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Fiber Photometry during Sleep

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Protocol status: Working

We use this protocol and it's working



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Abstract

Measurement of photometry during sleep using AAVs

Fiber Photometry- Virus Injection

1 **Preparation:**

- 1.1 Prepare the adenovirus solution at the desired concentration.
We use a dopamine and acetylcholine sensor (AAV-Ach-SnFR addgene #137955 and AAV-rGRAB-DA addgene #140557) with a ratio of 1:1.
- 1.2 Set up the surgical area in a clean and sterile environment.
We recommend using a quartz beads sterilizer for all procedures.
- 1.3 Prepare the anesthesia setup according to institutional guidelines.

2 **Anesthesia and Animal Preparation:**

- 2.1 Induce anesthesia using isoflurane in an induction chamber.
We recommend using between 2-3% Isoflurane in O₂.
- 2.2 Confirm the depth of anesthesia by checking the pedal reflex.
- 2.3 Once adequately anesthetized, transfer the mouse to a stereotaxic frame and secure its head using ear bars.
We use the digital control Kopf stereotaxic instrument (69100 series).
- 2.4 Place the heating pad or lamp to maintain the mouse's body temperature throughout the procedure.

3 **Surgical Site Preparation:**

- 3.1 Shave the scalp area over the skull to expose the surgical site.
We use epilation cream to provide better recovery.
- 3.2 Clean the exposed area with alternating iodine (50% in water).

4 **Injection Site Determination:**

4.1 Use stereotaxic coordinates to determine the injection site in the brain based on the desired region.
Here inject a total of 500nl of viral mix in the dorsomedial striatum (coordinates from the Bregma: AP 0.5mm, ML 1.1, DV 3.0mm).

4.2 Mark the coordinates on the skull surface using a surgical marker.

5 **Syringe Preparation:**

5.1 Load the Neuros7001 syringe with the adenovirus solution. As there is no dead volume, it is recommended to load only 5-10% more than the required volume.

5.2 Ensure there are no air bubbles in the syringe.

6 **Injection Procedure:**

6.1 Attach the syringe to the stereotaxic arm, ensuring stability and precision.

6.2 Lower the needle to the marked coordinates on the skull surface.

6.3 Slowly insert the needle into the brain to the desired depth.

6.4 Inject the adenovirus solution at a controlled rate using a microinjector.
We use 50-75 nl/min.

6.5 Wait for a few minutes to allow for diffusion of the solution. It is recommended to wait at least 50% of the injection time (*i.e.* Injection 10min, diffusion at least 5 minutes).

6.6 Slowly retract the needle from the brain.

7 **Post-injection Care:**

7.1 Apply pressure to the injection site with sterile gauze to minimize bleeding and leakage.

7.2 Clean the surgical site with sterile saline solution.

7.3 Close the incision with sutures.
We use 4.0 Vicryl suture with PGA to allow better recovery.

7.4 Monitor the mouse closely during recovery from anesthesia.

7.5 Return the mouse to its cage once fully recovered.

8 **Monitoring and Follow-up:**

8.1 Monitor the mice regularly for any signs of distress or complications.

8.2 Provide appropriate postoperative care, including analgesics if needed.

8.3 Follow institutional guidelines for postoperative monitoring and euthanasia procedures if necessary.

Fiber photometry- Implantation (2-3 weeks after AAV injection)

9 **Preparation:**

9.1 Prepare the surgical area in a clean and sterile environment.

9.2 Prepare the anesthesia setup according to institutional guidelines.

9.3 Ensure all surgical tools are sterilized and ready for use.

10 **Anesthesia and Animal Preparation:**

10.1 Induce anesthesia using isoflurane in an induction chamber.
We recommend using between 2-3% Isoflurane in O₂.

10.2 Confirm the depth of anesthesia by checking the pedal reflex.

10.3 Once adequately anesthetized, transfer the mouse to a stereotaxic frame and secure its head using ear bars.
We use the digital control Kopf stereotaxic instrument (69100 series).

10.4 Place the heating pad or lamp to maintain the mouse's body temperature throughout the procedure.

11 **Surgical Site Preparation:**

11.1 Shave the scalp area over the skull to expose the surgical site.
We use epilation cream to provide better recovery.

11.2 Clean the exposed area with alternating iodine (50% in water).

12 **Craniotomy:**

12.1 Use stereotaxic coordinates to determine the craniotomy site relative to the injection site.
As the implantation is 2-3 weeks after injection if the previous hole from AAV injection are visible and clean, you can ignore these steps.

12.2 Mark the coordinates on the skull surface using a surgical marker.

12.3 Make an incision in the scalp along the marked coordinates.

- 12.4 Use a micro drill or dental drill to create a small craniotomy window above the intended implantation site, being careful not to damage the dura.

13 **Implantation of Optic Fiber:**

- 13.1 Place the ferrule from the optic fiber on the holder.
We use a 0.50NA, 400µm, 4mm long, Thorlabs.
- 13.2 At various places, insert surgery screws (Agntho's #MCS1X2).
We recommend using at least 2 screws. Here, we are measuring sleep combined with photometry, the 2 anchor screws are for the sleep telemetry devices. Surgery is similar as the one for sleep, with the exception of the implantation of the fiber photometry.
- 13.3 Lower the optic fiber implant to the target depth using stereotaxic coordinates, ensuring it is positioned approximately 100-200 µm above the injection site.
- 13.4 Secure the optic fiber in place using dental cement or adhesive applied around the base of the fiber.
- 13.5 Allow the cement to dry completely.

14 **Postoperative Care:**

- 14.1 Apply topical antibiotic ointment to the surgical site to prevent infection.
- 14.2 Close the scalp incision using surgical adhesive or sutures.
- 14.3 Monitor the mouse closely during recovery from anesthesia.
- 14.4 Return the mouse to its cage once fully recovered.

15 **Monitoring and Follow-up:**

- 15.1 Monitor the mice regularly for signs of distress or complications.
- 15.2 Provide appropriate postoperative care, including analgesics if needed.
- 15.3 Ensure proper functioning of the optic fiber implant and patch cable setup before commencing experiments.

Fiber photometry- Habituation and Calibration

16 Day 1: Habituation

Preparation:

- 16.1 Set up the experimental room in a quiet, dimly lit area with minimal distractions.
- 16.2 Prepare the fiber photometry setup, ensuring all components are properly connected and functional.
- 16.3 Ensure the mice are acclimatized to the room for at least 1h before beginning the habituation.

17 Mouse Handling:

- 17.1 Gently handle each mouse to minimize stress and transfer it to the experimental cage.
- 17.2 Allow the mouse to freely explore the open lids cage for a designated period to habituate to the new environment.

18 Fiber Connection:

- 18.1 After habituation, connect the implanted optic fiber to the fiber photometry system without activating the light source.
We are using the Doric lenses FPS_2S_GCAMP+RedFluo system that allow acquisition of GCaMP and RCaMP signal combined with 405nm isosbestic. Connection is done with ceramic sleeves. The system collects 3 channels isosbestic channel (405nm), GCaMP

(460-490), and RCAMP (555-570) and collect isosbestic, Ach-SnFr, and rGRAB-DA signals at 1017Hz.

18.2 Ensure the connection is secure but does not restrict the mouse's movement.

19 **Monitoring:**

19.1 Observe the mouse for any signs of distress or discomfort during the habituation period.

19.2 Note any abnormal behaviors or reactions.
It is recommended to leave the mice alone in the room for 10-20min until the mouse seems to be sleeping, we are using webcam to monitor the animal behavior.

20 **Data Logging:**

20.1 Begin data logging on the fiber photometry system to capture baseline activity. Progressively increase each LED power until calcium spikes are visible. When one LED is set, turn OFF the LED and proceed to the next one. You need to see signals only in the dedicated signal to avoid cross talks.

20.2 After calibration, decrease progressively each LED power until no calcium spikes are visible, yet calcium oscillation is still present. This will avoid having strong and high amplitude calcium spike that might alter the analyses. For each mouse, note the signal amplitude as well as their dedicated LED power. In average we decrease for each channel the excitation power by 10%.

21 **Post-session Handling:**

21.1 After the habituation session, return the mouse to its home cage.

21.2 Clean the experimental cage and any equipment used with appropriate disinfectants. Repeat the experiment for 2-3 days.

22 Following this three-day habituation and calibration protocol will help ensure that the mice are acclimated to the experimental environment and that the fiber photometry system is properly calibrated for accurate data collection during subsequent experiments.

Fiber Photometry- Recording

- 23 **On the day of the recording, it is VERY IMPORTANT to proceed as early as possible to record as much sleep as possible.**

We record the animals ~1h after the switch from dark to light phase. We record bouts of 30 minutes and repeat the recording as many times as necessary.

- 24 **Day 4: Post-Experimental Recording and Analysis**
Post-Experimental Recording:

- 24.1 Repeat the setup process as on previous days. This time turn ON the EMG/EEG device at the same time as the photometry system. We are using the computer clock to synchronize both systems.

- 24.2 Record sleep parameters and fluorescence signals to assess post-experimental recovery and baseline levels.

- 25 **Data Analysis:**

- 25.1 Analyze recorded data from both sleep recordings and fiber photometry to extract relevant parameters.

- 25.2 Compare sleep patterns with neural activity recorded via fiber photometry to investigate correlations or effects of experimental manipulations.

- 25.3 Nota bene for analyses:

We processed sleep scoring as described, by extracting timestamps of Wake, REM, NREM, and RBD events. Using a custom-made Matlab script, photometry data were downsampled to 500Hz (average smooth window function), then aligned with the sleep scoring. For each 1-minute window, each channel of the photometry was normalized using the z-score function and then averaged over the 20s window corresponding to the sleep scoring. For each animal, photometry data were then averaged for specific sleep stages and compared across groups.