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# Title: Dynamic Clamp Methods to Investigate Impaired Neuronal Excitability Associated with Autism

**Authors and Affiliations:**
**Samuel P. Brown, Joseph L. Ransdell**

**Department of Biology, Miami University**

**Corresponding Authors:**
Joseph L. Ransdell (ransdejl@miamioh.edu)

**Email Addresses for All Authors:**
Samuel P. Brown (brownsp2@miamioh.edu)
Joseph L. Ransdell (ransdejl@miamioh.edu)

# Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? Yes

   **Yes, all done**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, all done**

3. **Filming location:** Will the filming need to take place in multiple locations? **no**

4. **Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **no**

**Current Protocol Length**
Number of Steps: 25
Number of Shots: 59

# Introduction

***Videographer: Obtain headshots for all authors available at the filming location.***

**INTRODUCTION:**

1.1. **Joey Ransdell:** Our lab studies how ion channels and ion channel regulation control the electrical activity of neurons in the central nervous system.
   1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: Figure 3*

1.2. **Joey Ransdell:** Because ion channel function is typically characterized using voltage-clamp methods, it is difficult to directly link a change in ion channel function with an effect on neuronal action potential firing.
   1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

**CONCLUSION:**

1.3. **Joey Ransdell:** The TSC1 gene, implicated in tuberous sclerosis and autism spectrum disorders, when selectively deleted in mouse cerebellar Purkinje neurons, leads to impaired action potential firing and reduced Nav channel expression at axon initial segments.
   1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: figure 2*

1.4. **Joey Ransdell:** Using dynamic clamp electrophysiology, we can directly test if the loss of Nav channels in Tsc1 mutant Purkinje neurons causes reduced action potential firing of these cells.
   1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 3.8.1., 3.8.2*

1.5. **Joey Ransdell:** Dynamic clamp methods can be used to directly test how changes or impairments in ion channel function affect the electrical outputs of neurons.
   1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.12.1.*

***Videographer: Obtain headshots for all authors available at the filming location.***

**Ethics Title Card**

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at Miami University

# Protocol

NOTE: LAB MEDIA/SCREEN/SCOPE timestamps for protocol were added at the postshoot stage. Please contact the postshoot note integrator (Sulakshana Karkala) for queries regarding lab media.

## 2. Configuring a Markov-based Conductance Model in Dynamic Clamp

**Demonstrator:** Samuel Brown

2.1. To begin, open the Dynamic Clamp Editor window in the data acquisition software **[1]** by selecting the **SutterPatch** *(Sutter-Patch)* tab and choosing **Dynamic Clamp Editor [1]**.

2.1.1. WIDE: Talent seated at the workstation.

2.1.2. SCREEN: 2.1.2.mp4 00:00-00:08

2.2. In the Conductance Pool interface, click **New** to create a pool and assign a name to it **[1]**. On the right side of the window, in the Headstage selector interface, select the model type for the conductance pool **[2]**. Choose when the model conductance should be applied, for example, during a routine or protocol **[3]**. In the same interface, set the **Update Rate** and select the **Voltage Signal Source** by choosing the headstage that is recording the voltage signal **[4]**.

2.2.1. SCREEN: 2.2.1.mp4 00:01-00:08

2.2.2. SCREEN: 2.2.2.mp4 00:00-00:02
*Video Editor: Please freeze frame here*

2.2.3. SCREEN: 2.2.3.mp4 00:00-00:06

2.2.4. SCREEN: 2.2.4.mp4 00:00-00:10

2.3. In the Headstage selector panel, for the Nav conductance Markov model, select **Markov Model** under **Model**, **During Sweeps** under **Active Mode**, **200 kilohertz** under **Update Rate**, and **Headstage 1** under **Voltage Signal Source [1]**. Check the boxes for **Apply** and **Command Signal to AuxOUT1** *(Command-Signal-to-Aux-OUT-One)* to begin model calculations ~~based on the voltage signal and enable dynamic clamp current injection for current-clamp protocols~~ **[2]**.

2.3.1. SCREEN: 2.3.1.mp4 00:00-00:10

2.3.2. SCREEN: 2.3.2.mp4 00:00-00:04
*Video Editor: Please freeze frame here*

2.4. Then, click **Edit Model Parameters** in the Headstage selector interface to access the state matrix editor **[1]**. ~~In the newly opened **Dynamic Clamp Markov Model Parameters** window, enter the state matrix equations corresponding to the Markov model conductance **[2].**~~

2.4.1. SCREEN: 2.4.1.mp4 00:00-00:08

~~2.4.2. SCREEN: Display the Dynamic Clamp Markov Model Parameters window. Type the state matrix equations into the appropriate fields.~~

**AUTHOR'S NOTE: Skip 2.4.2**

2.5. ~~In the **Channel Settings** panel, select the saved conductance models to be applied for the dynamic clamp **[1-TXT].**~~ On the right side of the **Channel Settings** panel, enter 55 millivolts as the **V reversal** potential ~~to reflect the sodium conductance value based on the artificial cerebrospinal fluid and the patch-electrode internal solution~~ **[2]**.

2.5.1. ~~SCREEN: Cursor selecting a saved conductance model from the Channel Settings panel. **TXT: Alternatively, select an empty channel to create a new conductance model**~~
**AUTHOR' S NOTE: There are no "saved conductance models", there is just a default channel (Channel 1)**

2.5.2. SCREEN: 2.5.2.mp4 00:00-00:04

2.6. In the same ~~Channel Settings~~ panel, enter the number of kinetic states for the selected conductance model **[1]**.

2.6.1. SCREEN: 2.6.1.mp4 00:00-00:06

2.7. In the **State Equations** panel, input the state transition rate constant equations into the **gating state matrix** to match the topological structure of the Markov model. ~~Assign each equation an ID starting from "S0" *(S-Zero)* **[1-TXT]**~~. Click **Edit State Matrix** on the right side of the panel to populate the matrix with the defined rate constant variables **[2]**.

2.7.1. SCREEN: 2.7.1.mp4 00:00-00:06
**TXT: Assign each equation an ID starting from S0**

2.7.2. SCREEN: 2.7.2.mp4 00:00-00:08

2.8. In the **Conductance Equations** panel, define the amplitude of the model conductance applied during dynamic clamp **[1-TXT]**. ~~Select the open or conducting states in the Markov model and assign a conductance value to them **[2].**~~

2.8.1. SCREEN:2.8.1.mp4 00:00-00:03

**TXT: Choose open or conducting states and assign a conductance value**

2.8.2. ~~SCREEN: Talent selecting the appropriate conducting states and entering a conductance value.~~
**AUTHOR'S NOTE: Combined with 2.8.1**

2.9. Enter 400 nanosiemens into the G7 box ~~to assign a peak Nav current amplitude consistent with prior voltage-clamp data from mouse Purkinje neurons~~ **[1]**.

2.9.1. SCREEN: 2.8.1.mp4 00:03-00:08

2.10. Once all model parameters are set, click **Load** to prepare the model for dynamic clamp application when a current-clamp routine is started **[1]**.

2.10.1. SCREEN: 2.10.1.mp4 00:00-00:08

2.11. To apply the dynamic clamp conductance, create a current-clamp routine using the **Routine Editor [1]**. In the Routine Editor, choose the **Parent Output Channel** to open the configuration panel **[2]**. Set the Recording Mode to **CC_Mode** *(C-C-Mode)* to enable current-clamp operation **[3]**.

2.11.1. SCREEN: 2.11.1.mp4 00:00-00:12

2.11.2. SCREEN: 2.11.2.mp4 00:00-00:04

2.11.3. SCREEN: 2.11.2.mp4 00:05-00:08

2.12. In the **Routine Editor Input Channels**, define the input signals to be recorded during the experiment **[1]**. Include the voltage signal, the routine's command current injection labeled **Current1**, and the dynamic clamp current injection labeled **AuxIN1** *(Aux-In-One)* **[2]**.

2.12.1. SCREEN: 2.12.1.mp4 00:00-00:03

2.12.2. SCREEN: 2.12.1.mp4 00:04-00:17

## 3. Acquiring Current-Clamp Recordings from Mouse Purkinje Neurons in Parasagittal Cerebellar Slices

3.1. Equip the electrophysiology rig with a tissue slice chamber perfused with artificial cerebrospinal fluid warmed to 34–36 degrees Celsius **[1]**. Carefully place a parasagittal cerebellar slice into the chamber and use a slice harp to secure it in a fixed, submerged position **[2]**. Submerge a chlorided ground electrode and a temperature probe into the chamber and confirm they are stable **[3]**.

3.1.1. WIDE: Talent setting up the tissue slice chamber and confirming fluid perfusion.

3.1.2. Talent placing the cerebellar slice into the chamber and positioning the slice harp.

3.1.3. Talent inserting and adjusting the chlorided ground electrode and temperature probe.

3.2. Then, locate the Purkinje neuron layer using a 40x *(Forty-Axe)* immersion objective lens **[1]**.

3.2.1. SCOPE: 3.2.1-3.3.2; 3.7.2-3.8.2.mp4 00:00-00:01

3.3. Once a healthy Purkinje neuron is identified for patch-clamp recording, raise the microscope objective lens **[1]**. Bring the tip of a glass microelectrode, mounted on an electrode holder connected to the headstage, into focus **[2]**. Control the microelectrode using a 3-axis robotic micromanipulator **[3]**.

3.3.1. SCOPE: 3.2.1-3.3.2; 3.7.2-3.8.2.mp4. 00:01-00:05

3.3.2. SCOPE: 3.2.1-3.3.2; 3.7.2-3.8.2.mp4. 00:06-00:15

3.3.3. Talent using the micromanipulator controls to move the microelectrode in 3 axes.

3.4. Next, add 2 to 3 milliliters of positive air pressure to the electrode holder pressure port before advancing toward the Purkinje neuron **[1]**.

3.4.1. Talent injecting positive air pressure into the electrode holder using a syringe or pressure control system.

3.5. Switch the amplifier to VC-mode using the amplifier control panel **[1]**. Click **Auto** next to the electrode compensation and voltage offset options to apply automatic settings **[2]**.

3.5.1. SCREEN: 3.5.1.mp4 00:00-00:08

3.5.2. SCREEN: 3.5.2.mp4. 00:00-00:07

3.6. Then, start a continuous membrane seal test protocol. Set the sweep duration to 5 milliseconds and apply a voltage step of either 5 or 10 millivolts **[1]**. Set the holding potential to 0 millivolts **[2]**.

3.6.1. SCREEN: 3.6.1.mp4 00:00-00:16

3.6.2. SCREEN: 3.6.2.mp4 00:00-00:10

3.7. Now, advance the microelectrode toward the target Purkinje neuron while adjusting the focal plane to stay aligned with or slightly below the electrode tip **[1]**. Position the microelectrode slightly above the neuron's plasma membrane **[2]**. Use the micromanipulator and the positive pressure at the electrode tip to displace extracellular debris, cleaning the surface of the cell soma **[3]**.

3.7.1. Talent maneuvering the electrode toward the neuron using the micromanipulator while adjusting the microscope focus.

3.7.2. SCOPE: 3.2.1-3.3.2; 3.7.2-3.8.2.mp4. 00:16-00:23

3.7.3. SCOPE: 3.2.1-3.3.2; 3.7.2-3.8.2.mp4. 00:42-00:53

3.8. Once the target neuron is cleared of extracellular debris, bring the microelectrode tip close to the membrane surface at the broad end of the Purkinje neuron soma ~~where the axon extends from the cell body~~ **[1]**. As the tip contacts the membrane, observe for the formation of a dimple due to the applied positive pressure **[2]**. At that point, release the positive pressure and apply a small amount of negative pressure using mouth suction **[3]**.

3.8.1. SCOPE: 3.2.1-3.3.2; 3.7.2-3.8.2.mp4. 00:53-00:56

3.8.2. SCOPE: 3.2.1-3.3.2; 3.7.2-3.8.2.mp4. 00:56-01:02

3.8.3. SCOPE: 3.2.1-3.3.2; 3.7.2-3.8.2.mp4. 01:03-end

3.9. On the **Amplifier Control** panel, change the **V-holding potential** to minus 80 millivolts **[1]**. ~~While monitoring the membrane seal test,~~ When the pipette resistance reaches 1 gigaohm or higher, apply additional negative pressure and click **Do Zap** ~~in the Membrane Seal Test panel~~ to rupture the membrane ~~and establish whole-cell configuration~~ **[2]**.

3.9.1. SCREEN: 3.9.1.mp4 00:00-00:08

3.9.2. SCREEN: 3.9.2.mp4 00:00-00:08

3.10. After switching the system to **current-clamp mode**, apply bridge balance compensation to at least 70 percent **[1]**.

3.10.1. SCREEN: 3.10.1.mp4 00:00-00:08

3.11. Then, apply the modeled conductance by clicking **Load** in the **Dynamic Clamp** settings window **[1]**. A small **DynC** *(Dyn-C)* label will appear on the dPatch controller, overlaid on **CC** *(C-C)* to confirm activation **[2]**.

3.11.1. SCREEN: 3.11.1.mp4 00:00-00:08.

3.11.2. SCREEN: 3.11.1.mp4 00:09-00:12

3.12. While the current-clamp protocol is running, observe the dynamic clamp current injection on the scope window that corresponds to the **AuxIN1** input signal **[1]**.

3.12.1. LAB MEDIA: 3.12.1.png

# Results

## 4. Results

4.1. Spontaneous firing frequency **[1]** and the amplitude of voltage-gated sodium currents are significantly reduced in Tsc1 *(T-S-C-One)* mutant Purkinje neurons compared to wild-type controls **[2].**

4.1.1. LAB MEDIA: Figure 2A and B. *Video editor: Highlight the blue traces and squares for Tsc1mut*

4.1.2. LAB MEDIA: Figure 2C. *Video editor: Highlight the blue curve*

4.2. Using dynamic clamp, we found that adding 400 nanosiemens of a modeled Nav conductance significantly increases and partially rescues the spontaneous firing frequency of *Tsc1* mutant Purkinje neurons **[1].**

4.2.1. LAB MEDIA: Figure 3 C1 and D1**.** *Video editor: Highlight the lower traces labeled as "400ns dynamic clamp" in 3C1 and connected black and gray points showing an upward trend in firing frequency in 4D1*.

4.3. Alternatively, subtracting 400 nanosiemens of modeled Nav conductance from wild-type Purkinje neurons significantly reduces spontaneous firing frequencies, mimicking the deficits previously identified in Tsc1 mutant Purkinje neurons **[1].**

4.3.1. LAB MEDIA: Figure 3 C2 and D2**.** *Video editor: Highlight the lower traces labeled as "400ns dynamic clamp" in 3C2 and connected black and gray points showing an upward trend in firing frequency in 4D2*.

**Pronunciation Guide:**
**Tuberous**
**Pronunciation link:** https://www.merriam-webster.com/dictionary/tuberous merriam-webster.com
**IPA: /ˈtuːbərəs/**
**Phonetic Spelling: TOO-buh-rus**
**Sclerosis**
**Pronunciation link:** https://www.merriam-webster.com/dictionary/sclerosis merriam-webster.com
**IPA: /sklə-ˈroʊsɪs/**
**Phonetic Spelling: skleh-ROH-sis**
**Purkinje**
**Pronunciation link:** https://www.merriam-webster.com/medical/purkinje merriam-webster.com
**IPA: /pərˈkɪn dʒi/**
**Phonetic Spelling: per-KIN-jee**
**Neuron**
**Pronunciation link:** https://dictionary.cambridge.org/us/pronunciation/english/neuron **(not cited but reliable)**
**IPA: /ˈnʊɑn/ or /ˈnjʊɑn/**
**Phonetic Spelling: NUR-ahn**
**Electrophysiology**
**Pronunciation link:** https://www.merriam-webster.com/dictionary/electrophysiology merriam-webster.com
**IPA: /i-ˌlɛk-trō-ˌfɪ-zē-ˈäl-ə-jē/**
**Phonetic Spelling: ee-LEK-troh-fy-zee-AH-luh-jee**
**Conductance**
**Pronunciation link:** https://www.merriam-webster.com/dictionary/conductance merriam-webster.com+1
**IPA: /kənˈdʌktəns/**
**Phonetic Spelling: kun-DUK-tans**
**Markov**
**Pronunciation link:** https://www.howtopronounce.com/markov howtopronounce.com
**IPA: /ˈmɑːrkɒf/ (in US: /ˈmɑːrkɔːf/)**
**Phonetic Spelling: MAR-kof**
**Dynamic (as in dynamic clamp)**
**Pronunciation link:** https://www.merriam-webster.com/dictionary/dynamic merriam-webster.com
**IPA: /daɪˈnæmɪk/**
**Phonetic Spelling: dy-NAM-ik**
**Clamp (as in dynamic clamp)**
**Pronunciation link:** https://www.merriam-webster.com/dictionary/clamp merriam-webster.com

**IPA: /klæmp/**
**Phonetic Spelling: klamp**
**Axon**
**Pronunciation link: https://www.merriam-webster.com/dictionary/axon merriam-webster.com**
**IPA: /ˈæksɑn/**
**Phonetic Spelling: AK-son**
**Voltage**
**Pronunciation link: https://www.merriam-webster.com/dictionary/voltage merriam-webster.com**
**IPA: /ˈvoʊltɪdʒ/**
**Phonetic Spelling: VOL-tij**
**Patch-clamp**
**Pronunciation link: (Compound word — patch and clamp individually: patch: https://www.merriam-webster.com/dictionary/patch merriam-webster.com ; clamp above)**
**IPA: /ˈpætʃ - klæmp/**
**Phonetic Spelling: PATCH-klamp**