FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 67833
Scriptwriter Name: Debopriya Sadhukhan
Project Page Link: https://review.jove.com/account/file-uploader?src=20689113

Title: Identification and Classification of Position-specific $GABA_A$ Receptor Subunit Missense Variants for Their Role In Hippocampal Pyramidal Neurons

Authors and Affiliations:

Ayla Arslan, Pınar Öz

Department of Molecular Biology and Genetics, Faculty of Engineering and Natural Sciences, Üsküdar University

Corresponding Authors:

Ayla Arslan ayla.arslan@alumni.uni-heidelberg.de

Email Addresses for All Authors:

Ayla Arslan **ayla.arslan@alumni.uni-heidelberg.de, aylaarslan@yahoo.com**
Pınar Öz **pinar.oz@uskudar.edu.tr**

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?

No.

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

Yes.

3. Filming location: Will the filming need to take place in multiple locations? **Yes**

How far apart are the locations? **Approximately 500 meters.**

Current Protocol Length
Number of Steps: 13
Number of Shots: 23

Introduction

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

REQUIRED:

1.1. **Ayla Arslan:** Our study is based on the idea that epileptogenic and proximal predicted mutations in GABA(A) receptor subunits may similarly affect CA1 pyramidal neuron model. We explore this through multiscale framework.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: LAB MEDIA: Figure 11.*

What are the current experimental challenges?

1.2. **Ayla Arslan:** Laboratory experiments are essential for discovering the truth, but they can't fully capture life's diversity and complexity at all scales —from molecules to organisms. There are just too many possibilities. That's the problem. NOTE: This statement was slightly edited during the shoot.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

How will your findings advance research in your field?

1.3. **Pınar Öz:** Our protocol and findings pave the way for a computational setting that can be used for exploring the impact of polymorphisms on neural function.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.1.*

What new scientific questions have your results paved the way for?

1.4. **Ayla Arslan:** Our study raises new questions regarding the extent to which predicted pathogenic variants and epileptogenic mutations show similar properties and how these relationships can be effectively captured and simulated.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What research questions will your laboratory focus on in the future?

1.5. **Pınar Öz:** Our current focus is on the EC-hippocampus microcircuit function. We will pursue in vivo animal models and computational microcircuit models to explore septal control on this circuitry in neuropsychiatric disorders.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

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

Protocol

2. Variant Data Organization

Demonstrator: Ayla Arslan or Pınar Öz Author's NOTE: To complement the software footage recordings, the videographer captured a shot of Demonstrator Ayla Arslan working at the computer, as originally planned in the script. Additionally, a shot of the second author, Pınar Öz, was recorded as supplementary material. **JoVE may choose either of the authors to demonstrate the software. In general one Demonstrator is enough for all software footage recordings.**

2.1. To begin, open the original Excel file containing the genetic data **[1]** and remove the unnecessary columns from the file, leaving only four columns **GRCh38Chromosome** *(G-R-CH-thirty-eight-chromosome)*, **GRCh38Location** *(G-R-CH-thirty-eight-Location)*, **Name**, and **Protein Change** before saving the file as data1.xlsx *(data 1 X-L-S-X)* under the working directory relevant to R software **[2]**.

2.1.1. An over-the-shoulder shot whilst the talent works on the Excel file. Videographer's NOTE: This shot is 67833_Prot_Ext_1, Slate: 2.1.1-E.

Videographer's NOTE: Two extra shots were taken whilst the talent Pınar Öz was working on the coding terminal -> -A wide shot. Video name 67833_Prot_Ext_2, Slate: 3.E.1. And over-the-shoulder shot whilst the talent works on the Excel file. Video name 67833_Prot_Ext_3, Slate: 3.E.2.

NOTE: Use any one of the shots where the talent is opening an Excel file. **Make sure to use the correct demonstrator's name in the protocol title accordingly.**

2.1.2. SCREEN: 67833_1.2.1.mp4 00:02-00:33.

2.2. For further data cleaning and formatting, open R software and R Studio **[1]**. Then, open the R script "Data_GABAA.R" *(data gaba A R)* **[2]**. Set the working directory **[3]** and load the necessary libraries by clicking the **Run** button **[4]**. Load the data file **[5]** and start data cleaning for columns that require this process. Further clean and combine the data in one column, separated by a single space. Create a new data frame for the combined output and add the desired ENSEMBLE *(Ensemble)* transcript variant ID *(I-D)* **[6]**. Write the result to a new Excel file called data1_output.xlsx *(Data 1 output)* **[7].**

2.2.1. SCREEN: 67833_1.2.2.mp4 00:02-00:07.

2.2.2. SCREEN: 67833_1.2.2.mp4 00:08-00:16.

2.2.3. SCREEN: 67833_1.2.2.mp4 00:20-00:29. *Video Editor: Emphasize the line the talent is selecting and "C:/Users/user/Desktop/GABAA_data".*

2.2.4. SCREEN: 67833_1.2.2.mp4 00:30-00:37.

2.2.5. SCREEN: 67833_1.2.2.mp4 00:38-00:40.

2.2.6. SCREEN: 67833_1.2.2.mp4 00:44-01:25.

2.2.7. SCREEN: 67833_1.2.2.mp4 01:26-01:42.

3. Synapse and Neuron Model Construction

3.1. To build a biophysical model of a GABAergic *(Gaba-ergic)* synapse on a multi-compartmental conductance-based hippocampal pyramidal neuron, install Brian2 *(Brian-two)* and import the required packages **[1]**.

3.1.1. SCREEN: 67833_2.3_2.7.3.mp4 00:00-00:21.

3.2. Design the conductance-based model by defining ion channel gating kinetics, passive and active parameters, and postsynaptic conductances **[1]**. Use the modified Hodgkin-Huxley type conductances for hippocampal pyramidal neurons. Adjust the density distribution of voltage-gated Na+ *(sodium)* channels for soma, axon initial segment, nodes of Ranvier, and dendrites. Set Na *(sodium)* and K *(potassium)* channel conductances as 0 *(zero)* in myelinated segments **[2]**.

3.2.1. SCREEN: 67833_2.3_2.7.3.mp4 00:22-00:48.

3.2.2. SCREEN: 67833_2.3_2.7.3.mp4 00:49-00:55.

3.3. Build ion channel gating kinetics for voltage-gated Na *(sodium)* and K *(potassium)* channels **[1]**.

3.3.1. SCREEN: 67833_2.3_2.7.3.mp4 00:44-00:48.

3.4. Introduce synaptic currents as the summation of all glutamatergic and GABAergic synapses in a compartment. Include both fast AMPA *(A-M-P-A)* receptor-mediated current and slow NMDA *(N-M-D-A)* receptor-mediated current in the glutamatergic current. Include only fast GABAA receptor-mediated current in GABAergic current. Assume that a constant amount of glutamate is released to the synapse for every presynaptic spike; therefore, the activation of receptors is **s_AMPA and s_NMDA** *(spike-time-dependent),* and the total receptor conductances, g_AMPA *(G-A-M-P-A)* and g_NMDA, reflect the amount of glutamate that is released by every event **[1]**.

3.4.1. SCREEN: 67833_2.3_2.7.3.mp4 00:56-01:14.

3.5. Set the morphological parameters by using the experimentally measured diameter for soma and neurites and the length of each neurite compartment and branching patterns. Reduce the real neuron morphology into a multi-compartmental model by

dividing the cell into multiple compartments that accurately preserve the main branching structure and maintain bilateral symmetry **[1]**.

3.5.1. SCREEN: 67833_2.3_2.7.3.mp4 01:15-01:38.

3.6. Determine the biophysical parameters for the GABAergic synapse model by evaluating the wild-type control measurements obtained previously and importing them to use in the model **[1]**. Define rise and deactivation time constants for GABA-A receptor-mediated postsynaptic current **[2]**.

3.6.1. SCREEN: 67833_2.3_2.7.3.mp4 01:50-02:12.
3.6.2. SCREEN: 67833_2.3_2.7.3.mp4 02:13-02:54.

3.7. Design the topology of the neuron model and assign morphological and biophysical parameters, which include specifying the spatial arrangement and interconnections of the compartments, based on the previously obtained morphological and branching information. Assign the appropriate morphological parameters, such as, segment length and diameter, and biophysical parameters to each compartment of the model **[1]**.

3.7.1. SCREEN: 67833_2.3_2.7.3.mp4 02:55-04:07.

3.8. Create the presynaptic activity using SpikeGeneratorGroup **[1]**. Connect the spike generator to the target compartment of the model neuron using the Synapses class to model synaptic connections **[2]**.

3.8.1. SCREEN: 67833_2.3_2.7.3.mp4 04:10-04:33.
3.8.2. SCREEN: 67833_2.3_2.7.3.mp4 04:33-04:47.

3.9. Set a sustained constant current of 0.85 nanoampere and place the electrode at the soma to mimic the subthreshold activity driven by baseline ionic current load at a given time **[1]**.

3.9.1. SCREEN: 67833_2.3_2.7.3.mp4 04:55-05:09.

3.10. To build recording monitors, record voltage traces from target compartments using StateMonitor **[1]**.

3.10.1. SCREEN: 67833_2.3_2.7.3.mp4 05:10-05:13.

3.11. Finally, build the network and run it **[1]**.

3.11.1. SCREEN: 67833_2.3_2.7.3.mp4 05:14-end.

Results

4. Results

4.1. Neuronal spike trains under single distal glutamatergic input and somatic GABAergic inhibition revealed the firing outcomes of wild-type and mutant GABAA receptors **[1]**.

4.1.1. LAB MEDIA: Figure 11. *Video Editor: Highlight the top plot.*

4.2. The β3N110D *(Beta-three-N-one-one-zero-D)* mutation impaired inhibition, causing neuron firing to lock onto the excitatory GluS1 *(Glu-S-one)* input after the 4th presynaptic spike **[1]**, with a short postsynaptic delay **[2]**.

4.2.1. LAB MEDIA: Figure 11. *Video Editor: Emphasize the top plot.*

4.2.2. LAB MEDIA: Figure 11. *Video Editor: Emphasize the β3N110D voltage plot (middle plot).*

4.3. The γ2K328M *(Gamma-two-K-three-two-eight-M)* mutation also impaired inhibition, with neuron firing occurring around the 5th GluS1 spike **[1]** and with a longer postsynaptic delay than β3N110D **[2]**.

4.3.1. LAB MEDIA: Figure 11. *Video Editor: Emphasize the top plot.*

4.3.2. LAB MEDIA: Figure 11. *Video Editor: Emphasize the β3N110D and γ2K328M voltage plots (middle and bottom plots).*

4.4. Under dual synaptic input, the γ2P302L mutation caused firing nearly synchronized with the medial apical GluS2 input, likely reflecting delayed summation from GluS1 **[1]**.

4.4.1. LAB MEDIA: Figure 12. *Video Editor: Show the top plot. Highlight the label γ2P302L.*

4.5. The β3T288N mutation exhibited a similar pattern, with spikes aligned to GluS2 inputs and a second spike appearing in near synchrony **[1]**.

4.5.1. LAB MEDIA: Figure 12. *Video Editor: Show the top plot. Highlight the label β3T288N.*

4.6. The β3N110D mutation under dual input conditions triggered spikes for nearly all excitatory inputs except the first two, with noticeably shortened interspike intervals **[1]**.

4.6.1. LAB MEDIA: Figure 12. *Video Editor: Show the top plot. Highlight the label β3N110D.*

4.7. The γ2K328M mutant showed a comparable firing pattern but failed to respond to the second and third excitatory inputs **[1]**.

4.7.1. LAB MEDIA: Figure 12. *Video Editor: Show the top plot. Highlight the label*

γ2K328M.

4.8. Under triple excitatory input conditions, both β3N110D and γ2K328M mutants responded to nearly all presynaptic spikes **[1]**, firing spike pairs in response to cumulative excitatory drive **[2]**.

4.8.1. LAB MEDIA: Figure 13. *Video Editor: Emphasize the top plot.*

4.8.2. LAB MEDIA: Figure 13. *Video Editor: Emphasize the voltage plots (middle and bottom plots).*

Pronunciation Guides:

1. **GABAergic**
   Pronunciation link:
   https://www.howtopronounce.com/gabaergic
   IPA (American): /ˌgæbəˈɜːrdʒɪk/
   Phonetic Spelling: gab-uh-UR-jik
2. **Hodgkin-Huxley**

- **Hodgkin**
  Pronunciation link:
  https://www.collinsdictionary.com/dictionary/english/hodgkin
  IPA (American): /ˈhɑːdʒkɪn/
  Phonetic Spelling: HAJ-kin
- **Huxley**
  Pronunciation link:
  https://www.youtube.com/watch?v=PcyqMTg4-q4
  IPA (American): /ˈhʌksli/
  Phonetic Spelling: HUKS-lee

(Combined: Hodgkin-Huxley = HAJ-kin HUKS-lee)

3. **AMPA** (A-M-P-A)
   Pronunciation link:
   No confirmed link found
   IPA (American): /ˈæmpə/
   Phonetic Spelling: AM-puh
4. **NMDA** (N-M-D-A)
   Pronunciation link:
   No confirmed link found
   IPA (American): /ˌɛn ɛm diˈeɪ/
   Phonetic Spelling: EN-EM-dee-AY
5. **β3N110D** (Beta-three-N-one-one-zero-D)
   Pronunciation link:
   No confirmed link found

IPA (approx.): /ˌbeɪtə θri ɛn wʌn wʌn ziː roʊ diː/
Phonetic Spelling: BAY-tuh three en one one zero dee

6. **γ2K328M** (Gamma-two-K-three-two-eight-M)
   Pronunciation link:
   No confirmed link found
   IPA (approx.): /ˌgæmə tu keɪ θri tu eɪt ɛm/
   Phonetic Spelling: GAM-uh two kay three two eight em
7. **ENSEMBLE**
   Pronunciation link:
   https://www.merriam-webster.com/dictionary/ensemble
   IPA (American): /ɑːnˈsɑmbəl/
   Phonetic Spelling: ahn-SOM-buhl