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# Protocol for extracellular recordings in the external globus pallidus of the behaving/awake monkey

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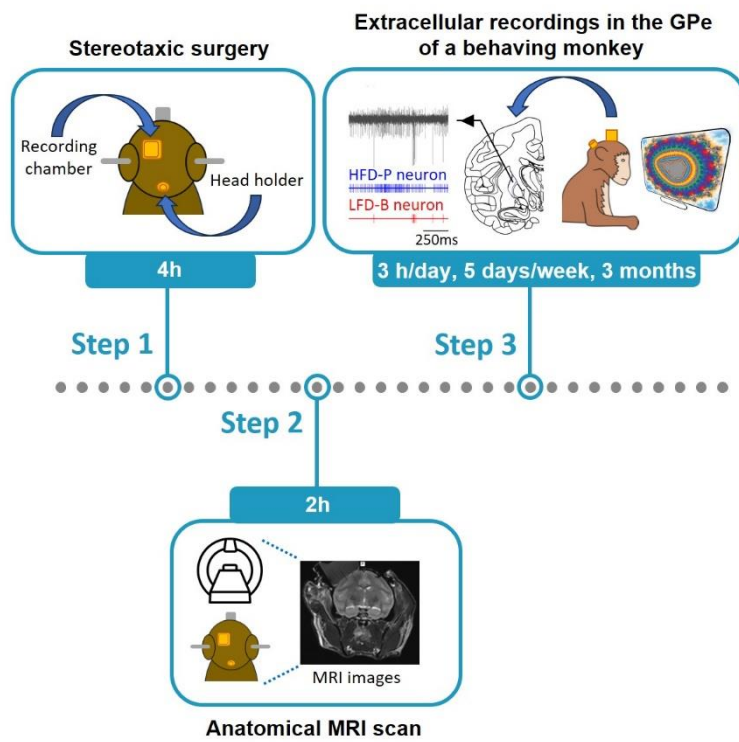
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## SUMMARY

Extracellular recordings in behaving animals are useful for establishing associations between neuronal activity and behavior. Here, we describe how to record in the external globus pallidus (GPe) of monkeys engaged in a behavioral task. We detail the stereotaxic surgery for chamber and head-holder implantation, the post-operative MRI scan to ascertain the GPe coordinates and validate the position of the chamber, and the data collection. This protocol makes it possible to examine the electrophysiological features of GPe neurons in behaving monkeys. For complete details on the use and execution of this protocol, please refer to Katabi et al. (2023)<sup>1</sup>.

## GRAPHICAL ABSTRACT



## BEFORE YOU BEGIN

### Institutional permissions

All the experimental protocols described here are in accordance with the National Research Council's *Guide for the Care and Use of Laboratory Animals* and the Hebrew University guidelines for the use and care of laboratory animals in research, as supervised by the Institutional Animal Care and Use Committee. The Hebrew University is an internationally accredited Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) institute. Any protocol should be first approved by the animal ethics committee of the affiliated research institution. Such approval may take several months (between 1 and 3 months) depending on the animal ethics committees.

For the total duration of the experiments described here, the monkeys were group-housed in the conventional animal facility of the Hebrew University under standard conditions (12 h light/dark cycle with light on from 7.00 am to 7.00 pm, ~ 60% humidity and ~ 22°C). The animals had free access to water, food, and an enriched environment (e.g., toys, music). They were also monitored daily by the experimentalists in cooperation with the professional team (including a vet) of the animal facility to ensure animal welfare and provide care whenever necessary.

To conduct extracellular recordings in the external globus pallidus (GPe) of behaving/awake monkeys, the following essential instruments need to be prepared:

### Manufacturing glass-coated tungsten microelectrodes

This procedure is described in a previous study<sup>2</sup>. Here, we simply present a summary of the main steps.

#### Timing: 15 min per electrode

1. Electrochemical etching of the tungsten rod to sharpen the tip.
  - a. Attach a tungsten rod (127  $\mu\text{m}$  in diameter and 152.4 mm in length, A-M Systems) to the electrode etcher (Metal Electrode Etcher, model EE1D, BAK Electronics) with an alligator clip so that it is maintained above a beaker containing a Levick solution<sup>3</sup> and two carbon rods (corresponding to the cathode and anode) used for electrolysis (Figure 1A).
  - b. Position the rod so that its lower part can be immersed in and removed from the Levick solution.
  - c. Set the number and duration of submersion and withdrawal cycles (dips) on the electrode etcher.
  - d. Start the etching process.

**Note:** Tests must be carried out beforehand to determine the number/duration of dips and the intensity/voltage of the current needed to obtain the desired tip shape.

- e. After the etching process is complete, wash the tungsten rod in distilled water to remove the etching residue and verify the macroscopic shape under a light optical microscope.

**Note:** The electrode tip should look like a pencil.

## 2. Glass coating of the etched tungsten rod (Figure 1B).

- a. Attach both ends of a glass capillary tube (1 mm inner diameter, 2 mm outer diameter, 150 mm long single barrel borosilicate capillary glass without microfilament, A-M Systems) to the horizontal micropipette puller so that it passes through the center of the heater coil.
- b. Insert the sharpened tungsten rod into the glass capillary tube so that its blunt end protrudes a few mm beyond the glass capillary tube.
- c. Set the correct electrical parameters of the horizontal micropipette puller and turn on the heater coil.

**Note:** The optimal electrical parameters depend on the design/model of the horizontal micropipette puller and how the experimenter will use it. Therefore, the electrical parameters must be determined by testing.

- d. When the heating coil is incandescent (~45 s), start to pull the glass capillary tube smoothly and evenly along the tungsten rod.
- e. Detach the glass-coated microelectrode from the horizontal micropipette puller and carefully remove the excess glass at both ends.

**Note:** 2-3 mm of glass should be removed at the blunt end of the microelectrode to obtain a short, uninsulated segment that will be used to connect to electrophysiological recording equipment.

- f. Rinse the glass-coated microelectrode in distilled water and verify under a microscope that the tungsten rod is evenly (no air bubbles or lumps) covered with glass that thins at the tip of the electrode.

## 3. Beveling and exposure of the electrode tip (Figure 1C).

- a. Place an abrasive disc (World Precision Instruments) on the beveller (SYS-48000 MicroBeveler, World Precision Instruments).
- b. Turn on the beveller and touch the abrasive disc gently with the electrode tip held at an angle of 35°-40°, while rolling the electrode between your fingers.

**Note:** Contact with the disc should remove the glass at the edge of the electrode and expose the tip.

- c. Verify the edge of the electrode under the microscope to make sure the tip is exposed.

4. Measuring the impedance of the microelectrode (Figure 1D).
  - a. Connect the short uninsulated segment of the electrode to an impedance conditioning module (FHC, 1 kHz sine wave) so that the exposed electrode tip is immersed in saline.
  - b. Measure the impedance of the microelectrode during and after beveling.

**Note:** Electrode impedance is affected by the length and shape of the exposed electrode tip. Glass-coated electrodes should have an impedance of more than 10 M $\Omega$  before tip exposure, and between 0.4 and 0.8 M $\Omega$  after tip exposure. Repeat the measurement several times to avoid random errors. We have been using electrodes with impedance typically between 0.4 and 0.8 or even 1 M $\Omega$  for decades in our laboratory and obtain very high-quality neuronal recordings with very good signal-to-noise in the GPe where neurons are sparse. Higher impedance electrodes (1-3 M $\Omega$ ) can better isolate neurons recorded in brain structures with higher neuronal density. Figures 1E, F, G, and H, show the tungsten rod/electrode tip before and after the etching/sharpening, glass coating, and beveling, respectively.

**Note:** Ready to use glass-coated tungsten microelectrodes can be purchased from commercial vendors (e.g., AlphaOmega Engineering, Israel).

### Calculating the stereotaxic coordinates of the external globus pallidus

#### Timing: 1 h

5. Determine the stereotaxic coordinates of the GPe from the atlas.

**Note:** The theoretical coordinates of the caudal GPe (in mm, corresponding to the posterior/motor territory) in macaques or similar non-human primate species (e.g., African green monkeys - vervets) from the vertical plane passing through the interaural line (AP), the midline (ML) and the horizontal plane passing through the interaural line (DV) are: AP:12, ML:12, DV:12 (Figure 2A and see<sup>4</sup>). Keep in mind that actual stereotaxic coordinates may differ from those given in any atlas due to inter-individual variability and the position of the head in the stereotaxic frame. Therefore, an MRI scan is highly recommended after surgery for the implantation of the chamber (see below) to verify its exact location and confirm the stereotaxic coordinates of the neuronal recordings.

6. Calculate the coordinates of the general zero (AP:0, ML:0, DV:0) on the stereotaxic frame (Figure 2B).
  - a. Attach and secure the stereotaxic frame to the operating table, making sure it is completely horizontal.
  - b. Insert the two ear bars into the corresponding slots in the stereotaxic frame so that they meet exactly in the center of the frame.

**Note:** The bars must be symmetrical. The exact center point between the two bars is the general zero (AP:0, ML:0, DV:0).

- c. Secure the first stereotaxic tower on the arm of the frame corresponding to the left or right side of the monkey (depending on whether the chamber is implanted over the right or left hemisphere, respectively) without tilting it, so that it is completely vertical.
  - d. Attach a pointer (a small pointed rod) to the end of the tower and orient it in all 3 dimensions (AP, ML and DV) so that it points precisely at the general zero point of the stereotaxic frame.
  - e. Read the exact position of the tower for each axis/dimension on the stereotaxic frame (all the measurements are in mm).
7. Calculate the final coordinates of the GPe on the stereotaxic frame.
- a. Add the stereotaxic coordinates of the GPe from the atlas to the coordinates of the general zero (AP:0, ML:0, DV:0) on the stereotaxic frame.
  - b. Move the stereotaxic tower attached to the arm of the frame in each axis/dimension so that the pointer is oriented toward the final coordinates of the GPe on the stereotaxic frame.
8. Calculate the final coordinates of the recording chamber on the stereotaxic frame.
- a. Secure the second stereotaxic tower with a longer pointer attached to the arm of the frame corresponding to the desired (right/left) side of the monkey.
  - b. Tilt it 40° to 50° laterally in the coronal plane.
  - c. Move the second tower antero-posteriorly until the second pointer is in front of the first pointer and move it up or down at the set angle until it touches the first pointer.
  - d. Read the exact position of the second tower for each axis/dimension on the stereotaxic frame.

**Note:** These measurements are the coordinates to position the chamber. There is no need to measure the height, as it does not affect the trajectory since the exact distance depends on the size of the monkey's head.

- e. Keep the configuration of the second stereotaxic tower as it will be oriented during surgery to properly position the chamber.
- f. Replace the second pointer by the rod holding the chamber, which must be cold-sterilized prior to installation.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
NaNO <sub>2</sub>	Sigma-Aldrich-Merck	Cat#S2252
KOH	Sigma-Aldrich-Merck	Cat#06103
Isoflurane	Sigma-Aldrich-Merck	Cat#Y0000858
Dental acrylic cement	D2DHealthco	N/A
C&B Metabond Quick Adhesive Cement	Parkell Inc	N/A
Medetomidine	Sigma-Aldrich-Merck	Cat#M-252
Ketamine	Sigma-Aldrich-Merck	Cat#Y0000450
Antisedan	Sigma-Aldrich-Merck	Cat#A9611
Carprofren	Sigma-Aldrich-Merck	Cat#Y0000846
Ceftriaxone	Sigma-Aldrich-Merck	Cat#C0691000
Diazepam	Sigma-Aldrich-Merck	Cat#D0940000
Lidocaine Hydrochloride gel	Sigma-Aldrich-Merck	Cat#L0600000
Poldine solution or Betadine surgical scrub	Sigma-Aldrich-Merck	Cat#PVP1
Chloramphenicol 5%	Rekah Pharmaceutical Industry	N/A
Bupivacaine/Lidocaine	Sigma-Aldrich-Merck	Cat#B1160000
Adrenaline	Sigma-Aldrich-Merck	Cat#Y0000882
Atropine	Sigma-Aldrich-Merck	Cat#Y0000878
Dexamethasone	Sigma-Aldrich-Merck	Cat#D-085
70% ethanol	Sigma-Aldrich-Merck	Cat#470198N/A
Saline solution	Sigma-Aldrich-Merck	CatS8776
Agar	Sigma-Aldrich-Merck	Cat#1296
Experimental models: Organisms/strains		
Macaque fascicularis monkeys	The B virus Free Cynomolgus (BFC) monkey Breeding Farm. Mazor Farm, Israel	N/A
Software and algorithms		
Alpha-Map	AlphaOmega Engineering	N/A
Electrode Positioning System	AlphaOmega Engineering	N/A
Multi-Channel Processor	AlphaOmega Engineering	N/A
Alpha Spike Detector	AlphaOmega Engineering	N/A
Matlab	Mathworks	R2007b (7.5)
Other		
Metal electrode etcher	BAK Electronics	Model EE1D
Horizontal micropipette puller	Bergman's lab	Custom made
Beveler	World Precision Instrument	SYS-48000 MicroBeveler
Impedance conditioning module	FHC	Cat#55-70-0
Tungsten rod	A-M Systems	Cat#716200
Carbon rods	Alfa Aesar	Cat#40768
Glass capillary tube	A-M Systems	Cat#629500
Place abrasive disc (delicate)	World Precision Instrument	Cat#48016-03
Place abrasive disc (rough)	World Precision Instrument	Cat#48076-10
Stereotaxic equipment	Kopf Instruments	Model 1404 heavy-duty research stereotaxic
Bair Hugger warming system	Bair Hugger	N/A
ChiroPro 980, Dental, Electric Console and Motor System with Foot Pedal	Bien-Air Dental	N/A



MRI-compatible Cilux head holder	Crist Instrument	N/A
27 mm (inner edge) square Cilux recording chamber	AlphaOmega Engineering	N/A
Ground screw	Crist Instrument	6-YCX-06L Cranial Fasteners, w/nuts & washer 6-32 x 5/8" (15.87 mm)
Titanium dental screws	D2DHealthco	N/A
Primate chair	Crist Instrument	N/A

**STEP-BY-STEP METHOD**

**Stereotaxic surgery for chamber and head-holder implantation**

**Timing: 4 h**

This step describes how to surgically expose the skull, attach an MRI-compatible Cilux head holder (Crist Instruments, MD) (Figure 3A, Bottom) and a 27 mm (inner edge) square Cilux recording chamber (AlphaOmega Engineering, Israel) (Figure 3A, Top) that enables access to the GPe under deep anesthesia and aseptic conditions. In some laboratories, the craniotomy is performed when the animal is fully recovered from the stereotaxic surgery for chamber and head holder implantation to reduce the risk of infection. The approach described below involves performing the craniotomy and chamber implantation during the same surgical procedure to reduce the risk associated with two anesthetic procedures. This one-step approach therefore requires extreme caution regarding aseptic conditions.

**Note:** Withhold food for 12 h and access to water for 2 h prior to surgery.

1. Induce anesthesia.
  - a. Inject medetomidine (0.1 mg/kg IM) and wait 15 min.
  - b. Inject ketamine (10 mg/kg IM) and wait until the monkey is asleep.
- Note:** Veterinarians recommend injecting a mixture of ketamine and diazepam (Valium).
  - c. Shave the monkey’s legs to prep for the intravenous infusion, and the monkey’s head and ears for chamber implantation.
  - d. Attach intravenous line.
  - e. Administer analgesics (e.g., Carprofen, 4 mg/kg SC) and antibiotics (e.g., Ceftriaxone, 35 mg/kg IV or IM).
  - f. Place and secure the endotracheal tube.
2. Place the monkey in the stereotaxic frame.
  - a. Insert the ear bars into the ear canals.
    - i. Move them simultaneously without releasing the monkey’s head until the numbers on the scales (in mm) on both sides are the same.
    - ii. Lock the ear bars.

**Note:** The monkey’s head should be in the center of the frame.

**Note:** Place local anesthetic (e.g., Lidocaine Hydrochloride gel) on the ear bars to prevent pain and locate the ear canals with a cotton swab before inserting the bars.

- b. Place the mouth grip into the animal's mouth, so that it is positioned against the hard palate.
  - i. Hold it in place such that the animal's head is straight and its eyes are facing forward.
  - ii. Secure the eye grips such that they fit tightly into the ventral grooves of the orbital socket.

**Note:** Use eye ointment (e.g., Chloramphenicol 5%) to keep the cornea moist during the procedure.

- c. Ensure that ear bars, mouth grip and eye grips are all secured.
3. Start administering isoflurane and N<sub>2</sub>O gas anesthesia.
4. Set up the monitoring equipment for end-tidal CO<sub>2</sub>, arterial O<sub>2</sub> saturation, ECG, blood pressure and rectal temperature.
5. Turn on the Bair Hugger warming system to keep the animal warm during surgery and cover the machine with a sheet.
6. Prepare for the first incision.
  - a. Swab the monkey's head with Poldine solution or Betadine surgical scrub (Providine-iodine 7.5%) and alcohol.
  - b. Inject the local anesthetic (e.g., Bupivacaine/Lidocaine) at the incision site (SC along the scalp midline).
  - c. Scrub, then put on a disposable sterile surgical gown and gloves.
  - d. Spread a large sheet over the monkey and place sterile drapes around the surgical field.
  - e. Cover the suction tube and the wire of the drill (e.g., ChiroPro 980, Dental, Electric Console and Motor System with Foot Pedal, Bien-Air Dental, Switzerland) with sterile plastic sleeves and prepare the diathermy system.
7. Make a scalpel incision along the midline of the scalp (anterior-posterior).
  - a. Pull the sides of the skin down.
  - b. Scrape away the connecting tissue above the skull.
  - c. Insert weighted hooks into the soft tissue on either side of the incision to expose the skull.

**Note:** From this stage on, the skull should be moistened (with moist gauze) as often as possible. Be careful not to wet the drapes around the surgical field, as this will compromise sterility.

8. Attach the stereotaxic tower with the chamber to the stereotaxic frame.
  - a. Move it to the final coordinates of the recording chamber.

b. Mark the inside of the chamber with a sterile marker on the skull.

9. Shape the edges of the chamber with flame drill heads to fit the curvature of the skull.

10. Drill (using 21/23 drill heads) around the marked area on the skull and remove the bone flap to expose the dura (Figure 3B). **Troubleshooting 1**

**Note:** During drilling, flush saline solution over the drilling area to prevent overheating.

11. Place moist gauze over the dura.

12. Mark the positions of the head holder and transcranial (ground) screws on the skull.

13. Drill holes in the skull to anchor the ground screws and insert titanium dental screws using the appropriate screwdrivers.

14. Mount the head holder on the skull in the correct position and wrap titanium wire around the titanium dental screws, the chamber and the head holder (Figure 3C).

**Note:** Be careful not to connect the two ends of the titanium wire, to avoid ground loops that can lead to noisy recordings.

15. Using a pen-brush, apply a thin layer of dental acrylic cement to connect and solidify the ground screws, titanium dental screws, chamber and head holder.

**Note:** Gradually add dental acrylic cement to the remaining areas, paying attention not to cover the inner surface of the chamber or the head holder.

**CRITICAL:** It is crucial at this stage to make sure that the bone is dry and free of blood and soft tissue. Because acrylic bonds poorly to healthy bone, a capillary gap can form between the acrylic headcap and the skull surface that provides an ideal environment for bacterial colonization. Once infected, the bone becomes necrotic, eventually resulting in expulsion of the retaining screws and the failure of the implant<sup>5</sup>. Therefore, before applying dental acrylic cement, we recommend carefully using bone scrapers to remove remnants of soft tissue, apply hydrogen peroxide to the surface of the skull for 1-2 minutes, rinse with saline and betadine, and dry with gauze. If necessary, the coagulation mode of the diathermy system can be used before applying hydrogen peroxide to stop minor bleeding on the surface of the skull caused by bone scraping. Furthermore, a thin layer of C&B Metabond Quick Adhesive Cement (Parkell Inc., USA) can be applied to the contours of the craniotomy before placing the chamber. This will seal the interface between the chamber wall and the bone. The cement can be extended to some of the bone screws to provide an anchor. Then, allow the cement to dry for several minutes and finally apply the standard acrylic dental cement to complete the coverage of the chamber, head holder and screws.

**Note:** The outer edge of the acrylic cement should be as smooth and continuous as possible.

16. Fill the chamber with saline solution to check for leaks. Use suction to empty the chamber.

17. Apply topical ointment (e.g., Chloramphenicol 5% ointment) to the upper edge of the chamber and seal it with its cover.

18. Discontinue the isoflurane and N<sub>2</sub>O gas anesthesia while continuing oxygen support.
19. Release the animal.
  - a. Take the monkey out of the stereotaxic frame.
  - b. Allow the animal to recover on the operating room table until extubation.
  - c. Remove all remaining monitoring equipment (i.e., infusion, thermistor, ECG and pulse oximeter).
  - d. Return the monkey to its home cage lined with sheets or pads. **Troubleshooting**

## 2

**Note:** Once the monkey is fully awake after surgery, restore access to water and place fresh soft food into the cage. Administer the following post-surgical analgesics (Carprofen, 4 mg/kg SC) and antibiotics (Ceftriaxone, 35 mg/kg IV or IM) for 3 days. If the animal shows no signs of pain or any other surgical complications (e.g., epileptic seizures) the next day, restore normal diet and release the animal in the yard with its peers. Two days after surgery, place the monkey in its primate chair, fixate its head in the experimental setup and flush the chamber with saline solution. Make sure there is nothing unusual during the first chamber assessment: the chamber cover remains intact and is easily removed, and the dura appears undamaged. Three days after surgery, flush the chamber and keep the monkey attached by the head holder for 15 to 30 min so that it can get used to it. This timeline corresponds to the scenario where the animal shows no signs of pain or other surgical complications. A longer recovery period may be necessary.

### **Anatomical MRI scan (Whole-body 3T, 32-channel head coil)**

Four days after surgery, perform an MRI scan to verify that the position of the chamber is correct and to confirm the stereotaxic coordinates of the neuronal recordings.

#### **Timing: 2 h**

**Note:** Withhold food for 12 h and access to water for 2 h prior to surgery.

20. Prepare the chamber for imaging.
  - a. Place the monkey in its primate chair and fixate its head in the experimental setup. **Troubleshooting 3**
  - b. Flush the chamber with saline solution.
  - c. Insert five tungsten electrodes into the animal's brain at the equally spaced coordinates of the recording chamber [Y,X = (-6,0), (0,-6), (0,0), (0,6) and (6,0) mm from the center of the chamber] so that 10 mm of each electrode penetrates the brain.

**Note:** An electrode can be mounted into a cannula to facilitate passage through the dura. After inserting the electrode, carefully remove the cannula so that the electrode remains in place. Repeat this step for each electrode.

d. Prepare 3% agar (i.e., mix 3 g of agar powder with 100 ml saline solution) and heat the solution for 30 s in a microwave.

i. After the solution is cold, fill the chamber as much as possible.

ii. Cover it with parafilm.

iii. Detach the monkey's head from the head holder.

**Note:** Filling the chamber with agar was effective in stabilizing the electrodes in our experiments. This can also be done using gelatin or silicone.

21. Induce anesthesia.

a. Half an hour before scanning, inject medetomidine (0.1 mg/kg IM) and take the animal to the MRI facility on a wheeled cart.

b. In the MRI procedure room, inject ketamine (10 mg/kg IM) and wait until the monkey is asleep.

22. Scan the animal.

a. Place the monkey's head in the MRI-compatible stereotaxic frame.

b. Position the head coil directly above and parallel to the monkey's head, so that it is centered on the scan area of interest.

c. Move the animal to the center of the magnet.

**Note:** At this point, everything is ready to start the acquisition. The MRI sequences include a high-resolution T2-weighted TSE sequence of: TR = 4232 ms, TE = 63 ms, flip angle = 180°. 70\*1 mm coronal slices (no gap) with a field of view (FOV) of 240\*240 mm and matrix 320\*313 (in-plane resolution of 0.75\*0.77 mm). Four averages are used to improve the signal-to-noise ratio. The coronal slices are positioned on a T2-weighted TSE sagittal sequence (TR = 2200 ms, TE = 63 ms, flip angle = 180°, 35 slices of 1 mm with 10% gap, FOV = 240\*240 mm, matrix = 320\*313).

23. Release the animal.

a. When the scan is complete, the monkey can be removed from the magnet, placed back on the wheeled cart and taken to the experimental setup room.

b. Fix the monkey's head in the experimental setup and carefully remove the agar and the electrodes.

c. Flush the chamber with saline solution and seal it with topical ointment (e.g., Chloramphenicol 5% ointment).

d. Detach the head holder, allow the monkey to wake up gradually, and then return it to its home cage, as was done after surgery.

24. Load, display the coronal MRI slices, and mark the edges of the chamber to assess the extent to which and where it covers the GPe (Figure 4).

**Note:** An additional MRI scan can be performed at the final stage of the recordings to re-verify the location of the recording sites and rule out significant brain shifts.

## Extracellular recordings

**Timing: 3 h/day, 5 days/week, 3 months**

This step details how to record extracellular neuronal activity in the GPe of behaving monkeys daily.

25. Prepare the animal and the electrophysiological recording equipment.

a. Place the monkey in its primate chair and fixate its head in the experimental setup. **Troubleshooting 3**

b. Flush the chamber with saline solution.

c. Connect the electrode manipulator (Microdrive, AlphaOmega Engineering, Israel) to the chamber.

**Note:** The electrode manipulator enables X and Y control of several electrodes, as well as micron-level precision when driving the electrode in the Z plane during acute and restricted animal experiments.

d. Place the electrodes in the correct X and Y coordinates to access the GPe.

**Note:** The stainless-steel guide tube containing the electrodes must be lowered to the surface of the dura.

e. Fill the chamber with 3% Agar.

f. Connect electrodes to the headstage preamplifiers.

g. Turn on the data acquisition system (Alpha-Map, AlphaOmega Engineering, Israel) and load the settings.

h. Start lowering the eight glass-coated tungsten microelectrodes (Electrode Positioning System, AlphaOmega Engineering, Israel) until they penetrate the brain. **Troubleshooting 4**

i. Measure the impedance of the electrodes to make sure they did not break as they passed through the dura.

**Note:** If broken, retract the electrode. Depending on the number of broken electrodes, the condition of the animal and other factors, replace the broken electrodes and restart the downward movements.

26. Data collection. **Troubleshooting 5**

a. Advance the glass-coated tungsten microelectrodes in steps of 10 to 20  $\mu\text{m}$  toward and through the GPe.

**Note:** Electrical activity must be amplified at 5000, band-pass filtered from 1 to 6000 Hz using a four-pole hardware Butterworth filter and sampled at 25 kHz by a 12-bit ( $\pm 5\text{V}$  input range) Analog/Digital (A/D) converter (Multi Channel Processor, AlphaOmega Engineering, Israel). This band-pass filtered signal (1-6000 Hz) contains the (1-250 Hz) local field potential (LFP, which probably represents the global signature of the synaptic inputs and dendritic processing)

and the (250-6000 Hz) multi-unit activity (MUA, which reflects the efferent/output activity of the local neuronal population) (Figures 5A and B).

- b. Reduce the step size (5 to 1  $\mu\text{m}$ ) when approaching the GPe and look for well-characterized spiking activity of the GPe.

**CRITICAL:** The GPe border is characterized by sudden high frequency discharge activity below the striatum. The border between GPe and GPi is identified based on the depth of the electrode within the pallidum, the detection of pallidal border cells (characterized by their typical regular firing pattern and broad action potential) and the differences in the activity patterns of GPe and GPi cells<sup>6,7</sup>. In the GPe of the awake behaving monkey, neurons exhibit a high-frequency discharge interrupted by pauses (HFD-P neurons) or a low-frequency discharge with occasional brief high-frequency bursts (LFD-B neurons). In contrast, in the GPi, almost all neurons display continuous (no pauses) high-frequency discharge.

- c. Detect and sort the GPe spiking activity online from the MUA using a template-matching algorithm (Alpha Spike Detector, AlphaOmega Engineering, Israel) or any other real-time spike detection method.

**Note:** In our setup, up to three different units can be isolated from the same electrode, and the detection timestamp of the sorted units is sampled at 40 kHz (Figure 5B).

#### 27. Stop recording and release the animal.

- a. Once the recording session is over, retract all electrodes, disconnect the electrode manipulator and turn off the data acquisition system.
- b. Carefully remove the agar.
- c. Flush the chamber with saline solution and seal the chamber cover with topical ointment (e.g., Chloramphenicol 5% ointment).
- d. Disconnect the head holder and return the monkey to its home cage.
- e. Clean the electrodes with hot water and replace the broken ones with new electrodes.

#### 28. Data preprocessing.

- a. Examine (offline visual inspection) the firing rate stability of each sorted unit over its recording span and manually select the longest stable period for further analysis.

**Note:** The remainder of the data are discarded.

- b. Quantify the isolation quality of the sorted units (only for the selected stable period) by calculating their isolation scores<sup>8</sup>.

**Note:** The isolation score ranges from 0 (i.e., multi-unit activity/noise level) to 1 (i.e., perfect isolation). In our recent study in Cell Reports<sup>1</sup>, only units that exhibited a stable firing rate for at least 15 min and an isolation score  $\geq 0.7$  were included in the database.

## EXPECTED OUTCOMES

Successful multi-electrode extracellular neuronal recordings in the GPe of NHPs enable the collection of the activity of well-isolated units and the identification of high- and low- frequency neurons based on their firing rate and pattern. The collection of neuronal activity at different spatial resolutions (LFP, spiking multi- and single-unit activity) provides a unique physiological database (composed of hundreds of neurons and neuronal pairs within the GPe) that allows for a variety of analyses in the time and frequency domains, starting with spiking (output) activity (e.g., peri-stimulus histograms, spike-to-spike correlations or auto- and cross-spectral density analyses) up to multi-spatial resolution analyses (e.g., spike-triggered average of the LFP or spike-field coherence analysis) depending on the project design.

## LIMITATIONS

This experimental method (extracellular recordings in the GPe of awake behaving monkeys) can be used to analyze the electrophysiological features of both NHP GPe LFD and HFD neurons. However, it cannot be used to characterize their biochemical, structural or network properties. Other studies using a juxtacellular recording-labeling technique and/or optogenetics need to be conducted to obtain these data. Another major limitation is the use of acute recording systems, which make it possible to advance the electrodes into the GPe on a daily basis, thus increasing the likelihood of pinpointing the neurons. This technique is critical for collecting large numbers of well-isolated neurons in the GPe, which is extremely important when examining spontaneous and behavioral task-related neuronal firing discharges and patterns. Nevertheless, unlike high-density chronic neural recordings and/or calcium imaging techniques, these acute recordings cannot monitor the activity of the same neurons over several days/weeks and do not permit a longitudinal examination of neuronal activity.

## TROUBLESHOOTING

### Problem 1

Accidental dural tears during craniotomy. (related to Step 10)

### Potential solutions

When the dura is torn or damaged, the decrease in cerebrospinal fluid (CSF) pressure leads to a series of clinical symptoms often followed by complications such as infections and neurological dysfunction<sup>9</sup>. The first action to take is to rinse thoroughly with saline solution (~500 ml) to assess the extent of the damage and discuss with the veterinarian the possibility of suturing the dura. Instead, Gelfoam (absorbable gelatin compressed sponge) can be used to cover a small dural incision. The most common remedy for dural tears or small defects is primary suturing using absorbable suture materials. If the damage is too large for direct repair,



dura mater substitutes can be considered. Veterinarians recommend anti-inflammatory treatment and the systemic administration of antibiotics as a preventive measure.

### **Problem 2**

Inflammation and infection of the brain subsequent to chamber and head-holder implantation surgery. (related to Step 19d)

#### **Potential solutions**

After surgery, the animals should be housed in individual cages under supervision until fully awake. After reintroduction to the group, the animals should be carefully monitored for the first postoperative week for any signs of discomfort or distress (excessive withdrawal from peers, abnormal posture, excessive vocalization, weight loss) that may be due to underlying inflammation and/or infection. Two days after surgery, when flushing the chamber for the first time, the dura should be assessed for damage. If necessary, antibiotics (Ceftriaxone, 30 mg/kg IM) and steroids (Dexamethasone, 0.25-1.0 mg/kg IM) should be given in consultation with the veterinarian. Flushing of the chamber and assessment of the dura should be done daily thereafter.

### **Problem 3**

Accidental detachment of the acrylic headcap from the skull. (related to Steps 20a and 25a)

#### **Potential solutions**

When the acrylic headcap is partially or fully detached from the skull, the animal must be anesthetized and the acrylic headcap removed completely. Based on the damage to the skull, a decision must be made as to whether the experiment should continue. If the decision is to continue the recordings, a new surgical procedure must be carried out to reimplant a new chamber over the existing craniotomy and attach a new head holder.

### **Problem 4**

Scar tissue growth and dura thickening. Since an inflammatory wound-healing response begins soon after the dura mater is first surgically exposed, scar tissue forms and the dura thickens, causing the recording electrodes to break during transdural penetration. (related to Step 25h)

#### **Potential solutions.**

To curtail connective tissue growth that can distort and/or break the recording electrodes, the dura must be stripped of this growth during surgery under aseptic conditions and mild sedation.

These surgical interventions interrupt the daily recording routine, interfere with the animal's performance on the behavioral task, and adversely affect its general well-being. Therefore careful removal of soft connective tissue and clotted blood from the previous day, using a sterile cotton swab, will slow down the solidification process. Alternatively, the application of an antimitotic compound, 5-fluorouracil (5-FU) to the exposed dura can also be used to suppress the growth of connective tissue over the exposed dura and reduce the vascularization of this tissue in the recording chamber<sup>10</sup>.

### **Problem 5**

Noise in the electrophysiological recordings. (related to Step 26)

### **Potential solutions**

To limit noise, the animal and the equipment should be placed in a Faraday cage to reduce environmental electromagnetic fields as much as possible. The power supply for the equipment should be located outside the Faraday cage, and ideally only DC power cables should enter the room. The Faraday cage and equipment must be properly grounded. When the animal is sitting in the primate chair it must be connected to the common ground to avoid interference when touching the equipment (e.g., joystick, touchscreen, etc.). If the noise persists, aluminum foil or mesh can be used to cover the microdrive and electrodes.

## **RESOURCE AVAILABILITY**

### **Lead contact**

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Shiran Katabi ([shiran.katabi@gmail.com](mailto:shiran.katabi@gmail.com))

### **Technical contact**

Questions about the technical specifics of performing the protocol should be directed to and will be answered by the technical contact, Shiran Katabi ([shiran.katabi@gmail.com](mailto:shiran.katabi@gmail.com))

### **Materials availability**

This study did not generate unique reagents.

### **Data and code availability**

This study did not generate new datasets or code.

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## AUTHOR CONTRIBUTIONS

S.K., H.B. and M.D. designed the original research. S.K. and Z.I. performed the surgery described here. S.K. and M.D. recorded and collected the data as described in this protocol. S.K., H.B. and M.D. wrote the manuscript. All authors read and commented on the final version of the manuscript.

## DECLARATION OF INTERESTS

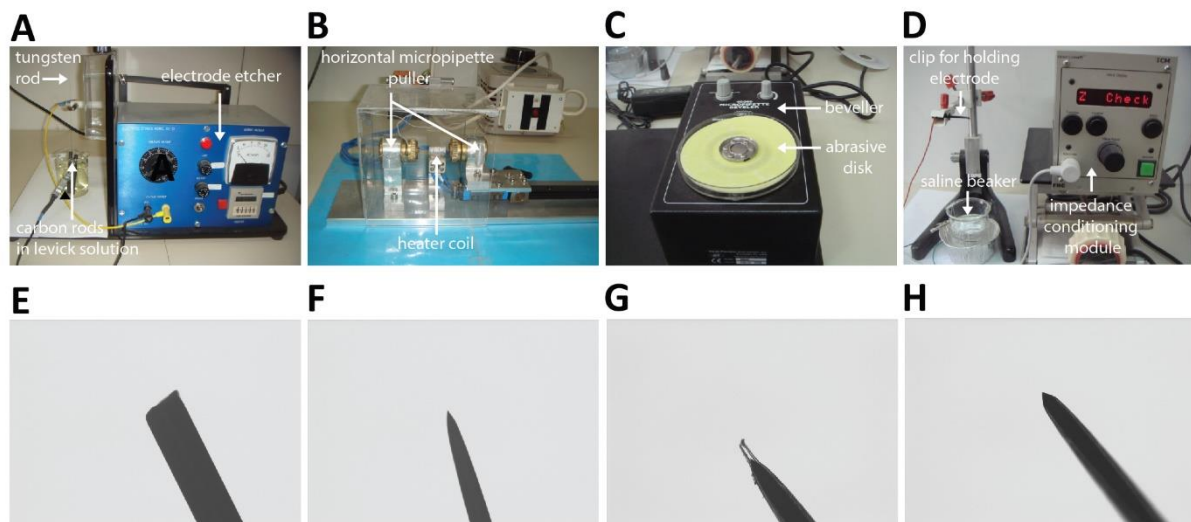
The authors declare no competing interests.

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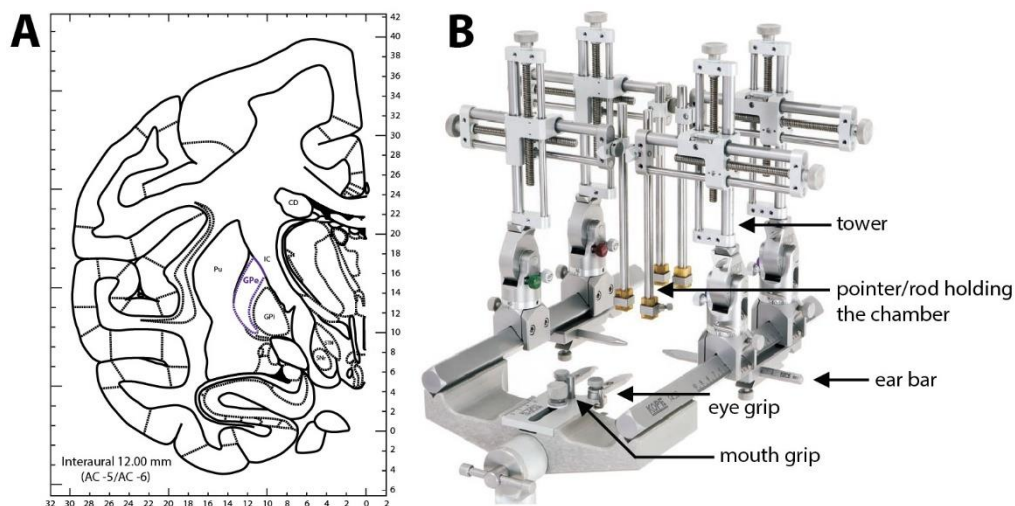
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## FIGURES



**Figure 1. Electrode manufacturing equipment and steps**

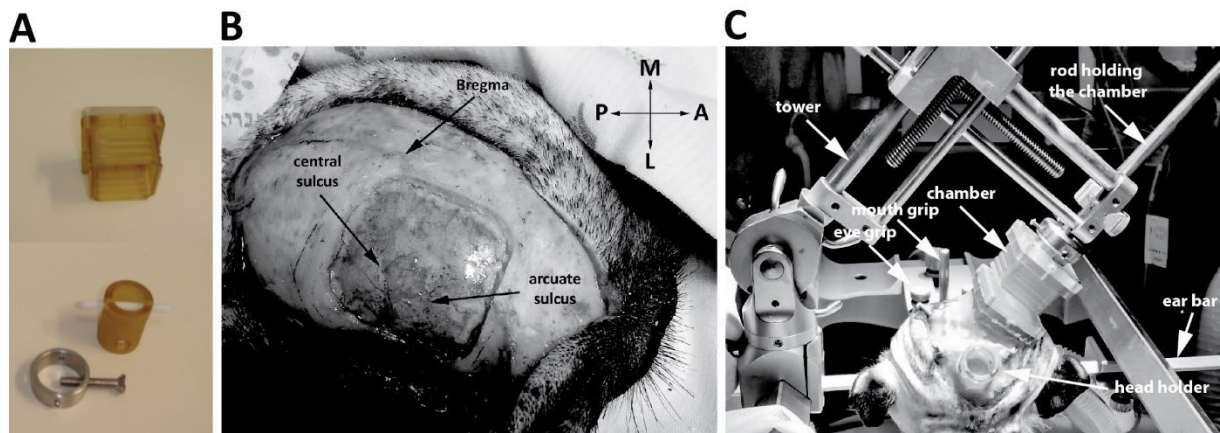
- (A) Electrode sharpening equipment.
- (B) Horizontal puller used for electrode coating with a glass capillary.
- (C) Beveling instrument used to exposing the tip of the electrode.
- (D) Electrode impedance meter.
- (E) Tungsten rod before sharpening (x20).
- (F) Tungsten rod after sharpening (x10).
- (G) Glass-coated microelectrode before beveling (x10).
- (H) Glass-coated microelectrode after beveling and tip exposure (x10).



### Figure 2. Stereotaxic targeting of the external globus pallidus

(A) Representative coronal section at +12 mm from the interaural line adapted from Paxinos et al. (2008). CD: caudate nucleus; Pu: putamen; GPe-i: external and internal segment of the globus pallidus; IC: internal capsule; STN: subthalamic nucleus; SNr: substantia nigra pars reticulata.

(B) Stereotaxic frame with its towers (David Kopf Instruments, CA).

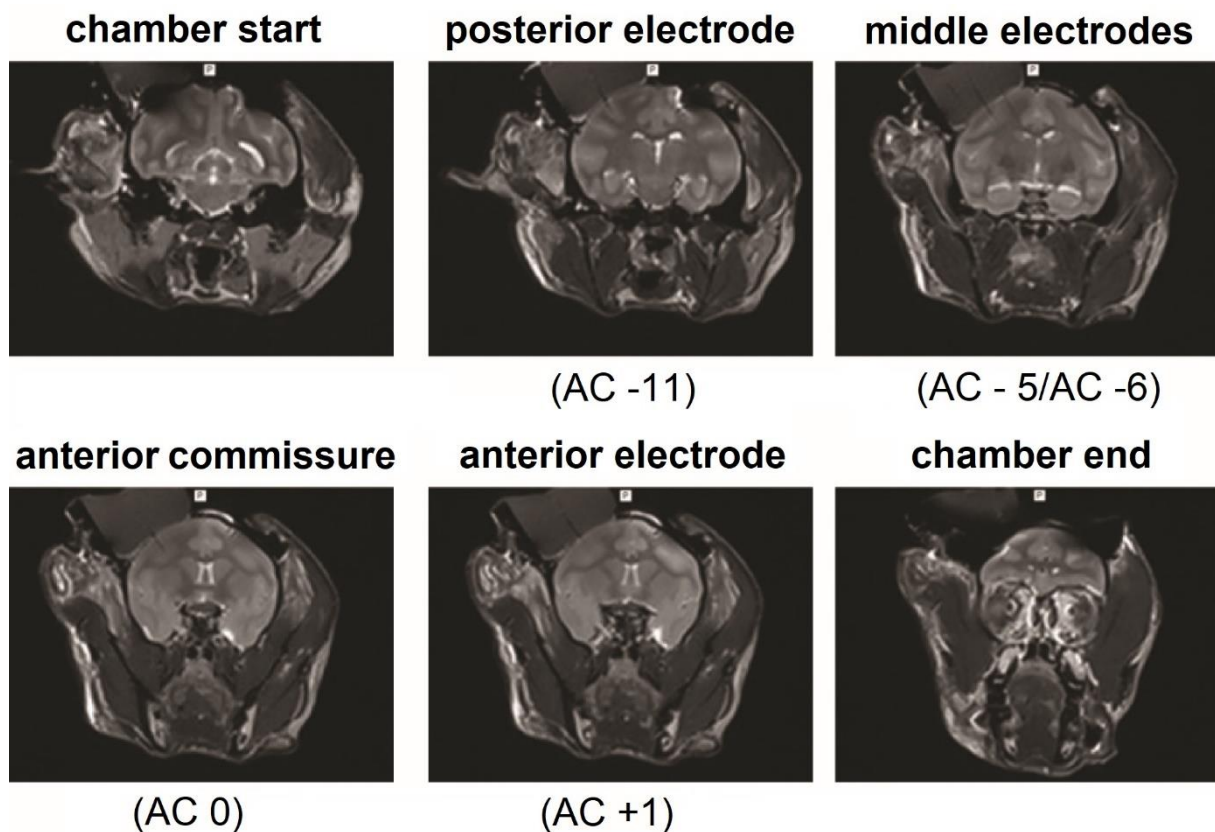


### Figure 3. Chamber and head-holder implantation

(A) Top, 27 mm (inner edge) square Cilux recording chamber (AlphaOmega Engineering, Israel). Bottom, MRI-compatible Cilux head holder (Crist Instruments, MD).

(B) Surface of the dura after craniotomy.

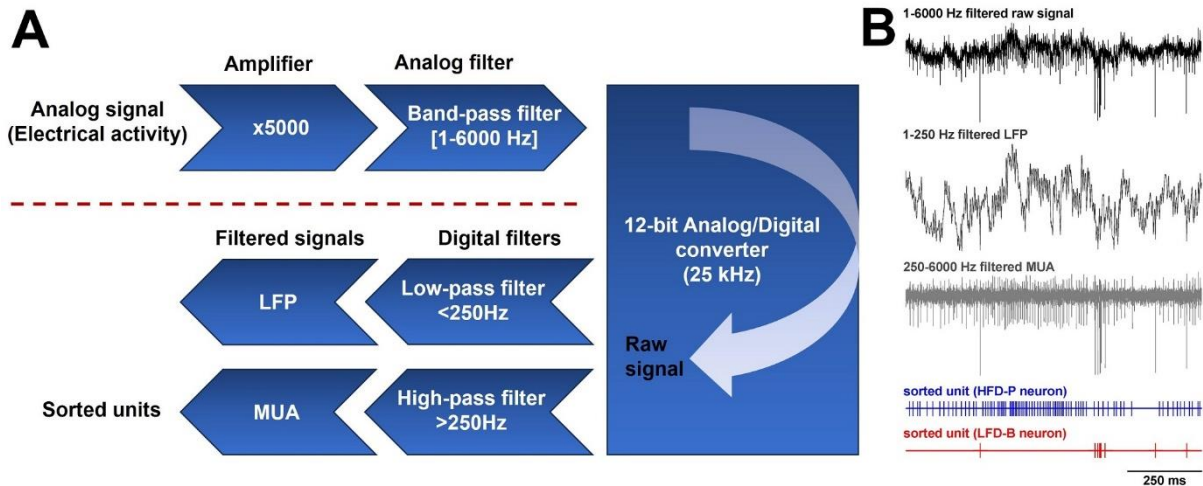
(C) View of the back of the monkey's head fixed in the stereotaxic frame. The square chamber is attached to the stereotaxic tower and positioned just above the burr hole.



**Figure 4. Post-operative MRI scan**

Coronal sections of post-operative MRI scan of a monkey's brain showing the location of the recording chamber and five tungsten electrodes inserted into the animal's brain at equally spaced coordinates matching the recording chamber [Y, X = (-6,0), (0,-6), (0,0), (0,6) and (6,0) mm from the chamber center]. The chamber position covers the entire GPe.





**Figure 5. Recording of LFP and spiking activity in the GPe of a non-human primate.**

(A) Electrical signal processing pipeline. The diagram shows the main signal processing steps. (B) The traces depict the different properties of the filters applied to the signal recorded by the electrode. The upper trace depicts the raw (1-6000 Hz) band-pass filtered trace. The second trace was low-pass filtered with a cut-off frequency of 250Hz and depicts the local field potential (LFP). The third trace was high-pass filtered with a cut-off frequency of 250Hz and depicts the multi-unit activity (MUA). The digital display of the spikes of two distinct neurons detected and sorted from the MUA is shown below the 250-6000 Hz filtered MUA.