FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 61282
Scriptwriter Name: Anastasia Gomez
Project Page Link: https://www.jove.com/account/file-uploader?src=18691058

Title: Combined In Vivo Anatomical and Functional Tracing of Ventral Tegmental Area Glutamate Terminals in the Hippocampus

Authors and Affiliations:

Amita Shrestha[1], Philip A. Adeniyi[1], and Olalekan M. Ogundele[1]

[1]Department of Comparative Biomedical Sciences, Louisiana State University School of Veterinary Medicine, Baton Rouge, LA70803, Louisiana, USA

Corresponding Authors:

O. M. Ogundele (ogundele@lsu.edu)

Email Addresses for All Authors:

shrestha1@lsu.edu
padeni1@lsu.edu
ogundele@lsu.edu

Author Questionnaire

1. **Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **Yes**

   If **Yes**, can you record movies/images using your own microscope camera?

   No

   If **No**, JoVE will need to record the microscope images using our scope kit (through a camera port or one of the oculars). Please list the make and model of your microscope.

   NIKON Ni-U (Motorized XYZ for 3D imaging)

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**

   If **Yes**, how far apart are the locations? **N/A**

protocol: **11 steps, 25 shots**

Introduction

1. Introductory Interview Statements

NOTE: Interviews 1.3, 1.4, and 7.1 are uploaded on the AWS project page: https://www.jove.com/account/file-uploader?src=18691058

REQUIRED:

1.1. **Olalekan M. Ogundele:** The current protocol highlights an affordable and straight forward method for combined neuroanatomical and electrophysiological tracing of neural circuits *in vivo.*

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

1.2. **Olalekan M. Ogundele:** The technique permits mapping of axon terminals from specific neuron population to a target site. Likewise, the anatomical map for these terminals can be verified by optogenetic stimulation during extracellular recording at the target site.

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

OPTIONAL:

1.3. **Amita Shrestha:** This protocol can be applied for studying multiple neural circuits associated with sensory, motor, and cognitive function.

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

1.4. **Philip A. Adeniyi:** Performing the procedure requires a sound knowledge of rodent stereotaxic surgery and handling of delicate neural electrodes. The researcher should also check the recording system connections to prevent noise in recordings.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Videographer: This statement is optional. If you don't have time, skip it.*

1.5. **Olalekan M. Ogundele:** Visual demonstration highlights basic steps in setting up an affordable LED system for *in vivo* optogenetics. Also, it shows how to connect the amplifier with the LED driver for a synchronized recording and stimulation in an anesthetized mouse.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Videographer: This statement is optional. If you don't have time, skip it.*

Ethics Title Card

1.6. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at the Louisiana State University School of Veterinary Medicine.

Protocol

2. Craniotomy and Animal Preparation

2.1. Place a heating pad on the stereotaxic frame so that the body of the mouse is lying on it, which will help maintain its body temperature throughout the procedure **[1]**, then gently fix the head on the stereotaxic apparatus **[2]**.

2.1.1. Talent putting a heating pad under the mouse. NOTE: The order of 2.1.1 and 2.1.2 was switched during the shoot, I updated the VO narration and shot descriptions according to author's notes.

2.1.2. Talent fixing the mouse's head. *Videographer: Obtain multiple usable takes of this because it will be reused in 4.1.1.*

2.2. Apply topical lidocaine to block sensation on the scalp **[1]**, clean the scalp with iodine **[2]**, then use a scalpel to make a midline incision extending from the frontal to the occipital region **[3]**.

2.2.1. Talent applying topical lidocaine.

2.2.2. Cleaning site with iodine solution

2.2.3. Talent making the incision.

2.3. For ventral tegmental area, or VTA, injection, position an ultrafine blunt-point needle **[1]** syringe at -3.08 millimeter anterior-posterior and 0.5 millimeter medial-lateral coordinates relative to the bregma **[2-TXT]**. Use a drilling tool to bore a 1-millimeter hole in the skull at the marked coordinate **[3]**.

2.3.1. Talent determining the Anterior-Posterior (AP) and medial-Lateral (ML) coordinates of the VTA on the skull using the bregma as reference.

2.3.2. Added shot: Talent positioning a needle tip at the approximate AP/ML location of the VTA. **TEXT: Use an ultrafine micromanipulator**

2.3.3. Talent drilling the hole.

3. AAV Cocktail Injection

3.1. Follow the manufacturer's instructions to fix the syringe holder on a micromanipulator **[1]** and fill the syringe with double distilled water to clean and test the flow of fluid **[2]**. Then, dispense the water to test the flow of the syringe **[3]**.

3.1.1. Talent adjusting manual injector that is fixed on a micromanipulator.

3.1.2. Talent turning the knob of the injector counterclockwise to fill the syringe barrel with distilled water.

3.1.3. Added shot: Talent turning the knob of the injector clockwise to dispense the distilled water.

3.2. Thaw aliquots of adeno-associated virus, or AAV, cocktail on ice **[1-TXT]**. Fill the mounted syringe with 1,000 nanoliters of AAV solution **[2]** and dispense 10 nanoliters of the solution to confirm the flow of the liquid **[3]**.

3.2.1. Aliquots on ice. **TEXT: AAV should be stored at -80 °C**

3.2.2. Added shot: Close up view of talent dispensing liquid from needle tip. NOTE: Authors added this shot here, but seems to me that it should be shown after or before 3.2.4.

3.2.3. Talent filling the syringe with AAV.

3.2.4. Talent dispensing the solution.

3.3. Use the micromanipulator to lower the needle to the injection site **[1]** and inject 600 to 800 nanoliters of AAV into the VTA, delivering the solution at 60 nanoliters per minute **[2]**. *Videographer: This step is difficult and important!*

3.3.1. Talent lowering the needle to the injection site.

3.3.2. Talent injecting the solution.

4. In Vivo Neural Recordings with Optogenetics

4.1. Affix the head of the animal on a stereotactic frame as previously described and perform a craniotomy to expose the dura. Use a drilling tool to remove part of the parietal bone **[2-TXT]**.

~~4.1.1. *Use 2.1.1.*~~

4.1.2. Talent using the drilling tool to expose the bone. **TEXT: 3 mm x 4 mm wide craniotomy**

4.2. Under a dissection microscope, use a bent, 27-gauge needle tip to excise the exposed dura, taking care to not pull apart the delicate pial covering and cortical tissues in this area **[1]**. Apply drops of artificial cerebrospinal fluid over the craniotomy area to prevent dryness **[2]**.

4.2.1. SCOPE: Talent excising the dura. NOTE: The order of 4.2.1 and 4.2.2 was switched during the shoot, I updated the VO narration and shot descriptions according to author's notes.

4.2.2. SCOPE: Talent applying drops to the craniotomy area.

4.3. Bore a hole in the occipital bone to hold the ground screw **[1-TXT]** and connect a stainless-steel ground wire **[2]**. Before lowering the cannula, connect the fiber optic cable to a fiber-coupled LED source **[3]**. *Videographer: This step is important!*

4.3.1. Talent drilling a hole. **TEXT: bit size: 0.8 mm, speed: 10,000 rpm ; pan head Phillips screw: M 0.6 x 2.0 mm**

4.3.2. Talent connecting a stainless-steel ground wire.

4.3.3. Talent connecting the fiber optic cable to the LED source.

4.4. Lower the 400-micrometer diameter optic fiber into the VTA at co-ordinates of AP -3.08 millimeters and ML 0.5 millimeters **[1]**. Using a micro-manipulator, position the electrode contact sites in the pyramidal cell layer of the CA1 **[2-TXT]**. *Videographer: This step is important!*

4.4.1. Talent positioning the optic fiber to the depth of the VTA.

4.4.2. Talent positioning neural probe and moving probe shank to the depth of the CA1 pyramidal cell layer. **TEXT: AP: -1.94mm, ML: +1.0mm; DV: +1.1 to 1.2mm**

4.5. To synchronize the light pulse with the neural recording, connect the LED driver and recording controller digital **IN** port to a transistor-transistor logic pulser with a BNC splitter **[1]**. Adjust the knob to determine the effective intensity that can generate a response without producing photoelectric artifacts **[2]** and connect the ground on the skull to the ground on the adapter **[3]**. *Videographer: This step is important!*

4.5.1. Talent connecting the LED driver and IN port to the logic pulser.

4.5.2. Talent adjusting the knob.

4.5.3. Added shot: Talent connects the ground on the skull to the ground on the adapter.

4.6. Connect the BNC cable to the amplifier and the pulse generator **[1]**, then connect the pre-amplifier head stage to the recording controller via a serial peripheral interface cable **[2]** and check the LED color lights on the recording controller ports. Green and yellow LEDs indicate proper voltage on the connected amplifier board and the red LED indicates a working software-head stage control **[3]**. *Videographer: This step is important!*

4.6.1. Added shots: Talent connecting BNC cable to (a) Digital IN port of amplifier, (b) TTL pulse generator.

4.6.2. Talent connecting the pre-amplifier head stage to the recording controller.

4.6.3. Green, yellow, and red LED lights.

5. Amplifier and Filter Settings

5.1. After launching the software, select the data file format and change the file name **[1]**. Switch to the port for the connected head stage and click on **disable all on port** if the electrode has fewer channels than the head stage. Select the appropriate number of channels to be displayed on the screen **[2]**.

5.1.1. SCREEN: Shrestha et al_INTAN Set_X (1).mp4. 0:00 – 0:16.

5.1.2. SCREEN: Shrestha et al_INTAN Set_X (1).mp4. 0:16 – 0:34.

5.2. To capture the TTL time stamp for synchronized neural recording and light pulse trigger timestamp, click on the **digital in ports** and enable DIGITAL-IN-01. This must correspond with the BNC connection on the amplifier DIGITAL IN ports **[1]**.

5.2.1. SCREEN: Shrestha et al_INTAN Set_X (1).mp4. 0:34 – 0:39.

5.3. Adjust the time scale and voltage scale for appropriate waveform display and select the sampling rate, keeping in mind that a higher sampling rate and number of channels will increase the file size **[1]**.

5.3.1. SCREEN: Shrestha et al_INTAN Set_X (1).mp4. 0:40 – 1:03.

5.4. Next, set the amplifier bandwidth for single-unit recording. A 300 to 5,000 Hertz cut off frequency was used here **[1]**. For spike sound, click on **Analog Out-Audio** and enable the Analog port, then adjust the gain and silencer for optimum sound **[2]**.

5.4.1. SCREEN: Shrestha et al_INTAN Set_X (1).mp4. 1:04 – 1:12.

5.4.2. SCREEN: Shrestha et al_INTAN Set_X (1).mp4. 1:13 – 1:25.

5.5. To synchronize amplifier recording and light pulse train, click on the configure panel and enable **show marker**. Switch to the appropriate channel that corresponds with the BNC connection and re-inspect the ports to make sure that the recording channels and Digital IN channel are enabled to capture neural spikes and light pulse time stamps **[1]**.

5.5.1. SCREEN: Shrestha et al_INTAN Set_X (1).mp4. 1:26 – 1:39.

5.6. Click on spike scope to view waveforms that constitute the continuously recorded spike train. Use the mouse to set the threshold for the waveforms to be displayed, then inspect the RMS to determine the noise level in your recording **[1]**.

5.6.1. SCREEN: Shrestha et al_INTAN Set_X (1).mp4. 1:44 – 1:55.

Results

6. Results: VTA Glutamate Projections to the Hippocampus Modulate CA1 Activity

6.1. Adeno-associated virus expression was verified by immunofluorescence imaging of eYFP in the ventral tegmental area of mice 21 days post-injection **[1]**. Fluorescence imaging was also used to detect presynaptic VTA glutamate projections in the hippocampus layers DG, CA3, and CA1 **[2]**.

6.1.1. LAB MEDIA: Figure 2.

6.1.2. LAB MEDIA: Movie 2_fluor_in text.mp4.

6.2. Once extracellular voltage activity was detected, the baseline activity was recorded for approximately 10 minutes **[1]** before triggering the light pulse at the desired frequency **[2]**. This made it possible to compare the firing or burst rates of CA1 putative neurons before and after VTA glutamate photostimulation **[3]**.

6.2.1. LAB MEDIA: Movie 1_in text.mp4. 00:00 to 00:10

6.2.2. LAB MEDIA: Movie 1_in text.mp4. 00:10 to 00:17. *Video Editor: Emphasize the light blue lines that appear on the screen when start is clicked on the TTL software (left upper corner of movie screen). This depicts the time stamp for synchronized recording and photostimulation.*

6.2.3. LAB MEDIA: Figure 5 C.

6.3. To support this outcome, statistical comparison of the CA1 network firing rates before and after photostimulation revealed a significant increase for the post-stimulation period **[1-TXT]**. Subsequent analysis of the raster train to detect bursts also showed an increased burst rate for the CA1 putative pyramidal neurons after photostimulation **[2-TXT]**.

6.3.1. LAB MEDIA: Figure 7 A. *Video Editor: Add "***p = 0.0002***" as a text overlay.*

6.3.2. LAB MEDIA: Figure 7 B. *Video Editor: Add "***p = 0.0025***" as a text overlay.*

Conclusion

7. Conclusion Interview Statements

NOTE: Interviews 1.3, 1.4, and 7.1 are uploaded on the AWS project page: https://www.jove.com/account/file-uploader?src=18691058

7.1. **Philip A. Adeniyi:** Response to photostimulation must be validated by fluorescence of AAV reporter expression at the stimulus site. Also, the distribution of anatomical terminals should be correlated with the site of recording for responsive putative units.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: Movie 3_fluor_in text.mp4.*

7.2. **Olalekan M. Ogundele:** This method can also be performed in awake mice undertaking behavioral tasks. In this case, chronic implantable probes and fiberoptic cannula should be used.

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

7.3. **Olalekan M. Ogundele:** This technique will allow for robust tracing of neural circuits through a combination of presynaptic terminal distribution in a target site and modulation of presynaptic terminals to detect target site response *in vivo.*

7.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Videographer: This statement is optional. If you don't have time, skip it.*