

Project Page Link: <https://review.jove.com/account/file-uploader?src=21504683>

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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, all done**

- 3. Filming location:** Will the filming need to take place in multiple locations? **Two places.**
 - 1) Universitätsmedizin Rostock - Oscar Langendorff Institut für Physiologie**
Gertrudenstraße 9, 18057 Rostock
 - and**
 - 2) Institute of General Electrical Engineering**
Albert-Einstein-Str. 2, Room Ex138
18059 Rostock

- 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. **Yes**

Current Protocol Length

Number of Steps: 20

Number of Shots: 47

Interviews

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

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Rüdiger Köhling:** We investigate how globus pallidus deep-brain stimulation modulates cerebellar network activity and restores neuronal function in dystonia.

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

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Denise Franz:** High-density microelectrode arrays enable simultaneous motor loop recordings to analyze network-wide pallidal DBS mechanisms.

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

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Fabiana Santana Kragelund:** This protocol enables high-resolution measurement of cerebellar network activity and the effect of its modulation by deep brain stimulation.

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

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Fabiana Santana Kragelund:** Maintaining stable HD-MEA recordings through all measures, with proper grounding, good tissue-electrode contact, and low-noise experimental conditions, is critical.

1.4.1. 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.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

~~What problem in your field of research does your JoVE article address?~~

- 1.5. **Rüdiger Köhling, Professor and Director, Oscar Langendorff Institute of Physiology, University Medical Center Rostock:** (authors will present their testimonial statements live)

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

~~What is the key takeaway you hope viewers remember after watching your video article?~~

- 1.6. **Rüdiger Köhling, Professor and Director, Oscar Langendorff Institute of Physiology, University Medical Center Rostock:** (authors will present their testimonial statements live)

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

Ethics Title Card

This research has been approved by the ethics committee at the Oscar Langendorff Institute of Physiology, University Medical Center Rostock

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

2. Encapsulation of STELLA Brain Stimulation System for Implantation in Dystonic Hamsters

Demonstrator: Dr. Fabiana Santana Kragelund

- 2.1. To begin, house the electronics of the STELLA stimulation system in a custom 3D (*Three-D*)-printed polyethylene terephthalate glycol, or PETG (*pet-G*) capsule [1]. Use silicone to seal the area between the lid and the capsule [2] and the housing at the cable exit [3]. After drying, inspect the silicone for visible gaps and repeat the sealing if gaps are present [4].
 - 2.1.1. WIDE: Talent placing the STELLA stimulation system electronics into the custom 3D-printed capsule. *Videographer: Please obtain CU shot as well for this shot.*
 - 2.1.2. Talent applying silicone between the lid and the capsule.
 - 2.1.3. Talent applying silicone around the cable exit.
 - 2.1.4. Talent inspecting the dried silicone seal.
- 2.2. After full solidification of the silicone, prepare the epoxy resin at a 100 to 35 mixing ratio by weight [1]. Use the Vevor RS-1.5 (*Vee-vor-R-S-one-point-five*) to remove air bubbles from the resin under vacuum [2].
 - 2.2.1. Talent mixing epoxy resin at the specified ratio.
 - 2.2.2. Talent placing the resin under vacuum in the Vevor RS-1.5. **NOTE: This shot is not provided. Please add a missing media title card. Author's notes: Extra Footage from the authors.**
- 2.3. Before assembly, apply a thin layer of vaseline to the mating surface of the custom silicone mould [1]. Place the stimulator capsule into the mould [2]. Slowly fill it with epoxy resin until the stimulator and cable junctions are fully covered, avoiding air bubbles during filling [3].
 - 2.3.1. Talent applying vaseline to the mating surface of the silicone mould.
 - 2.3.2. Talent placing the stimulator capsule into the mould.
 - 2.3.3. Talent slowly pouring epoxy resin into the mould.
- 2.4. After resin curing, remove the capsule from the mould [1]. Use a fine file or polishing tool to smooth the epoxy surface and eliminate sharp edges [2].
 - 2.4.1. Talent removing the cured capsule from the mould.

- 2.4.2. Talent smoothing the epoxy surface with a fine file or polishing tool.
 - 2.5. Immerse the capsule in liquid silicone to apply a final thin silicone coating [1]. Use a plastic pipette to apply additional silicone around the cable near the junction area [2]. Use a stick to remove excess silicone before curing to avoid coating thickness [3].
 - 2.5.1. Talent immersing the cured epoxy capsule in liquid silicone.
 - 2.5.2. Talent applying additional silicone near the cable junction with a plastic pipette.
 - 2.5.3. Talent removing excess silicone with a stick.
 - 2.6. To perform a post-encapsulation functionality test before surgical implantation or experimental use, place the STELLA stimulation system in Ringer's solution [1] and activate the magnetic built-in self-test function [2]. Confirm the correct light-emitting diode, or LED (L-E-D), blink pattern of 2 by 2, indicating proper stimulation output and rule out short circuits, open circuits, or low battery conditions [3]. After testing, turn the device off to save battery [4]. Notes: VO is added for an additional shot.
 - 2.6.1. Talent placing the STELLA stimulation system in Ringer's solution.
 - 2.6.2. Talent activating the magnetic built-in self-test function.
 - 2.6.3. Close shot of the light-emitting diode showing the correct 2 by 2 blink pattern.
- Added shot: Talent turning off the device by applying 2 magnet signals. The first signal activates the modus, and the second signal during the fast-blinking indication successfully turns the device off.

3. Post-Stimulation Cerebellar Slice Preparation

- 3.1. Prepare the workplace with dissection tools, sucrose solution, Petri dishes, and the vibratome setup [1].
 - 3.1.1. Talent arranging dissection tools, sucrose solution, Petri dishes, and the vibratome setup on the workstation.
- 3.2. Use a sucrose solution at approximately 4 degrees Celsius for brain extraction and slicing with continuous carbogenation [1]. Prepare ice-cold slushy sucrose to maintain low tissue temperature during rapid dissection [2].
 - 3.2.1. Talent placing sucrose solution for use during extraction and slicing with carbogenation.
 - 3.2.2. Talent preparing ice-cold slushy sucrose.
- 3.3. Transfer the brain excised within 15 minutes following the 11-day deep-brain stimulation, or DBS (D-B-S) period, into a new Petri dish filled with ice-cold slushy sucrose, with a filter paper at the bottom to prevent the cerebellum from slipping during sectioning [1].

- 3.3.1. Talent transferring the brain into a new Petri dish with ice-cold slushy sucrose and filter paper at the bottom.
- 3.4. Use a blade to remove the cerebellum by making a coronal cut through the pons [1]. Then, remove the cerebellar hemispheres through parasagittal cuts [2].
 - 3.4.1. Talent cutting through the pons with a blade to remove the cerebellum.
 - 3.4.2. Talent making parasagittal cuts with a blade to remove the cerebellar hemispheres. Author's notes: Use the first take, it was better
- 3.5. Prepare the vibratome plate with a drop of super glue [1]. Carefully remove the cerebellar vermis from the Petri dish [2]. Dab the parasagittal section plane onto filter paper to dry it before gluing this side [3].
 - 3.5.1. Talent applying a drop of super glue to the vibratome plate. Author's notes: Onetake. There is no slate with the number 3.5.2/ 3.5.3
 - 3.5.2. Talent carefully removing the cerebellar vermis from the Petri dish.
 - 3.5.3. Talent dabbing the parasagittal section plane onto filter paper.
- 3.6. Place the vibratome plate into the tray [1]. Fill the tray with enough half-slushy sucrose solution to completely submerge the vermis [2]. Gas the tray with carbogen [3]. Notes: VO narration is also edited for inverted shots.
 - 3.6.1. Talent placing the vibratome plate into the tray.
 - 3.6.3. Talent filling the tray with sucrose mixture until the vermis is submerged.
 - 3.6.2. Talent gassing the tray with carbogen. Note: 3.6.3 is placed before 3.6.2
- 3.7. Cut parasagittal cerebellar slices with a thickness of 200 micrometers at a constant speed of 0.08 millimeters per second [1]. Store the slices in artificial cerebrospinal fluid, or ACSF (A-C-S-F), in a beaker constantly gassed with carbogen at room temperature [2].
 - 3.7.1. Talent cutting parasagittal cerebellar slices on the vibratome.
 - 3.7.2. Talent transferring the slices into ACSF in a carbogen-gassed beaker.
- 3.8. Allow the slices to recover in carbonated ACSF at room temperature for approximately 40 minutes before transfer to the high-density microelectrode array, or HD-MEA (H-D-M-E-A) for rewarming and stabilization before recording [1].
 - 3.8.1. Talent placing the slices in carbonated ACSF for recovery.

4. Preparing the CorePlate™ Single-Well of the BioCAM Duplex MEA System for Recording

- 4.1. Clean the gold-plated pads with 100 percent ethanol [1] and the electrode array with 70 percent ethanol and a soft brush [2]. Then, rinse the electrode array with MilliQ (Milli-q) water [3].

- 4.1.1. Talent cleaning the gold-plated pads with 100 percent ethanol.
- 4.1.2. Talent cleaning the electrode array with 70 percent ethanol and a soft brush.
- 4.1.3. Talent rinsing the electrode array with MilliQ water.
- 4.2. Prior to tissue slice placement, insert the CorePlate Single-Well into the recording setup [1].
 - 4.2.1. Talent inserting the CorePlate Single-Well into the recording setup.
- 4.3. Connect the HD-MEA with CorePlate™ Single-Well into the recording system [1]. Pipette approximately 2 milliliters of ACSF into the well without air bubbles [2].
 - 4.3.1. Talent connecting the high-density microelectrode array CorePlate Single-Well to the recording system (Duplex).
 - 4.3.2. Talent pipetting ACSF into the well.
- 4.4. Start live acquisition in **Brainwave 5**, or **BW5 (B-W-five)** software, and set the parameters like cockpit mode and tissue model [1]. Verify that the background noise falls within the optimal range of 9–20 microvolts [2].
 - 4.4.1. SCREEN: Noise_SRN_Jove-ID-70507.mp4. 00:35 – 00:40; 00:53 – 1:01. *Video Editor: At timestamp range 00:56, please highlight the selection of “Advanced” under Cockpit Mode, as shown in Shot 4.4.1_ref image in project page and at timestamp 00:59, highlight the selection of “Brain Slice” under Model menu, as shown in Shot 4.4.1B_ref.*
 - 4.4.2. SCREEN: Noise_SRN_Jove-ID-70507.mp4. 1:30–1:40. *Video Editor: Please emphasize the blue-colored Y-axis line on the left side and the blue noise lines on the screen. If possible, please add text “Baseline/Noise: 9–25 μ V” on top of the noise lines, as shown in the figure Shot 4.4.2_ref uploaded to the project page.*
- 4.5. Record for at least 30 seconds [1]. After stopping the recording, open the saved file for analysis [2].
 - 4.5.1. SCREEN: Noise_SRN_Jove-ID-70507.mp4. 1:55–2:00
 - 4.5.2. SCREEN: Noise_SRN_Jove-ID-70507.mp4. 2:01–2:10; 2:20–2:23
- 4.6. To analyze the noise interface, click on **Results** and select **Playback** [1]. Click on **Media Export** and under the **Raw Traces** dropdown menu, select **Activity Map** to analyze the Peak-to-Peak amplitude [2] and standard deviation to determine the baseline noise [3].
 - 4.6.1. SCREEN: Noise_SRN_Jove-ID-70507.mp4. 2:26–2:34
 - 4.6.2. SCREEN: Noise_SRN_Jove-ID-70507.mp4. 2:36–2:48
 - 4.6.3. SCREEN: Noise_SRN_Jove-ID-70507.mp4. 2:49–2:55
- 4.7. Next, use a Pasteur pipette to place the tissue slice in the center of the Coreplate Single well under a microscope and remove the ACSF [1]. Position the anchor on top of the

slice to fix it [2]. Use a 3-milliliter Pasteur pipette to apply and remove a single drop of water to wash the slice, repeating 5–10 times [3]. Use a twisted paper wick to remove the residual water [4].

4.7.1. SCREEN: Slice-position_Jove-ID-70507-.mp4. 14:37–14:42; 15:36–15:41

4.7.2. SCREEN: Slice-position_Jove-ID-70507-.mp4. 16:10–16:20

4.7.3. SCREEN: Slice-position_Jove-ID-70507-.mp4. 16:28–16:33; 16:47–16:50

4.7.4. SCREEN: Slice-position_Jove-ID-70507-.mp4. 19:53–19:57

4.8. Apply drops of ACSF to the center of the well to improve contact between the slice and electrodes for optimal recording [1]. Position the inflow line above the slice and the outflow line away from it [2]. Remove any bubbles from the ground electrode before proceeding with data acquisition and analysis [3].

4.8.1. SCREEN: Slice-position_Jove-ID-70507-.mp4. 22:00–22:12

4.8.2. SCREEN: Slice-position_Jove-ID-70507-.mp4. 22:32–22:35; 22:41–22:45

4.8.3. SCREEN: Slice-position_Jove-ID-70507-.mp4. 23:37–23:49

Results

5. Results

- 5.1. The analysis of the total number of spikes in molecular, Purkinje cell, and granular cell layers of the cerebellar cortex showed that dystonic tissue slices generated significantly fewer spikes [1], whereas pallidal DBS increased the number of spikes to levels comparable to those of healthy controls [2].
 - 5.1.1. LAB MEDIA: Table 3. *Video Editor: Please emphasize the ML, PL, and GL values for the “dt^{SZ}” column and the WT column under the row heading “Number of Spikes”*
 - 5.1.2. LAB MEDIA: Table 3. *Video Editor: Please emphasize the ML, PL, and GL values for the “dt^{SZ} - DBS” column and the WT column under the row heading “Number of Spikes”*
- 5.2. The Kruskal-Wallis H test further confirmed a statistically significant increase in the number of spikes in the molecular layer of cerebellar cortex for the dystonic DBS group compared to both the untreated dystonic and dystonic-Sham group [1].
 - 5.2.1. LAB MEDIA: Figure 6A. *Video Editor: Please highlight the height of the black bar, relative to the light grey and dark grey bars.*
- 5.3. The dystonic DBS group also showed an increase in spikes in the Purkinje cell layer [1] and the granular cell layer, reaching values comparable to those of the WT group [2].
 - 5.3.1. LAB MEDIA: Figure 6B. *Video Editor: Please highlight the heights of the black and white bars simultaneously.*
 - 5.3.2. LAB MEDIA: Figure 6C. *Video Editor: Please highlight the heights of the black and white bars simultaneously.*

Pronunciation guides:

1. Polyethylene terephthalate glycol
 Pronunciation link: <https://www.merriam-webster.com/>
 IPA: /ˌpɑːliˈeθəˌliːn ˌterəfˈθæleɪt ˈɡlaɪˌkɔːl/
 Phonetic Spelling: pah·lee·eth·uh·leen teh·ruhf·THAL·ayt GLY·kawl
2. Silicone
 Pronunciation link: <https://www.merriam-webster.com/dictionary/silicone>
 IPA: /ˈsɪləˌkoʊn/
 Phonetic Spelling: sil·uh·kohn
3. Epoxy
 Pronunciation link: <https://www.merriam-webster.com/dictionary/epoxy>

IPA: /ɪˈpɑːksi/

Phonetic Spelling: ih·PAHK·see

4. Vaseline

Pronunciation link: <https://www.merriam-webster.com/dictionary/Vaseline>

IPA: /ˈvæsəˌliːn/

Phonetic Spelling: vas·uh·leen

5. Encapsulation

Pronunciation link: <https://www.merriam-webster.com/dictionary/encapsulation>

IPA: /ɪnˌkæpsjəˈleɪʃən/

Phonetic Spelling: en·kap·syuh·LAY·shuhn

6. Ringer's

Pronunciation link: <https://www.merriam-webster.com/dictionary/Ringer%27s%20solution>

IPA: /ˈrɪŋərz/

Phonetic Spelling: RING·erz

7. Cerebellar

Pronunciation link: <https://www.merriam-webster.com/dictionary/cerebellar>

IPA: /ˌserəˈbelər/

Phonetic Spelling: sehr·uh·BEL·er

8. Vibratome

Pronunciation link: <https://www.howtopronounce.com/vibratome>

IPA: /ˈvaɪbrəˌtoʊm/

Phonetic Spelling: VY·bruh·tohm

9. Carbogenation

Pronunciation link: No confirmed link found

IPA: /ˌkɑːrboʊdʒəˈneɪʃən/

Phonetic Spelling: kar·boh·juh·NAY·shuhn

10. Parasagittal

Pronunciation link: <https://www.merriam-webster.com/medical/parasagittal>

IPA: /ˌpærəˈsædʒɪtəl/

Phonetic Spelling: par·uh·SAJ·ih·tuhl

11. Vermis

Pronunciation link: <https://www.merriam-webster.com/medical/vermis>

IPA: /ˈvɜːmɪs/

Phonetic Spelling: VUR·miss

12. Cerebrospinal

Pronunciation link: <https://www.merriam-webster.com/dictionary/cerebrospinal>

IPA: /səˌriːbroʊˈspainəl/

Phonetic Spelling: suh·REE·broh·SPY·nuhl

13. Microelectrode

Pronunciation link: <https://www.merriam-webster.com/medical/microelectrode>

IPA: /ˌmaɪkroʊˈlekˌtroʊd/

Phonetic Spelling: MY·kroh·ih·LEK·trohd

14. Ethanol

Pronunciation link: <https://www.merriam-webster.com/dictionary/ethanol>

IPA: /'eθə,nɔ:l/

Phonetic Spelling: ETH·uh·nawl

15. MilliQ

Pronunciation link: No confirmed link found

IPA: /,mɪli'kju:/

Phonetic Spelling: MIL·ee·Q

16. Kruskal-Wallis

Pronunciation link: <https://www.howtopronounce.com/kruskal-wallis-test>

IPA: /'krʊskəl 'wɑ:lɪs/

Phonetic Spelling: KRUHS·kuhl WAH·liss

17. Dystonic

Pronunciation link: <https://www.merriam-webster.com/medical/dystonia>

IPA: /dɪ'stɑ:nɪk/

Phonetic Spelling: dis·TAHN·ik

18. Pallidal

Pronunciation link: <https://www.howtopronounce.com/pallidal>

IPA: /'pælɪdəl/

Phonetic Spelling: PAL·ih·duhl

19. Purkinje

Pronunciation link: <https://www.merriam-webster.com/medical/Purkinje%20cell>

IPA: /pər'kɪndʒi/

Phonetic Spelling: per·KIN·jee

20. Granular

Pronunciation link: <https://www.merriam-webster.com/dictionary/granular>

IPA: /'grænjələr/

Phonetic Spelling: GRAN·yuh·ler