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Optical sensors 2-photon imaging

In 1 collection

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ABSTRACT

This protocol describes the steps to collect genetically encoded optical sensors fluorescence in mouse brain slices using 2-photon microscopy.
2-photon imaging was performed using a 2-photon laser scanning microscopy system, custom-built on a BX51WI microscope (Olympus). A Ti:Sapphire laser (Chameleon Ultra I; Coherent) was tuned to emit pulsed excitation at 920 nm and scanned using a pair of X-Y galvanometer mirrors (6215, Cambridge Technology). Emitted fluorescence was collected through a water-immersion objective (60X, Olympus), a dichroic mirror (T700LPXXR, Chroma) and filters (ET680sp and ET525/50 m-2P, Chroma), and was detected using a GaAsP photomultiplier tube (PMT, H10770PA-40, Hamamatsu). A current preamplifier (SR570, Stanford Research Systems) was used to convert the output to voltage, which was then digitized by a data acquisition card (PCI-6110, National Instruments).

MATERIALS

- 2-photon laser scanning microscopy system, custom-built on a BX51WI microscope (Olympus).
- Ti:Sapphire laser (Chameleon Ultra I; Coherent).
- X-Y galvanometer mirrors (6215, Cambridge Technology).
- Dichroic mirror (T700LPXXR, Chroma).
- Filters (ET680sp and ET525/50 m-2P, Chroma).
- GaAsP photomultiplier tube (PMT, H10770PA-40, Hamamatsu).
- Current preamplifier (SR570, Stanford Research Systems).
- Data acquisition card (PCI-6110, National Instruments).
- Toronado: https://github.com/StrowbridgeLab/Toronado-Laser-Scanning
- Axograph X (Axograph Scientific).

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BEFORE START INSTRUCTIONS

Viruses encoding for genetically encoded optical sensors are intracranially injected in the selected area of the brain by stereotaxic surgery. Imaging is performed 3-4 weeks after injections to ensure appropriate expression levels.

Acute brain slice preparation

1 Steps are described in the protocol linked below.

Protocol

NAME
Acute Brain Slices

CREATED BY
kelsey.barcomb

PREVIEW

Rig setup

2 Turn on required devices and software for acquisition (Toronado and Axograph).

- Toronado: https://github.com/StrowbridgeLab/Toronado-Laser-Scanning
- Axograph X (Axograph Scientific).

3 Set 1X ACSF (add drugs needed for particular experiments) in a jug or bottle and bubble it with O_2/CO_2.

1X ACSF
For 1L:

- 900 mL MilliQH2O + 100 mL stock 10X ACSF + 1.8 g NaHCO3 + 2 g D-Glucose

10X ACSF stock

	A	B	C
	Chemical	[mM]	10X Stock (g/4L)

	A	B	C
	NaCl	126	294.52
	KCl	2.5	7.44
	MgCl2*6H2O	1.2	9.75
	NaH2PO4*H2O	1.2	6.64
	CaCl2*2H2O	2.5	14.7
	NaHCO3	21.4	
	D-Glucose	11.1	

4 Place the intake line into the ACSF container and allow circulation. Wait until the fluid has entered the recording chamber, then turn on the in-line heater (Warner Instruments) and set it to desired temperature 32-34 °C

5 Electrical stimulation electrodes:

5.1 Pull electrodes (World Precision Instruments) using a puller (Narishige, PC-10).

5.2 Fill electrodes with 1X ACSF using a syringe with filter.

Image acquisition

6 Transfer brain slice from incubation vial to the recording chamber and secure down the slice using a harp.

7 Locate and focus on the desired region of the brain under IR-DIC using the low power (4x) objective.

8 Change the microscope lens to high power (60x) objective and focus on healthy neurons.

9 Turn off IR light source and switch to 2-photon laser scanning mode by sliding mirror to allow 2P laser excitation (wavelength: 920 nm), opening iris to PMTs, and start imaging using Toronado software and Axograph. Turn on PMT and dynode power sources.

10 Select a region of good sensor expression based on basal fluorescence using 'Focus' mode (Zoom 2.5) in Toronado.

11 If using electrical stimulation:
Position the stimulating electrode on the center of that region.
Set a protocol in Axograph with the appropriate stimulation intensity, duration and number of pulses depending on the experimental design.

12 Acquisition modes:

> **Note**
>
> Laser power must be tuned by adjusting attenuation through Pockels cells so that the fluorescence at baseline is bright enough but far from signal saturation.

- *Rasterized image/movie sequence*:

To measure spatial dynamics of fluorescence changes across the whole field of view (FOV), select an appropriate 'Zoom' and then press 'Movie' to acquire rasterized image sequences.
In order to evoke changes of fluorescence, electrically stimulate neurotransmitter/neuromodulator (i.e. ACh/DA) release and/or different drugs can be washed on after recording a baseline period. Timing of electrical stimulation is triggered by Axograph software.

- 2-*photon spot photometry:*

To measure high-speed changes of fluorescence, select an individual spot (ROI) from rasterized

image under appropriate 'Zoom' 'Morph' options. The 2P laser will be scanned across a circular path (diameter: 150 nm) centered at this spot, and emitted fluorescence will be continuously measured.
Set and run a protocol in Axograph with a 'Photometry' (TTL) pulse that must be equal to photometry duration on Toronado. PMT output is amplified, filtered and fed directly to A-D board (Digitizer).
In order to induce stimulatory events, electrical stimulation can be applied and/or different drugs can be washed on after recording a baseline period.

2-photon imaging GRABACh 3.0 recordings

1m

13

1m

- Rasterized image/movie sequence for electrically evoked ACh release:

Induce ACh release by electrical stimulation (estim: 25 µA, duration: 0.5 ms)
Record three to five movies per region. Wait 1-2 min between stimulations to ensure full recovery.
Using Fiji (Image J), stack the sequence of images from each recorded movie, average the movies from the same region, and obtain the change in fluorescence (ΔF/F0) in a square region of interest (25 µm x 25 µm). F0 was defined as the average of fluorescence in the images from baseline period.

Note: For slow pharmacological effects when drugs are bath applied, a time-lapse acquisition is preferred. Individual rasterized images were collected under 'Morph' mode every 00:01:00
Drugs were applied after 5-10 min of baseline.

- 2-photon spot photometry for paired-pulse (PPR) ratio experiments:

Induce ACh release by electrical stimulation (estim: 25 µA, duration: 0.5 ms, interstimulus interval: 100 ms). Analyze the change in fluorescence (ΔF/F0) in Axograph.