Recording solution

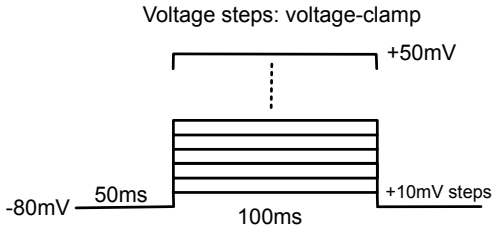
Extracellular solution	in mM
CH_3SO_3H (99%)	140
TEA-OH	145
HEPES	10
D-glucose	15
$CaCl_2$	10
TTX	100 nM
Lidocaine	100 µM
pH, adjusted with TEA-OH	7.4
Osmolarity ($mOsm.Kg^{-1}$)	~300

Intracellular solution	in mM
N-methyl-D-glucamine (NMDG)	120
TEA-OH	10
EGTA	11
HEPES	10
$CaCl_2$	1.1
Na-creatine PO_4	14
Mg-ATP	4
Na_2-GTP	0.3
HCl (to Balance out Cl)	20
pH, adjusted with CH_3SO_3H	7.2
Osmolarity ($mOsm.Kg^{-1}$)	~290

Intra- and extracellular solutions are optimized to isolate calcium currents

b) Recording protocols

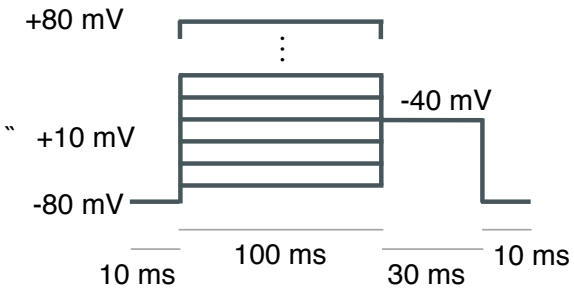
1. Hold DRG neuron at V_{hold} = -80 mV
2. Run a ramp from -80 to +80 mV (duration 200ms) every 10s over 5min
3. Record Ca^{2+} currents (I_{Ca})



1 s between Sweeps. Repeat 5times, Recovery time: 5s

Repeat 10 times, recovery time 5s

3. Generate I/V curves and record tail currents
   Use a high sampling rate and less filtering (e.g. 10 kHz)



Repeat 10 times, Recovery times: 5

4. LVA-I_{Ca} isolation:
   If LVA-Ca^{2+} current is present in the first pulse (it activates at hyperpolarized potential and inactivates at -40 mV), set V_{hold} = -40 mV
5. Run the protocol again
6. Run protocol again with V_{hold} = -90 mV

7. Supplementary protcol: V_{hold} = -80 mV, 10ms, V_{Step} to +80mV for 10ms (+10 mV increment) and step back to -80mV

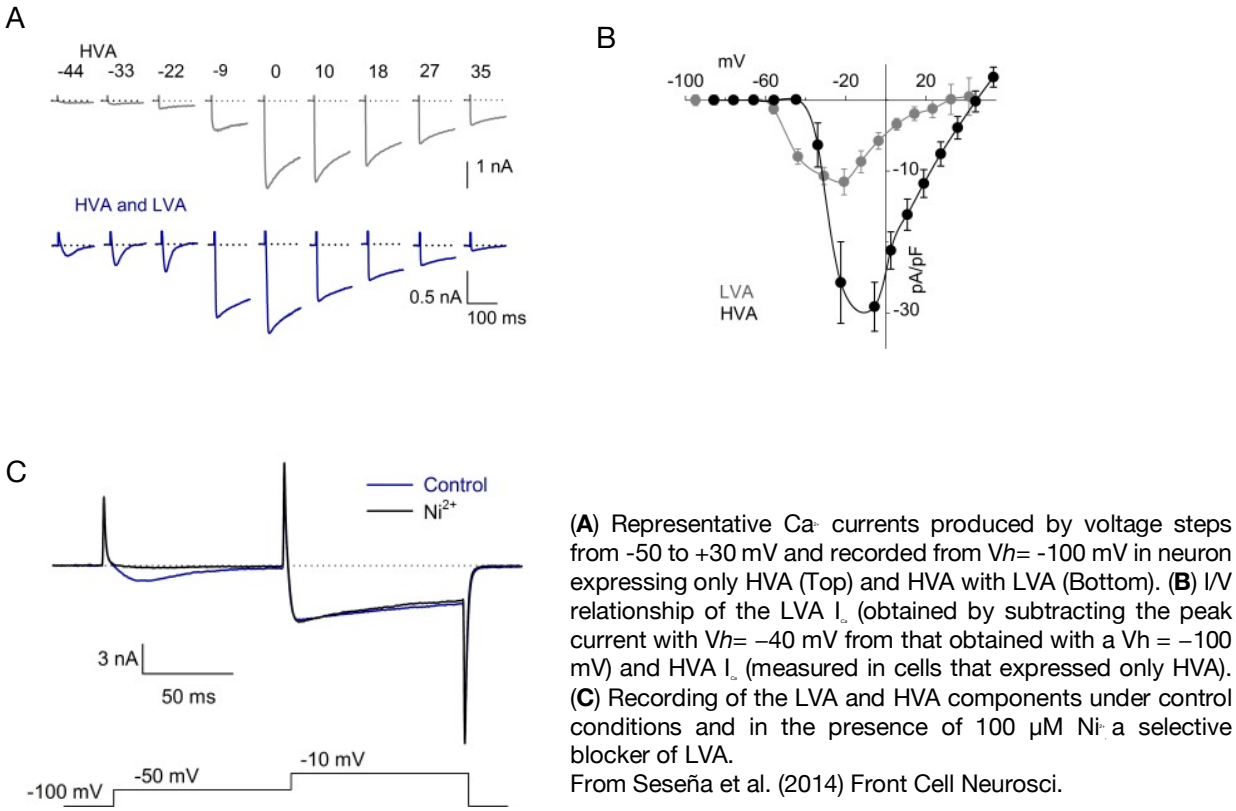
4- Extra (Time Permitting)

Look at Ca^{2+} dependent inactivation by comparing current recorded using solutions containing either Ca^{2+} or Ba^{2+}.

5- Analysis & Questions

1. Plot the I/V curve for I_{Ca}
2. Where is the current reversal potential?
3. Plot the activation curve for Ca^{2+} currents using the tail currents.
4. If LVA-Ca^{2+} currents are observed, plot the I/V curves for I_{Ca} using both V_{hold} values (-60 and -90 mV).

6- Useful Information/References



(**A**) Representative Ca currents produced by voltage steps from -50 to +30 mV and recorded from V*h*= -100 mV in neuron expressing only HVA (Top) and HVA with LVA (Bottom). (**B**) I/V relationship of the LVA I (obtained by subtracting the peak current with V*h*= –40 mV from that obtained with a Vh = –100 mV) and HVA I (measured in cells that expressed only HVA). (**C**) Recording of the LVA and HVA components under control conditions and in the presence of 100 µM Ni a selective blocker of LVA.
From Seseña et al. (2014) Front Cell Neurosci.

Scott MB, Kammermeier PJ (2017) CaV2 channel subtype expression in rat sympathetic neurons is selectively regulated by $\alpha 2\,\delta$ subunits. ***Channels (Austin).*** Nov 2;11(6):555-573.

Zamponi GW, Striessnig J, Koschak A, Dolphin AC (2015) The Physiology, Pathology, and Pharmacology of Voltage-Gated Calcium Channels and Their Future Therapeutic Potential. ***Pharmacol Rev***. Oct;67(4):821-70.

7- NOTES:

Lab #7 – Unitary glycine currents in HEK cells

Set up a rig to conduct single channel recording and record unitary current from Glycine receptors

1- Objectives

Single channel recordings are a classical way to describe the properties of ion channels (unitary channel conductance, kinetics of channel opening, etc…).
In this lab, we will record single channel currents from cell-attached patches of transfected HEK cells.

2- Procedure

1. Reduce noise in system ≤ 2 pA (acceptable for single channel recordings).
2. Set up epifluorescence illumination
3. Pull high resistance electrodes (>10 MΩ) and Sylgard electrode tip.
4. Record single channel glycine currents in cell-attached mode.

3- Methods

a) Recording solutions

Extracellular solution	in mM
NaCl	140
KCl	4
$CaCl_2$	2
$MgCl_2$	2
HEPES	10
D-glucose	10
pH, adjusted with NaOH	7.4
Osmolarity ($mOsm.Kg^{-1}$)	~300

Pipette solution
Use extracellular solution and add **1 mM glycine**

HEK cells have been transfected with cDNA constructs to express glycine receptors. GFP is also expressed to enable visualization of transfected cells.
Change configuration on amplifier to PATCH β=1 (change in headstage feedback resistor)

b) Recording protocols

1. Using epifluorescence illumination system, select a fluorescent HEK cell (i.e. expressing glycine receptors)
2. Switch back to a 4x or lower objective
3. Bring electrode to cell under the low power objective
4. Get a GΩ seal and **stay in cell-attached mode**
5. Compensate the pipette capacitance, turn up gain to 50-100x, open filter as wide as possible, sample at 5 kHz or more
6. Set command V*hold* (scope window) to -40 mV
7. Observe the changes in single-channel currents upon changing V*hold*
8. Run Glycine Ramp protocol (Ramp Loop)
9. Run Glycine Loop protocol at -80, -60, -40, -20, 0, +20, +40, +60, +80 mV

4- Extra (Time Permitting)

For something a little tougher – ask for HEK cells expressing potassium channels fused with YFP, for this you'll have to pull some inside-out patches (at this point seek advice from A. Plested).

5- Analysis & Questions

1. From the ramps, determine the E_{Cl}
2. Determine the single channel conductance and channel open time
3. Plot an "all point" amplitude histograms

6- Useful references

Plested AJ, Baranovic J (2016) Single-channel recording of glycine receptors in Human Embryonic Kidney (HEK) cells. ***Cold Spring Harb Protoc*** (8).

7- NOTES:

NOTES:

Lab #8 – Measuring I_h in hippocampal CA1 neurons *in vitro*

1- Introduction

Today, we will make patch-clamp recordings from CA1 neurons in acute slices of mouse hippocampus.
Whole-cell voltage- and current-clamp recordings will be made from CA1 neurons to measure HCN channel-mediated I_h currents and determine how they affect membrane potential dynamics and synaptic integration.

2- Objectives

1. Set up and become proficient with the 700B amplifiers (better amplifiers for I-clamp recordings).
2. Get used to looking at the structure of the brain slice as opposed to the isolated DRG or HEK cells
3. Get used to using a Platinum 'harp' to weigh down the slice.
4. Recognize healthy slices and cells – identify a region of the slice where there are healthy looking Pyramidal neurons.
5. Perform somatic voltage-clamp recordings from CA1 pyramidal neurons.
6. Record I_h current and determine its influence on membrane resistance and cell excitability.

3- Methods

a) Slice preparation

Acute horizontal slices of mouse hippocampus (250 µm thick) from P12-14 mice.

b) Recording solutions

Standard artificial cerebrospinal fluid (ACSF) and K-Gluconate-based internal solution

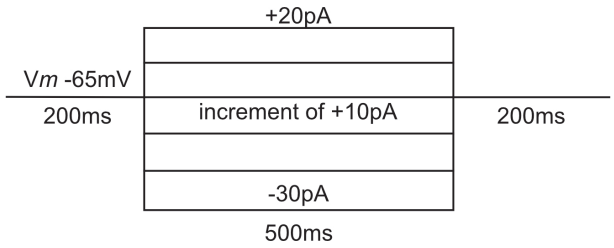
Extracellular solution	in mM
NaCl	125
KCl	2.5
$CaCl_2$	2
$MgSO_4$	1
Glucose	25
$NaHCO_3$	25
NaH_2PO_4	1.25
pH adjusted with 95% O_2/5% CO_2	7.4
Osmolarity (mOsm.Kg^{-1})	~300

Intracellular solution	in mM
K-Gluconate	115
KCl	20
Na-Phosphocreatine	10
HEPES	20
EGTA	10
$CaCl_2$	1
Mg-ATP	2
Na_2-GTP	0.3
pH adjusted with KOH	7.3
Osmolarity (mOsm.Kg-1)	~290

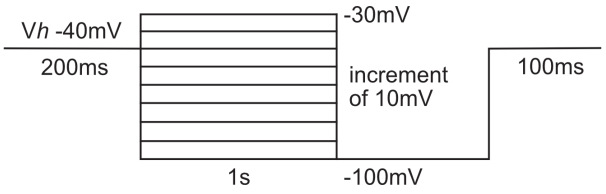
* CsCl & picrotoxin will be added to the ACSF

g- Recording protocols

5. Start recording in **Current-Clamp** mode with V_m around -65 mV
   ** *Inject some DC current if needed to bring neuron to the appropriate membrane potential.*
   ** *Depending of the neuron you <u>might</u> need to adjust the amount of DC current to be injected.*
   [lex mw xli wleti sj xli l}tivtspevm~exmsr wxitC
6. Ett p} GwGP xs xli fexl ex 8qQ
   [lex lettirw xs xli l}tivtspevm~exmsr wleti0 xli qiqfveri tsxirxmep0 xli qiqfveri viwmwxergiC



3. Switch to **Voltage-Clamp** and keep your neuron at V_h = -70mV
BUT before starting your recording bring it to -40mV for 5 to 10 s
4. Apply the protocol illustrated below



5. Repeat protocol 1 et 2 in the presence of 4 mM CsCl in the bath.
6. To monitor CsCl wash in:
- Go in Current-clamp mode
- Set membrane potential to -65 mV with DC current injection if needed
- Apply a DC current step of -30pA, 500ms (adjust amplitude, see above) every 10s
7. What effect does Cs^+ have on the sag and amplitude of Ih?

4- Extra (Time Permitting)

Perform dendritic patch at increasing distance from soma and inject -100 to -200 pA of DC current. How does the hyperpolarization voltage step looks like with distance?
Set up your stimulating electrode on manipulator 2

1. Apply a train of stimuli (stimulate on Schaffer Collateral - 5 stimuli @ 50 Hz)
2. In the presence of picrotoxin to examine summation of excitatory inputs.
3. Repeat the same experiments in the presence of Cs^+ to block HCN1 channels.
4. What happens?

5- Analysis & Questions

1. Measure reversal potential of the current and slow activating I_h current amplitude
2. Measure the 'sag' in V_m in the absence and presence of Cs^+
3. What is the effect of blocking I_h with Cs^+?

6- Useful references

Garden DL, Dodson PD, O'Donnell C, White MD, Nolan MF (2008). Tuning of synaptic integration in the medial entorhinal cortex to the organization of grid cell firing fields. ***Neuron*** 60(5): 875-89.

Nolan MF, Dudman JT, Dodson PD, Santoro B (2007). HCN1 channels control resting and active integrative properties of stellate cells from layer II of the entorhinal cortex. ***J Neurosci*** 27(46):12440-51.

Gasparini S, DiFrancesco D (1997). Action of the hyperpolarization-activated current (Ih) blocker ZD 7288 in hippocampal CA1 neurons. ***Pflugers Arch*** 435(1):99-106.

7- NOTES:

Lab #9 – Record miniature excitatory postsynaptic currents (*m*EPSCs)

1- Introduction

In the cerebellum, the principal neuron is the cerebellar Purkinje cell (PC) which receives feedforward excitation from granule cells and feedforward inhibition from molecular layer interneurons (MLIs). Purkinje cells are large ~30 μm in diameter while MLIs are ~10 μm.
You will record from either PCs or MLIs, which both receive excitatory input from parallel fibers – the axons of upstream granule cells. The *m*EPSCs will be small in amplitude so you will have to make sure that the baseline noise is ~8-10 pA in amplitude before starting. But after your single channel session with *A. Plested*, this will be simple.

2- Objectives

1. Set up and become proficient with the 700B amplifiers (better amplifiers for I-clamp recordings).
2. Get used to looking at the structure of the brain slice as opposed to the isolated DRG or HEK cells
3. Get used to using a Platinum 'harp' to weigh down the slice.
4. Recognize healthy slices and cells – identify a region of the slice where there are healthy looking Purkinje cells.
5. Patch a PC or MLI and record glutamatergic *s*EPSCs and *m*EPSCs.

3- Methods

a) Slice preparation

Acute sagittal slices of mouse cerebellum (250 μm thick) from P12-14 mice.

b) Recording solutions

Standard artificial cerebrospinal fluid (ACSF) and Cs-Meth-based internal solution

Extracellular solution	in mM
NaCl	125
KCl	2.5
$CaCl_2$	2
$MgSO_4$	1
Glucose	25
$NaHCO_3$	25
NaH_2PO_4	1.25
pH adjusted with 95% O_2/5% CO_2	7.4
Osmolarity (mOsm.Kg-1)	~300

Intracellular solution	in mM
$CsMeSO_3$	130
NaCl	10
$MgCl_2$	2
$CaCl_2$	0.16
EGTA	0.5
HEPES	10
MgATP	2
Na_2GTP	0.3
Na-phosphocreatine	10
Osmolarity (mOsm.Kg-1)	~290

c) Recording protocols

Patch electrodes should be in the 6-8 MΩ range.
If using a $CsCH_3SO_3$-based internal solution, hold the cell at **-70 mV**.
Try and get a 'clean' break into whole-cell configuration and then adjust the series resistance compensation.

During the experiment:

1. Record repeated 10 s sweeps (with a minimal inter-sweep interval)
2. You are trying to get a pseudo continuous record (2 kHz filter and 10 kHz sampling).
3. Record first in control ACSF (as many sweeps as needed to get a good number of synaptic currents; 10-100 sweeps).
4. Record next in ACSF with TTX (500 nM) – to get miniature (minis) synaptic events.
5. Record next in ACSF with picrotoxin (100 µM) – to block $GABA_A$-Rs.
6. At the end, record in ACSF with DNQX (10 µM) – to block AMPA-Rs.

4- Useful Information/References

1. Slices are good for about 6-7 hr after slicing
2. Recording pipettes
    - Type of glass: borosilicate, with filament
    - Make sure glass is clean, fire-polished
    - Minimize capacitance from electrode with:
        - Minimum internal solution
        - Minimum fluid level in recording chamber
        - Apply Sylgard or wax near tip

Zhuang X, Sun W, Xu-Friedman MA (2017). Changes in properties of auditory nerve synapses following conductive hearing loss. ***J Neurosci*** 37(2):323-332.

Xu-Friedman MA (2013). Illustrating concepts of quantal analysis with an intuitive classroom model. ***Adv Physiol Educ*** 37(1):112-6.

5- NOTES:

Lab #10 – Record miniature inhibitory postsynaptic currents (*m*IPSCs)

1- Introduction

In the cerebellum, the main cell types present are Purkinje cells, granule cells, Golgi cells and molecular layer interneurons (MLIs; stellate and basket cells). All are GABAergic except for the granule cells. They are identified from their positions in the layered structure of the cerebellum, by their shape, and by their electrophysiological properties. You will record from MLIs, which form GABAergic contacts with one another. The cells are 2-quite Objecsmall, tivesbut eminently 'clampable'.

2- Objectives

1. Get used to looking at the structure of the brain slice as opposed to the isolated DRG or HEK cells
2. Get used to using a Platinum 'harp' to weigh down the slice.
3. recognize healthy slices and cells – identify a region of the slice where there are healthy looking Purkinje cells.
4. Patch a MLI neuron and record GABAergic *m*IPSCs.

3- Methods

a) Slice preparation

Acute sagittal slices of mouse cerebellum (250 µm thick) from P12-14 mice.

b) Recording solutions

Standard artificial cerebrospinal fluid (ACSF) and CsCl-based internal solution

Extracellular solution	**in mM**
NaCl	125
KCl	2.5
$CaCl_2$	2
$MgSO_4$	1
Glucose	25
$NaHCO_3$	25
NaH_2PO_4	1.25
pH adjusted with 95% O_2/5% CO_2	7.4
Osmolarity ($mOsm.Kg^{-1}$)	~300

Intracellular solution	**in mM**
CsCl	130
$CaCl_2$	0.5
EGTA	5
QX-314 (Lidocaine)	4
HEPES	10
Na-Phosphocreatine	10
Mg-ATP	2
Na_2-GTP	0.3
pH adjusted with CsOH	7.3
Osmolarity ($mOsm.Kg^{-1}$)	~290

During the experiment:

Select your target cell and place a bipolar stimulus electrode ca. 100 µm lateral in L5.

1. Perform somatic voltage-clamp recording from cortical L5 pyramidal neurons at V_{hold} = -70 mV.
2. Stimulate recurrent synaptic inputs by adjusting the stimulus current amplitude (0-100 µA) until you measure stable triggered inputs.
3. Calculate E_{Cl} (theoretical) and repeat the recording at that potential to isolate excitatory synaptic inputs.
4. Perform somatic voltage clamp as in 1 (V_{hold}: -70 mV and 0 mV)
5. Start stimulating in L5 and subsequently move your stimulation electrode through the other cortical layers to measure the layer-specific inputs

Cortical excitability in the absence of inhibition:

1. Repeat experiment 1 at V_{hold} = -70 mV
2. Wash in SR95531 (20 µM) to pharmacologically isolate EPSCs. What happens?

4- Tips for electrical stimulation of synaptic inputs

1. While hunting for a synapse, deliver a stimulus repeatedly at 2 second intervals.
2. Set stimulus amplitude to zero when positioning stimulating electrode on the surface of the slice.
3. Tip should contact slice surface gently, making contact without moving tissue near the recorded neuron.
4. Slowly increase stimulus intensity, observing 1-3 traces before incrementing. You should not have to use >0.1 mA.
5. If a response is not observed at one polarity, set the amplitude back to zero and switch to the opposite polarity.

5- Useful references

Cohen JY, Amoroso MW, Uchida N (2015). Serotonergic neurons signal reward and punishment on multiple timescales. ***Elife*** 4.

6- NOTES:

Lab #11 – Cortical connectivity

1- Introduction

Today, we will examine synaptic inputs to pyramidal neurons in layer 5 of the cortex. Recordings will be in voltage-clamp to allow observation of excitatory and inhibitory synaptic currents. We will electrically stimulate presynaptic fiber to elicit post-synaptic currents (PSCs) in the recorded cell.
Because cortical circuitry is complex, the recorded responses will consist of a mixture of monosynaptic, di-synaptic, and polysynaptic excitatory and inhibitory inputs.

2- Objectives

1. Recording cortical Layer 5 pyramidal cells.
2. Set up extracellular electrical stimulation to induce postsynaptic current or potential.
3. Identify recurrent synaptic connectivity onto pyramidal cells.
4. Record and separate pharmacologically inhibitory from excitatory synaptic inputs.

3- Methods

a) Slice preparation

Acute coronal slices of cortex (250 µm thick) from P16 rats.

b) Recording solutions

Standard artificial cerebrospinal fluid (ACSF) and K-Gluconate-based internal solution

Extracellular solution	in mM
NaCl	125
KCl	2.5
$CaCl_2$	2
$MgSO_4$	1
Glucose	25
$NaHCO_3$	25
NaH_2PO_4	1.25
pH adjusted with 95% O_2/5% CO_2	7.4
Osmolarity (mOsm.Kg^{-1})	~300

Intracellular solution	in mM
K-Gluc	130
$CaCl_2$	0.5
EGTA	5
QX-314 (Lidocaine)	4
HEPES	10
Na-Phosphocreatine	10
Mg-ATP	2
Na_2-GTP	0.3
pH adjusted with KOH	7.3
Osmolarity (mOsm.Kg-1)	~290

c) Recording protocols

Set up the stimulation electrode on your second micro-manipulator and connect it to the isolated stimulation unit. This unit will deliver continuous current pulses through the extracellular stimulus electrode. Pre-synaptic cells/fibers are activated by delivering a brief (0.2 ms) shock to initiate an action potential in the axons. The position of the stimulus electrode determines which axons can be activated. The size of the stimulus electrode and the amplitude of the current delivered by the stimulus unit determine the area over which axons are stimulated.

c) Recording protocols

If using a CsCl-based internal solution, hold the cell at -70 mV.
Patch electrodes should be in the 4-6 MΩ range.
Try and get a 'clean' break into whole-cell configuration and then adjust the series resistance compensation.

During the experiment:

1. Record repeated 2s sweeps (with a minimal inter-sweep interval)
2. You are trying to get a pseudo continuous record (2 kHz filter and 10 kHz sampling).
3. Record first in control ACSF (as many sweeps as needed to get a good number of synaptic currents; 10-100 sweeps).
4. Record next in ACSF with TTX (500 nM) – to get minis.
5. Record next in ACSF with DNQX (10 µM) – to block AMPA-Rs.
6. At the end, record in ACSF with picrotoxin (100 µM) – to confirm the GABAergic nature of *m*IPSCs.

4- Extra (Time Permitting)

1. Use K-Gluconate-based internal solution and hold at -40 mV (can you tell why?)
2. Try the same protocol on a Purkinje cell (in this case you would need to use thin-walled glass and electrodes of 2-5 MΩ and the CsCl-based internal solution (again with series resistance compensation).

Intracellular solution	in mM
K-gluconate	130
$MgCl_2$	1
$CaCl_2$	1
HEPES	10
EGTA	10
Na-Phosphocreatine	10
Mg-ATP	4
Na_2-GTP	0.3
pH adjusted with kOH	7.3
Osmolarity ($mOsm.Kg^{-1}$)	~290

5- Useful Information/References

1. Slices are good for about 6-7 hr after slicing
2. Recording pipettes
    - Type of glass: borosilicate, with filament
    - Make sure glass is clean, fire-polished
    - Minimize capacitance from electrode with:
        - Minimum internal solution
        - Minimum fluid level in recording chamber
        - Apply Sylgard or wax near tip

Hull C (2017). *Cellular and synaptic properties of local inhibitory circuits.* **Cold Spring Harb Protoc** 2017(5):pdb.top095281.

Hull C (2017). Measuring feedforward inhibition and its impact on local circuit function. **Cold Spring Harb Protoc** 2017(5):pdb.prot095828.

Hull C, Regehr WG (2012). Identification of an inhibitory circuit that regulates cerebellar Golgi cell activity. **Neuron** 73(1):149-58.

6- NOTES:

Lab #12 – Synaptic plasticity & Long Term Potentiation (LTP)

1- Introduction

The aim of this lab is to evoke long-term potentiation (LTP) of Schaffer-collateral (SC) inputs from CA3 to CA1 pyramidal neurons in the hippocampus. LTP is a long-lasting enhancement in signal transmission between two neurons and is widely considered to be one of the major cellular mechanisms underlying learning and memory consolidation.

2- Objectives

1. Record from CA1 pyramidal neuron
2. Locate Schaffer collaterals (SC) and stimulate them electrically
3. Induction of LTP at SC synapses onto CA1 pyramidal neurons

3- Methods

a) Slice preparation

Acute horizontal slices of mouse hippocampus (250 µm thick) from P12-14 mice.

b) Recording solutions

Standard artificial cerebrospinal fluid (ACSF) and K-Gluconate-based internal solution

Extracellular solution	in mM
NaCl	125
KCl	2.5
$CaCl_2$	2
$MgSO_4$	1
Glucose	25
$NaHCO_3$	25
NaH_2PO_4	1.25
pH adjusted with 95% O_2/5% CO_2	7.4
Osmolarity ($mOsm.Kg^{-1}$)	~300

Intracellular solution	in mM
K-Gluconate	115
KCl	20
Na-Phosphocreatine	10
HEPES	20
Mg-ATP	2
Na_2-GTP	0.3
pH adjusted with KOH	7.3
Osmolarity ($mOsm.Kg^{-1}$)	~290

c) Recording protocols

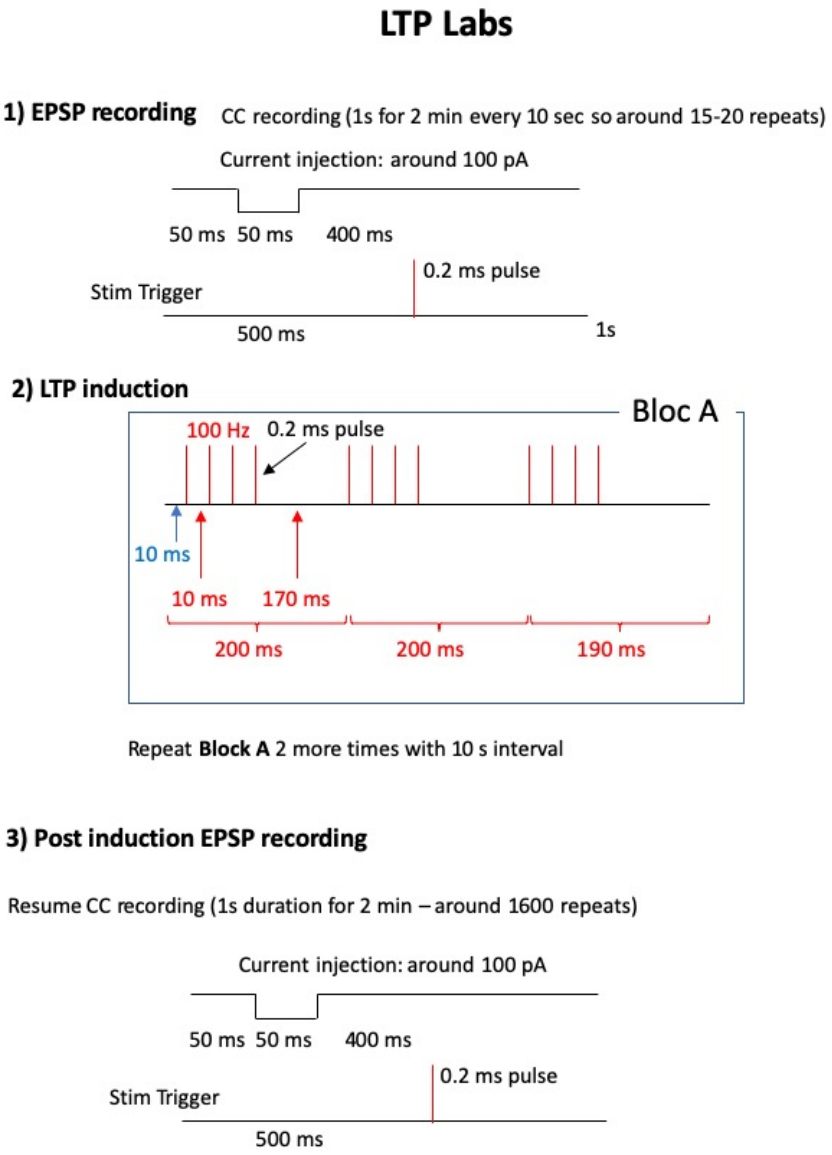
1. Patch a pyramidal cell in CA1 and find the optimum parameters to elicit stable synaptic responses while stimulating SC.
2. Record baseline activity and wait until you have a stable EPSP baseline (10 min) before inducing LTP
3. Apply a theta burst LTP induction protocol via your extracellular stimulation electrode.
4. Axons will be activated by delivering brief (0.2 ms) pulses and you will need to position your electrode and adjust the amplitude of the current delivered to locate strong presynaptic inputs.
5. Do this in current clamp so that you can record changes in EPSP amplitude after LTP induction.

The stimulation protocol will be:

- **Pre-induction Baseline:** [2 stim @ 30 Hz][wait 10 s] - [repeat 60 times]
- Add current step in current clamp to monitor Rs and R*input* changes

- **Induction:** [Theta burst stimulation – 4 stim @ 100 Hz, with 200 ms from start-to-start of each train (i.e. 5 Hz)] - [repeat 12 times]
- Wait 10s, repeat steps above a further 2 times
- Repeat theta burst stimulation protocol 3 times at 0.1 Hz

- **Post-induction Baseline:** [2 stim @ 30 Hz][wait 10 s][repeat 180 times]



6. **Optional** – postsynaptic current injection to generate coincident pre- and post-synaptic activity. If you would like to try this, we can discuss which protocol to use (e.g. 1nA postsynaptic current injection for 5ms).

4- Tips for electrical stimulation of synaptic inputs

1. While hunting for a synapse, deliver a stimulus repeatedly at 2 second intervals.
2. Set stimulus amplitude to zero when positioning stimulating electrode on the surface of the slice.
3. Tip should contact slice surface gently, making contact without moving tissue near the recorded neuron.
4. Slowly increase stimulus intensity, observing 1-3 traces before incrementing. You shouldn't have to use >0.1 mA.
5. If a response is not observed at one polarity, set amplitude back to zero and switch to opposite polarity.

5- Useful references

Simons SB, Escobedo Y, Yasuda R, Dudek SM (2009). Regional differences in hippocampal calcium handling provide a cellular mechanism for limiting plasticity. ***Proc Natl Acad Sci USA*** 106(33):14080-4.

Zhao M, Choi YS, Obrietan K, Dudek SM (2007). *Synaptic plasticity (and the lack thereof) in hippocampal CA2 neurons.* ***J Neurosci*** 27(44):12025-32.

Bliss TV, Collingridge GL (1993). A synaptic model of memory: long-term potentiation in the hippocampus. ***Nature*** 361(6407): 31–39.

Bliss T, Lømo T (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. ***J Physiol*** 232 (2): 331–

6- NOTES:

NOTES:

Lab #13 – Dendritic patching in pyramidal neurons *in vitro*

1- Introduction

Patch-clamp recordings from dendrites are used to investigate how synaptic inputs are integrated and how active processes in the dendrites shape these signals. Today, we will make whole-cell current-clamp recordings from pyramidal cell dendrites in acute slices of rat cortex or hippocampus.

2- Objectives

1. Set up and become proficient with the 700B amplifiers (better amplifiers for I-clamp recordings).
2. Optimize optics on your rig (Köhler illumination, setting for DIC or oblique illumination; **see Annex**).
3. record from pyramidal cell dendrites

3- Methods

a) Slice preparation

Acute horizontal or coronal slices of cortex or hippocampus (250 µm thick) from P12-14 mice**.**

b) Recording solutions

Standard artificial cerebrospinal fluid (ACSF) and K-Gluconate-based internal solution

Extracellular solution	in mM
NaCl	125
KCl	2.5
$CaCl_2$	2
$MgSO_4$	1
Glucose	25
$NaHCO_3$	25
NaH_2PO_4	1.25
pH adjusted with 95% O_2/5% CO_2	7.4
Osmolarity (mOsm.Kg^{-1})	~300

Intracellular solution	in mM
K-Gluconate	115
KCl	20
Na-Phosphocreatine	10
HEPES	20
EGTA	10
$CaCl_2$	1
Mg-ATP	2
Na_2-GTP	0.3
pH adjusted with KOH	7.3
Osmolarity (mOsm.Kg-1)	~290

c) Recording protocols

1. Identify a good dendrite projecting from a pyramidal neuron (flat, not contrasty, not blebby, not near other junk)
2. Put minimal positive pressure (0.4 psi) on pipette (~10 MΩ) as you approach the dendrite
3. Target the center of the dendrite with your pipette, push fairly hard until you see a dimple
4. Immediately reverse the pressure and attempt to seal and break in
5. Inject current, see dendritic spikes, publish a Science paper

4- Extra (Time Permitting)

1. Compare dendritic spikes with somatic action potentials
2. Stimulate synaptic inputs onto pyramidal cell
3. Make paired somatic-dendritic recordings.
4. Inject current at the soma and observe back-propagating APs in the dendrite.
5. Does it look different? Why?

5- Useful references

Poleg-Polsky A, Ding H, Diamond JS (2018). Functional compartmentalization within starburst amacrine cell dendrites in the retina. ***Cell Rep*** 22(11):2898-2908.

Davie JT, Kole MH, Letzkus JJ, Rancz EA, Spruston N, Stuart GJ, Häusser M (2006). Dendritic patch-clamp recording. ***Nat Protoc*** 1(3):1235-47.

Köhler illumination: Annex and http://en.wikipedia.org/wiki/K%C3%B6hler_illumination

6- NOTES:

NOTES:

Lab #14 – Free Lab

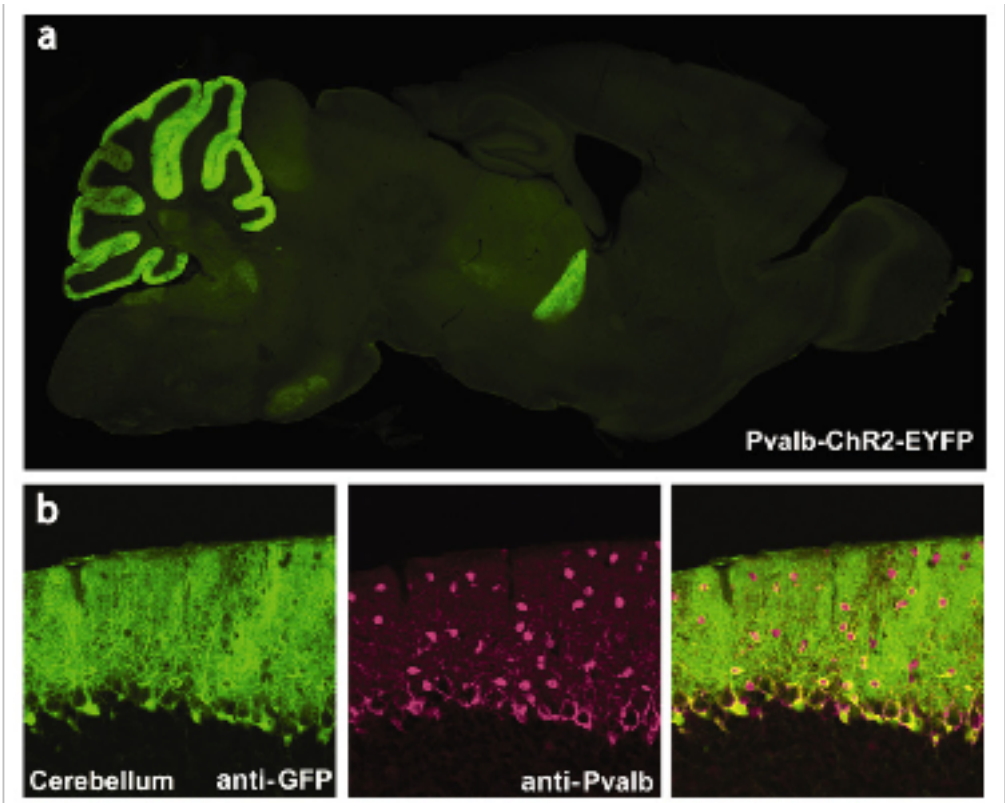
What experiment would you like to repeat/try

This is a free lab day where (within reason) the TAs will slice your brain region of choice and you try any experiment you wish.

Lab #15 – Optogenetics – ChR2 biophysics

1- Introduction

We will record from brain slices of cerebellum and/or thalamus obtained from transgenic mice expressing the light-activated cation channel Channel Rhodopsin 2 (ChR2) coupled to EYFP under control of the parvalbumin promoter/enhancer on the BAC transgene (Zhao et al., Nature Methods 2011). The mice were generated in the lab of G. Feng and as illustrated in the images below, ChR2 is widely expressed in cerebellar Purkinje cells and molecular layer interneurons. We will use fluorescence to locate and patch ChR2-expressing cells and then record light-evoked currents by varying the intensity and duration of the blue-light stimulation.



2- Objectives

1. Use LED illumination to visualize ChR2 expressing neurons
2. Record light-activated ChR2-mediated current
3. Test properties of the current and consequence on neuronal excitability

3- Methods

a) Slice preparation

Parasagittal sections of cerebellum (250 µm thick) or of thalamus (250 µm thick) from 5-7-week-old mice.

b) Recording solutions

Standard artificial cerebrospinal fluid (ACSF) and K-Gluconate-based internal solution

Extracellular solution	in mM
NaCl	125
KCl	2.5
$CaCl_2$	2
$MgSO_4$	1
Glucose	25
$NaHCO_3$	25
NaH_2PO_4	1.25
pH adjusted with 95% O_2/5% CO_2	7.4
Osmolarity (mOsm.Kg^{-1})	~300

Intracellular solution	in mM
K-Gluconate	115
KCl	20
Na-Phosphocreatine	10
HEPES	20
Mg-ATP	2
Na_2-GTP	0.3
pH adjusted with KOH	7.3
Osmolarity (mOsm.Kg^{-1})	~290

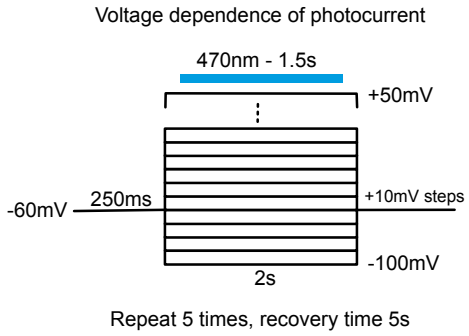
To characterize the ChR2 properties, an extracellular solution containing TTX 1 µM and TEA-Cl 5-20 mM will be used to block activation of Na^+ and K^+ voltage-dependent channels.
The standard extracellular solution will be used for current-clamp recordings.

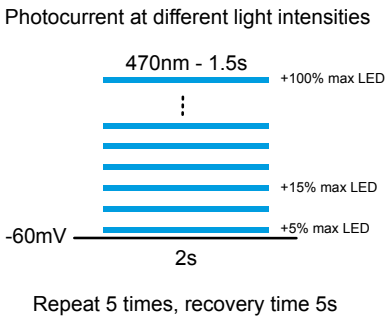
c) Recording protocols

Use epifluorescence illumination to locate a ChR2-expressing cell and establish a whole-cell recording.

In **<u>Voltage-clamp mode</u>**, set V_{hold} = -60mV

1. Evoke light-activated ChR2-mediated currents
2. Generate a current-voltage relationship
3. Generate an intensity-current relationship
4. Explore how long the photocurrent takes to recover from desensitization. This will involve changing the inter-stimulus interval between successive light pulses





In **<u>Current-clamp mode</u>** at resting membrane potential

1. Explore the effects of light stimulation on spiking – set up a protocol to investigate this in current clamp.

4- Useful references

Zhao S, Ting JT, Atallah HE, Qiu L, Tan J, Gloss B, Augustine GJ, Deisseroth K, Luo M, Graybiel AM, Feng G (2011). *Cell type–specific channelrhodopsin-2 transgenic mice for optogenetic dissection of neural circuitry function.* ***Nat Methods*** 8(9):745-52.

Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K (2005). *Millisecond-timescale, genetically targeted optical control of neural activity.* ***Nat Neurosci*** 8(9):1263-8.

Nagel G, Szellas T, Huhn W, Kateriya S, Adeishvili N, Berthold P, Ollig D, Hegemann P, Bamberg E (2003). *Channelrhodopsin-2, a directly light-gated cation-selective membrane channel.* ***Proc Natl Acad Sci USA*** 100(24):13940-5.

Nagel G, Ollig D, Fuhrmann M, Kateriya S, Musti AM, Bamberg E, Hegemann P (2002). *Channelrhodopsin-1: a light-gated proton channel in green algae.* ***Science*** 296(5577):2395-8.

5- NOTES:

NOTES:

Lab #16 – Optogenetics – synaptic integration and circuit interrogation

1- Introduction

We will record from brain slices of cerebellum and/or thalamus obtained from transgenic mice expressing the light-activated cation channel ChR2 coupled to EYFP under control of the parvalbumin promoter/enhancer on the BAC transgene (Zhao et al., Nature Methods 2011) (see details in Lab #13).

2- Objectives

We will use ChR2-YFP fluorescence to locate photoactivable cells/fiber and then record light-evoked synaptic currents by varying the intensity and duration of the blue-light stimulation. Use photo-activation of ChR2-expressing neuron to generate synaptic currents/ Potentiel and interrogate neuronal circuits.

3- Methods

a) Slice preparation

Parasagittal sections of cerebellum (250 µm thick), thalamus or cortex from 5-7-week-old mice.

b) Recording solutions

Standard artificial cerebrospinal fluid (ACSF) and K-Gluconate-based internal solution

Extracellular solution	in mM
NaCl	125
KCl	2.5
$CaCl_2$	2
$MgSO_4$	1
Glucose	25
$NaHCO_3$	25
NaH_2PO_4	1.25
pH adjusted with 95% O_2/5% CO_2	7.4
Osmolarity ($mOsm.Kg^{-1}$)	~300

Intracellular solution	in mM
K-Gluconate	115
KCl	20
MgCl2	2
CaCl2	1
EGTA	10
HEPES	10
Mg-ATP	2
Na_2-GTP	0.3
Na-phosphocreatine	10
Osmolarity (mOsm.Kg-1)	~290

c) Recording protocols

- Light-triggered firing activity
    1. Patch a ChR2-YFP positive neuron in the slice.
    2. Switch to current clamp and deliver brief light pulses (5-10ms) of varying amplitude to determine the light intensity required to evoke a spike.
    3. Deliver trains of light pulses to evoke multiple spikes, at low frequency (e.g., 10 pulses of 10ms at 10Hz).
    4. Determine how fast the cell can respond by increasing the frequency (shortening the interval between successive pulses).

- Synaptic connectivity and circuit mapping
    1. Patch a neuron (molecular layer interneurons or Purkinje cells) in a cerebellum slice in Voltage-clamp mode (V_{hold} = -70 mV).
    2. Use blue light to activate ChR2 and record synaptically-evoked responses.
    3. Record from a ChR2-YFP negative neuron in **Voltage-clamp mode** (V_{hold} = -70 mV) in thalamic or cortical slices.
    4. Deliver light pulses using the parameter that evoked spikes (see above).
    5. Look for synaptic responses in the recorded neuron (light stimulation of the presynaptic neuron).

- Temporal summation
    1. In **Voltage-clamp mode**, with the parameters eliciting synaptic response
    2. Apply 5-10 light pulses at 20, 50 and 100 Hz and observe the response.
    3. Repeat the protocol in **Current-clamp mode** and determine the frequency leading to neuron spiking.

4- Tips for electrical stimulation of synaptic inputs

1. Because of incomplete recovery from desensitization, the light intensity may need to be increased to reliably obtain spikes during high-frequency trains.
2. Given that both express parvalbumin and therefore ChR2, It is worthwhile considering how to distinguish light-evoked responses (either in voltage- or current-clamp) that arise from direct activation of ChR2 expressed in the recorded cell *vs.* responses that result from light-evoked spikes in neurons that are presynaptic to the recorded neuron.
3. If you get spiking with a single pulse, reduce light intensity to get subthreshold synaptic responses.

5- <w Useful references

Xiao L, Priest MF, Kozorovitskiy Y (2018). Oxytocin functions as a spatiotemporal filter for excitatory synaptic inputs to VTA dopamine neurons. ***Elife*** 7. pii: e33892.

Xiao L, Priest MF, Nasenbeny J, Lu T, Kozorovitskiy Y (2017). *Biased oxytocinergic modulation of midbrain dopamine systems.* ***Neuron*** 95(2):368-384.e5.

Zhao S, Ting JT, Atallah HE, Qiu L, Tan J, Gloss B, Augustine GJ, Deisseroth K, Luo M, Graybiel AM, Feng G (2011). *Cell type–specific channelrhodopsin-2 transgenic mice for optogenetic dissection of neural circuitry function.* ***Nat Methods*** 8(9):745-52.

6- NOTES:

In vivo Labs (Starting from June 15th)

1- Introduction

In this laboratory, you will learn how to perform whole-cell patch-clamp *in vivo* recordings from somatosensory cortex of anesthetized mice. We will make use of a "blind patch" approach where changes in pipette resistance are used as a guide to find neurons. We will focus on superficial and deep layers of barrel cortex to record synaptic responses to whisker stimulation in vivo.

2- Objectives

The goal is to measure how sensory driven synaptic inputs are converted into action potential output, i.e. to observe synaptic integration *in vivo*.

3- Methods

a) Surgical Methods

The course instructors and assistants will perform the surgical procedures ahead of your session. Mice will undergo general anesthesia using a suitable injectable anesthetic (e.g. Ketamine/Dexdomitor, 70-100mg/kg + 0.5-1 mg/kg, respectively). A small craniotomy (500-1000 µm) will be made on top of somatosensory cortex using a dental drill. The dura mater may be removed. A stainless steel head-post will be attached to the skull to assist head fixation during electrophysiological recording. The anesthetized animal will then be transferred to the in vivo recording set-up. The animal will be head-fixed, lying on a heating mat controlling the temperature of the anesthetized animal. Ketamine + Dexdomitor will be topped up approximately hourly to maintain anesthesia.

b) Establishing whole-cell recordings

We will use a "blind patch" approach to find and record from neurons in neocortex:

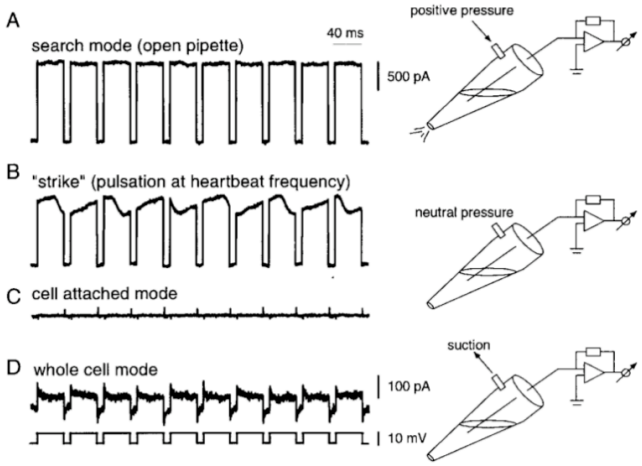


Fig. 1A–D Establishing whole-cell recordings in vivo. Once the electrode has penetrated the upper cortical layers and is positioned at the target depth, the positive pressure (>200 mbar) on the pipette is reduced to approximately 30 mbar. The electrode resistance should be monitored to ensure that the pipette has remained debris-free during the initial positioning phase (**A**). Step sizes of 2–3 μm were used to search for cells. A "strike" is apparent from the immediate and phasic shift in electrode impedance locked to the heartbeat frequency (**B**). Removing the remaining positive pressure and slowly clamping the patch to a hyperpolarized potential usually results in a gigaohm seal (**C**–**D**). The holding potential here is –70 mV and the seal resistance is approximately 10 GΩ. A slow ramp of negative pressure (up to 100 mbar) is applied to the pipette to achieve the whole-cell configuration

From Margrie TW, Brecht M, Sakmann et al., 2002.

Intracellular solution	in mM
K-Gluconate	115
KCl	20
Na2-Phosphocreatine	10
HEPES	20
Mg-ATP	2
Na-GTP	0.3
pH adjusted with KOH	7.3
Osmolarity (mOsm.Kg^{-1})	~290

c) Sensory stimulation

We will apply brief air puffs to whiskers. Air puffs will be triggered by TTL pulses that we record along with the membrane potential sweeps. We will then compute stimulus-triggered mean membrane potential responses to whisker stimulations.
Additional protocols could look at double puffs, reducing the inter puff duration as there is usually a long depression in activity after the first pulse so you can explore network wide effects on repetitive stimuli.

4- Useful References

Margrie TW, Brecht M, Sakmann B. In vivo, low-resistance, whole-cell recordings from neurons in the anaesthetized and awake mammalian brain. ***Pflugers Arch.*** 2002, 444(4):491-8

Lee D, Lee AK. In Vivo Patch-Clamp Recording in Awake Head-Fixed Rodents. ***Cold Spring Harbor Protoc***. 2017 doi:10.1101/pdb.prot095802

Lee D, Lee AK. Whole-Cell Recording in the Awake Brain. ***Cold Spring Harbor Protoc.*** 2017 doi:10.1101/pdb.top087304

5- NOTES:

Appendix

Koehler Illumination - what, why and how

Why do Koehler illumination?

Koehler Illumination is a process that provides optimum contrast and resolution by focusing and centring the light path and spreading it evenly over the field of view.

Sophisticated and well-equipped microscopes fail to yield quality images because of incorrect use of the light source. Illumination of a specimen should be bright, glare-free and evenly dispersed in the field of view.

To allow a microscope to be set up for Koehler, it must have two adjustable iris diaphragms: the aperture diaphragm at the substage condenser and the field diaphragm nearer to the lamp.

The aperture iris diaphragm controls the angular aperture of the cone of light from the condenser, while the field iris diaphragm controls the area of the circle of light illuminating the specimen.

Technical requirements

The substage condenser must be capable of being focused up and down and must be fitted with an aperture iris diaphragm that can be opened and closed by a lever or knob.

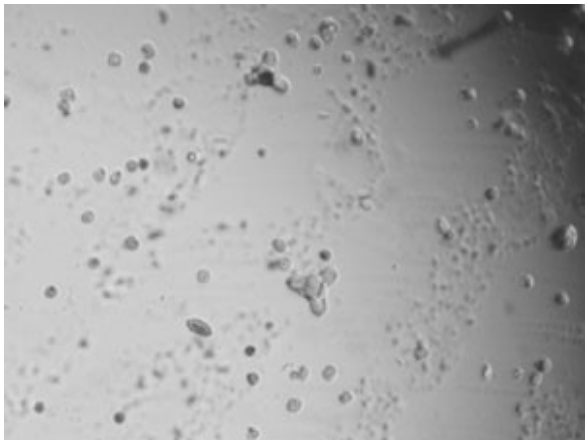
The light path must be fitted with a condensing lens, a collector lens and a field iris diaphragm that can be opened and closed.

A quick guide - 6 steps to Koehler illumination

Focusing the condenser

1) Place a thin sample on the stage and focus on it using a 4x or 10x objective.

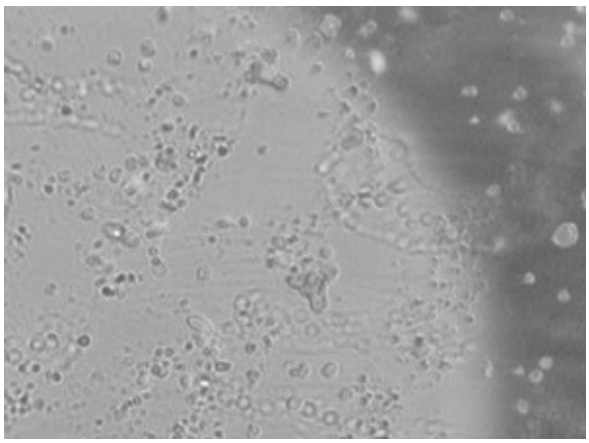
For an appropriate starting position, make sure the condenser front lens is approximately 0.5cm from the bottom of the coverslip.



2) Using the field iris diaphragm control, located on the front surface of the substage optics, close the diaphragm right down while looking at your monitor.

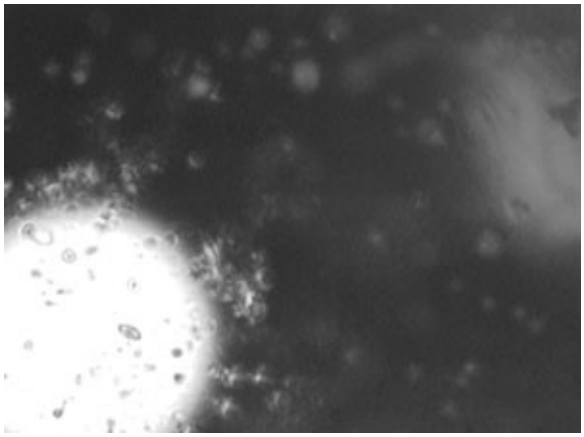
You will see a dark circle encroaching on the screen.

Note: If this dark circle does not fall within your field of view, you may need to use the two silver adjusting screws on the condenser arm to centre your condenser



3) Move the condenser up or down until the edge of the dark circle (the blades of the iris) appears in sharp focus on the monitor. Once you have positioned the condenser correctly switch the user interface back to position (I) to avoid accidental re-positioning of the condenser.

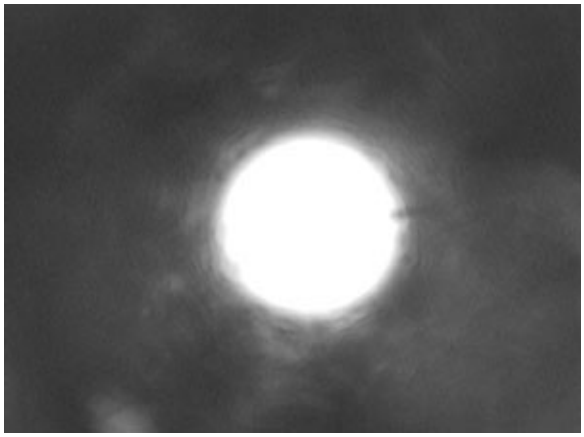
Note: Depending on the working distance of the condenser you may be in a close proximity to the sample.



Centring the condenser

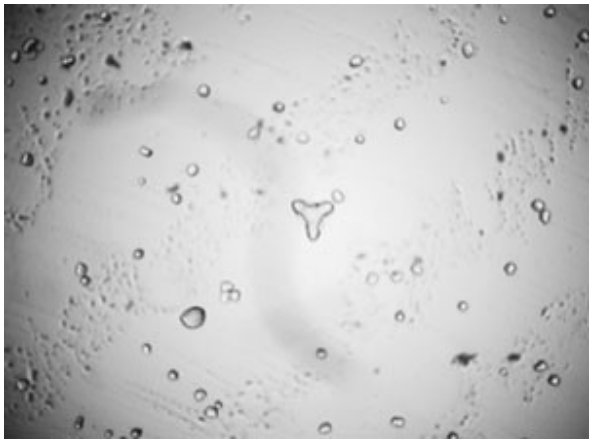
4) Located on the condenser arm are two silver adjusting screws used for centring the condenser. Turn the screws to centre the now multi-edged shape. It should now appear in the centre of the field of view.

This process is made easier in the final stages by opening the diaphragm almost to the edge of the monitor's field of view.



5) Once the condenser has been focused and centred in this way, the diaphragm can be opened so that it is just outside the field of view.

The condenser will remain centred when different objectives are selected, but the field iris diaphragm will have to be adjusted to just outside the field of view at different magnifications.



Adjusting the aperture iris

This important step is often neglected, leading to either suboptimal resolution and/or poor contrast.

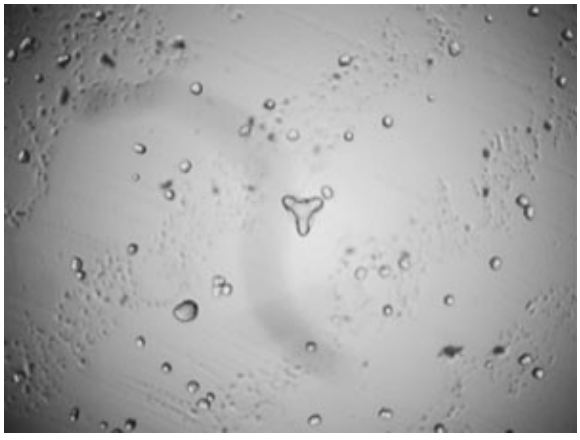
6) Locate the control for the aperture iris, which will often be a thin silver lever protruding from the condenser. With the condenser in place, focused and centred, the iris should be closed down so that it occupies the outer 20% or so of the field. This increases the contrast, making observation easier.

Although some specimens may need variation on the 20%, beware of closing the iris too much as resolution will be drastically reduced.

Although the human eye will accept sub-optimal images, once these images are captured by camera, the results will speak for themselves (take a look at some of the photomicrographs in scientific journals and judge for yourself!).

Note: A more accurate way of adjusting the aperture iris is to note the numerical aperture (or NA) on the objective, then set the NA on the condenser to 20% less.

For example, with a 40x objective with a numerical aperture value of 0.65, set the graduation on the condenser to 20% less, approximately 0.5



Take a little time to check your microscope before each use will be well worth the effort.

Need further help or advice?

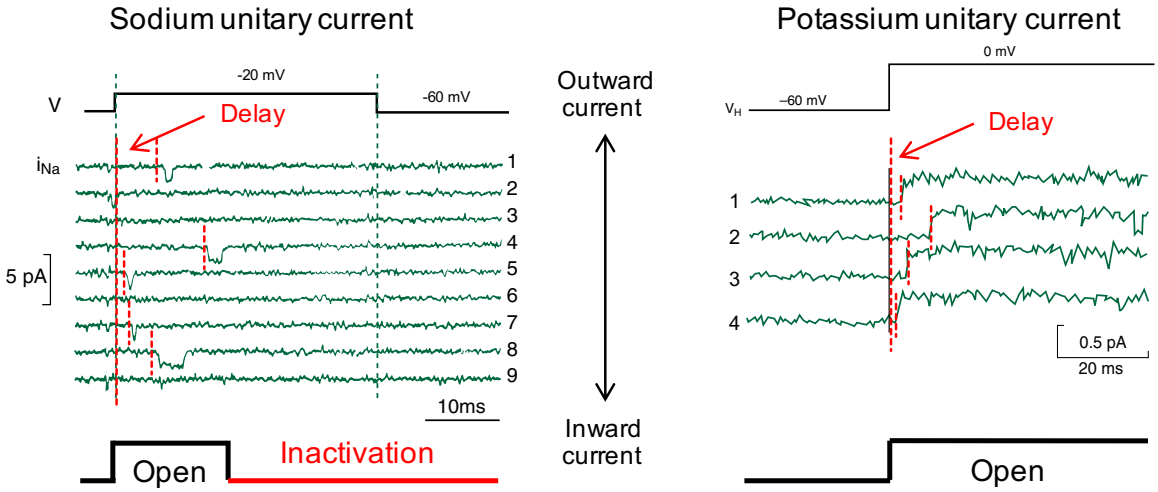
If you would like further assistance we have a wealth of experts at Scientifica who can help.

From unitary current to macroscopic ones

1) Properties of unitary current & current-voltage relationship

A

- Current activity at the single channel level is a stochastic/probabilistic phenomenon
- Every type of channel has a peculiar opening/closing pattern defined by the intrinsic properties of the protein (delay of the first opening, opening synchronicity, persistent vs. inactivation…)

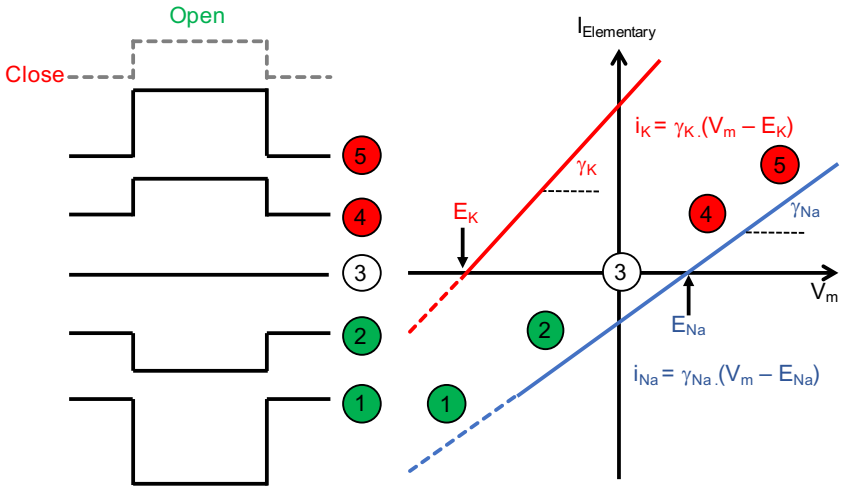


Sodium current:
Inward current with a short first opening delay followed by a short opening event before inactivation. Note that although the traces show 9 consecutive recordings of the same channel, the pattern of opening differs from trace to trace.

Potassium current:
Outward current with a longer first opening delay followed by a long lasting opening event without inactivation.

B

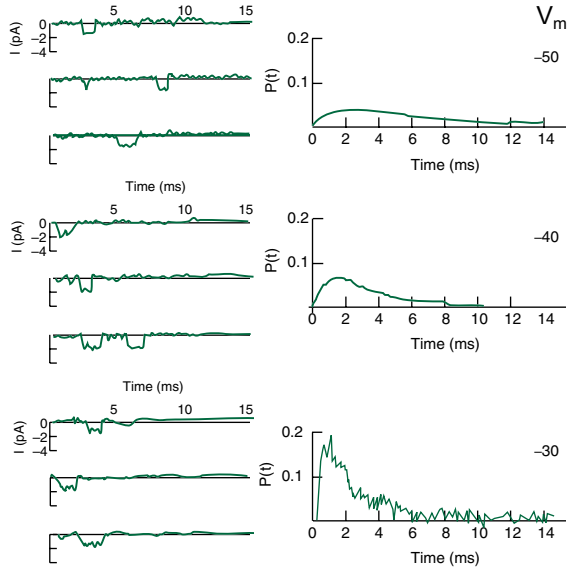
- For a given channel, it is either opened or closed and when opened its conductance is equal to the unitary channel conductance (g), a constant.
- The I-V relationship is defined by the current equation (see below) which is linear because it represents a linear regression function (y = ax +b) with a = g, the slope.
- In these conditions, the current amplitude ONLY depends on the driving force (Df, $V_m - E_{ion}$) as depicted below. When Df is negative, the current is inward and negative (1, 2) and when Df is positive, the current is outward and positive (4, 5). At the current reversal potential (3), the current is zero although the channel is opened.



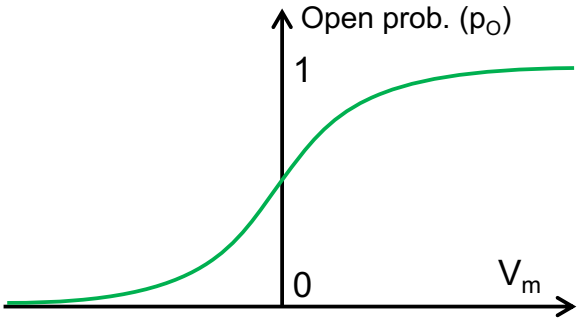
2) Channel voltage-dependence from single to macroscopic currents

A

- At the single channel level, the voltage-dependence is defined by the increase in open probability (p_O) as a function of voltage *i.e.* the more the membrane potential is depolarized the more the channel is found in its open configuration (see below, left).
- Depending on the channel properties, there is a 'threshold potential' for activation where the channel open probability becomes significantly high enough to see opening events in the course of the experiment.
- In any case, a channel can open at any potential it is just that at a hyperpolarized membrane potential the open probability is very low and you will have to get a couple of coffee breaks before seeing one event.
- At full activation, the open probability can reach the value of 1 but often, because of inactivation mechanisms, it is lower.
- Many channels get activated upon depolarization BUT there are also channels activated by hyperpolarization (e.g. I_h current carried by HCN channels)



Voltage-dependence of the sodium channel



Voltage-dependence of channel open probability

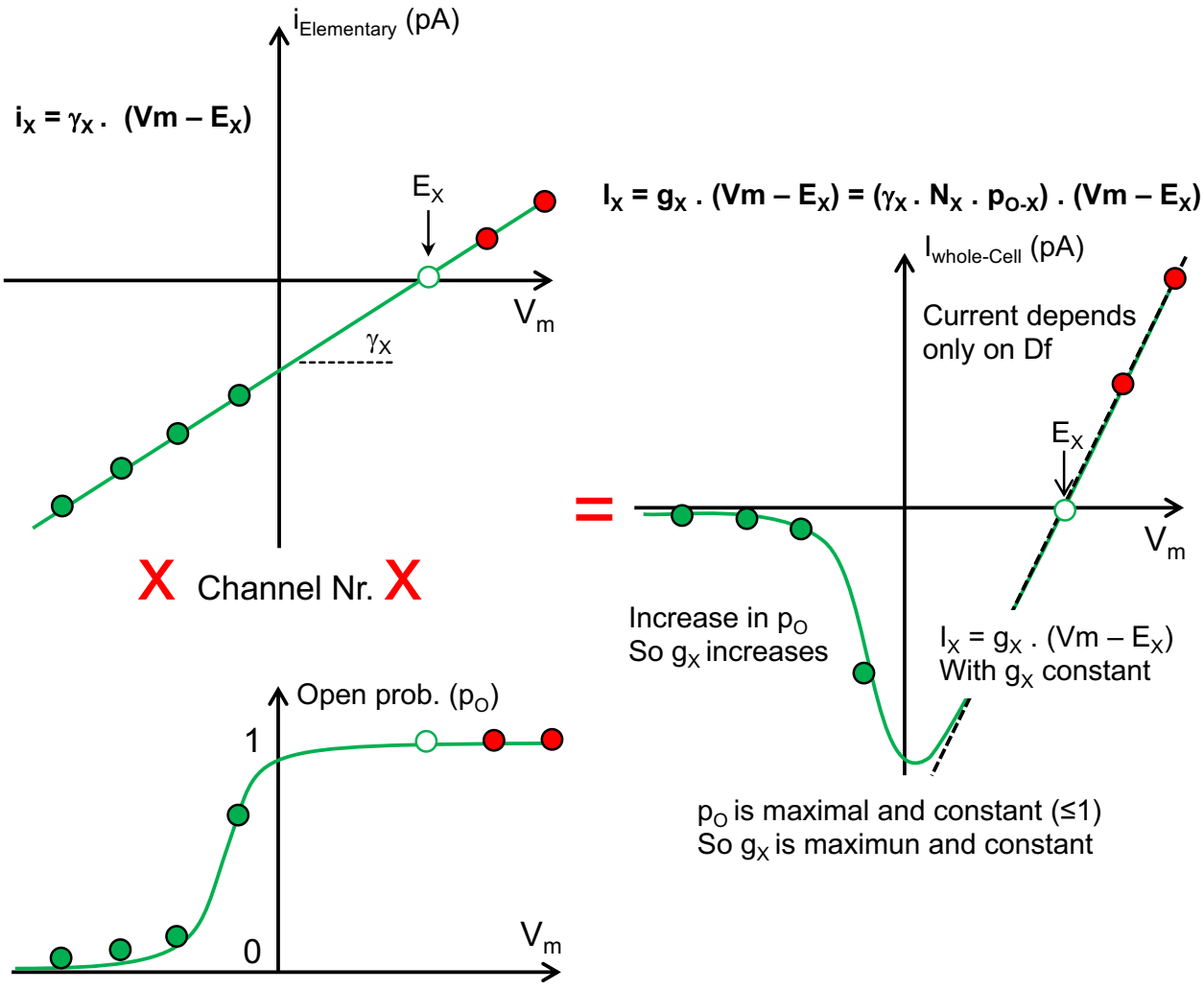
B

- At the macroscopic level, the current (I_X) recorded in whole-cell is the result of the activation of several channels (N_X) each behaving as described before and defined by their unitary conductance (γ_X) and their voltage-dependent open probability ($p_{O\text{-}X}$). Therefore:

$$I_X = g_X \,.\, (Vm - E_X) \text{ with } g_X = \gamma_X \,.\, N_X \,.\, p_{O\text{-}X}$$

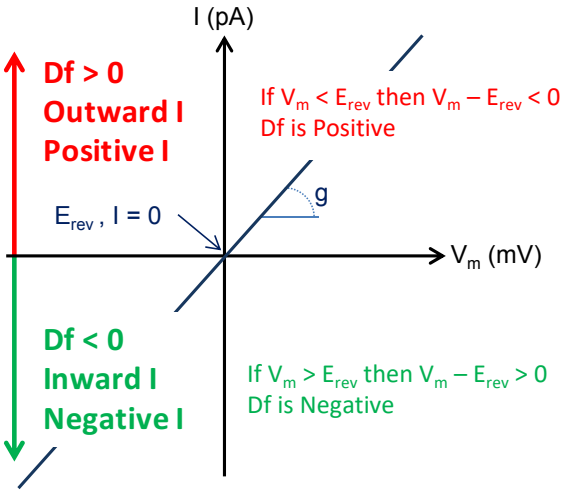
  where the whole-cell conductance (g_X) is the product of the elementary channel conductance (γ_X) and of the number of channels expressed in the neuron (N_X).
- Before the threshold potential, $p_{O\text{-}X}$ is very low or equal to 0 so g_X is equal to zero
  With the increase in $p_{O\text{-}X}$, g_X increases until the open probability gets maximal and constant (equal to 1 or lower, plateau on the graph above).
- Typically $g_X = \gamma_X \,.\, N_X$ and the I-V relationship becomes linear. The current amplitude depends only on Df.

Graphically, one can summarize the current voltage-dependance as follow:



Making sense of a Current

Current-Voltage Relationship, Current direction



Current equation

$$I = g (V_m - E_{rev})$$

I, Current (pA)
g Channel Conductance (pS, macroscopic)
V_m, Membrane potential (mV)
E_{rev}, Current reversal potential = Equilibrium potential (E_{ion}, from Nernst equation) (mV)

$V_m - E_{rev}$ = Driving force (Df)

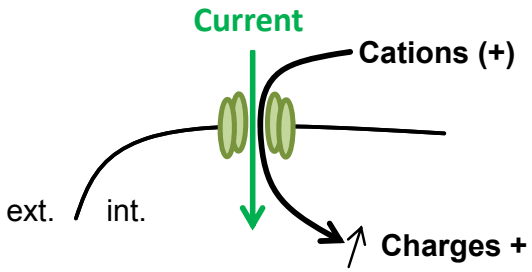
Whatever the charge of an ion carried by a given Current, the direction of this current will **ONLY** depend on the sign of Df.

(see lecture for I equation determination)

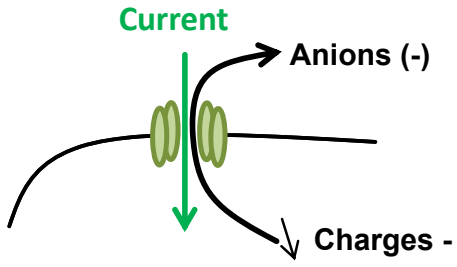
!!! As a rule Cations flow in the direction of the current, Anion flow in the opposite direction

INWARD I = NEGATIVE I
$V_m - Er_{ev} < 0$ and Df is Negative
Current will induce a **Depolarization**

1.1 – Current carried by **Cations**



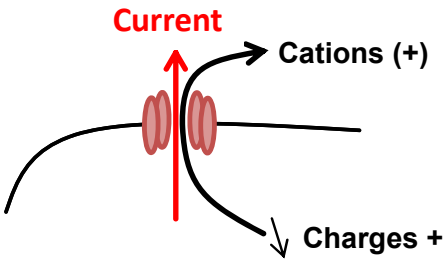
1.2 – Current carried by **Anions**



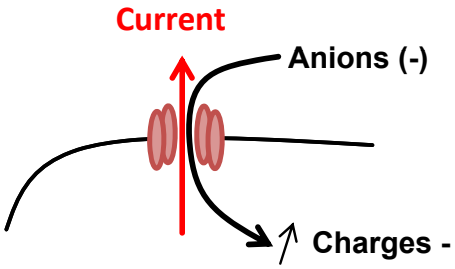
Intracellular **increase/accumulation** of **Positive** charges (1.1) or **decrease/loss** of **Negative** charges (1.2)
The **intracellular space** becomes **more positive** compare to the extracellular space: there is a **Depolarization of V_m**

OUTWARD I = POSITIVE I
$V_m - Er_{ev} > 0$ and Df is POSITIVE
Current will induce a **Hyperpolarization**

2.1 – Current carried by **Cations**



2.2 – Current carried by **Anions**



Intracellular **decrease/loss** of **Positive** charges (2.1) or **increase/accumulation** of **Negative** charges (1.2)
The **intracellular space** becomes **more negative** compare to the extracellular space: There is an **Hyperpolarization of V_m**