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GRAB sensor imaging in mouse striatal slices

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ABSTRACT

This protocol describes the steps to image GRAB sensors in mouse striatal slices.

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GRAB sensors, imaging, mouse ex vivo slices

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MATERIALS TEXT

Equipment:

- Iris 9 Scientific CMOS (sCMOS) camera Teledyne Photometrics
- Vibratome VT1200S Leica Biosystems
- Microscope BX50WI Olympus
- Microscope C-mount Camera Adapter U-CMAD-2
- Gilson MINIPULS 3 Peristaltic Pump (SKU: GFAM00051)
- Multichannel Systems stimulus generator (STG4004)
- Cairn Research OptoLED Light Source
- Digitimer DS3 Isolated Current Stimulator
- FHC stimulating electrode (SKU: 30200)

Virus:

- AAV5-hsyn-ACh3.0(ACh4.3) virus (WZ Biosciences, Cat #YL001003-AV5) - Titre: ~8x10^{12} GC/ml in saline

Reagents:

- Sodium Chloride (Sigma-Aldrich, Catalog #31434, CAS #7647-14-5)
- Sodium Bicarbonate (Sigma-Aldrich, Catalog #31437-M, CAS #144-55-8)
- HEPES (Sigma-Aldrich, Catalog #H3375, CAS #7365-45-9)
- HEPES Sodium Salt (Sigma-Aldrich, Catalog #H7006, CAS #75277-39-3)
- Potassium Chloride (Sigma-Aldrich, Catalog #P9541, CAS #7447-40-7)
- Calcium Chloride Solution (Sigma-Aldrich, Catalog #21114, CAS #10043-52-4)

Software:

- ImageJ (1.53q)
- ImageJ plugin: Micro-Manager Reader 1.3.38, May 3, 2009
- MATLAB (2020)

BEFORE STARTING

Stereotaxic intracranial injections were performed to deliver AAV-packaged GRAB sensors in the mouse striatum. After injection, mice were kept for 3 weeks to ensure appropriate construct expression.

Also note that this protocol describes the steps for recording changes in fluorescence signals measured with GRAB sensors resulting from electrically-evoked neurotransmitter release.

Slice Preparation

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1 Sacrifice mice by cervical dislocation and exsanguination, and collect the brain.

2 Cut 300 µm thick coronal slices using a vibratome in ice-cold HEPES-based buffer saturated with 95% O_2/ 5% CO_2, containing (in mM): 120 NaCl, 20 $NaHCO_3$, 6.7 HEPES acid, 5 KCl, 3.3 HEPES salt, 2 $CaCl_2$, 2 $MgSO_4$, 1.2 KH_2PO_4, 10 glucose.

3 Keep slices in a holding chamber for at least 1 hour at room temperature (20-22°C) in HEPES-based buffer before transferral to the recording chamber.

4 While slices are incubating, prepare the recording solution (artificial cerebrospinal fluid, aCSF). Bicarbonate-buffer based aCSF contains (in mM): 125 NaCl, 26 $NaHCO_3$, 3.8 KCl, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 1.2 KH_2PO_4, 10 glucose.

5 Clean the recording chamber and perfusion system with dH2O and then run aCSF through (perfusion rate ~ 2 ml/min).

Ensure that the recording chamber temperature is stable at 32°C (fluctuation range 31-33°C is acceptable).

6 Following 1 hour incubation, bisect slices and transfer to the recording chamber. Leave slice to equilibrate in the bath for for 30 minutes prior recording.

Check GRAB expression

7 Check GRAB sensor expression under a 10x immersion objective using a blue LED light (470 nm, ~ 10-11 mW) and an ImageJ plugin (MicroManager). We illuminate through the microscope turret and objective.

Image processing and data extraction

8 Acquire images under a 10x immersion objective using the ImageJ plugin MicroManager. We image using a CMOS camera.

9 After choosing a region of good sensor expression, position the stimulating electrode on

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tissue.

9.1 We electrically stimulate neurotransmitter release using a surface bipolar concentric Pt/Ir electrode (FHC Inc., outer/inner diameter 125/25 µm), applying pulses at 0.6 mA and for 200 µs.

9.2 If needed, change the camera acquisition rate. The camera default acquisition rate (30 frames per second, fps) can be modified to obtain higher sampling frequency. To achieve the highest sampling rate (100 fps), lower the camera exposure to 10 ms, and define a rectangular recording window smaller than the field of view. The recording window should include the tip of the stimulating electrode and be large enough to allow sampling from multiple regions.

10 Once the acquisition parameters have been finalised, proceed with acquisition. We generally electrically evoke neurotransmitter release every 2.5 minutes to ensure full recovery of terminals between pulses.

11 Open the image saved for each stimulation in ImageJ to extract fluorescence information.

12 Acquire one image for each stimulation condition applying continuous blue light for the time needed for the signal to reach baseline (depending on the off rates of different GRAB sensors).

13 Draw a square (100 µm x 100 µm) region of interest (ROI) and position 100 µm away from the tip of the stimulating electrode. Draw an ROI of the same size and position on the tip of the stimulating electrode to use as background.

14 Select Images > stacks > plot z-axis profile to extract fluorescence and frame values from the ROIs. Save the data in Excel.

15 It is possible to select multiple ROIs at different distances from the electrode to record signals further away from the stimulation source.

Data Analysis

16 Frame and fluorescence values were analysed with a custom written MATLAB code.

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17 First, convert the frames into time (s), knowing the acquisition sampling rate.

18 Subtract the background from the raw fluorescence values. Background was chosen as a ROI with no fluorophore expression (on the stimulating electrode) to account for non-specific changes in ambient light.

19 Fit a two-term exponential decay function ('exp2' function in MATLAB) to pre and post stimulation fluorescence values (F) to obtain baseline fluorescence values (F_0).

20 Calculate $\Delta F/F_0$ as $(F-F_0)/F_0$.

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