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Electrophysiology and 2-photon imaging of Ca^{+2}-transients V.2

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Abstract

This protocol describes the steps for imaging dendritic calcium transients evoked by backprogating action potentials. 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).

Before start

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)
	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	

	A	B	C
	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 Recording electrodes or patch pipettes:

5.1 Pull patch pipettes (1.5 – 2 MΩ) (World Precision Instruments) using an electrode puller (Narishige, PC-10).

5.2 Thaw an aliquot of the K-Gluconate internal solution for calcium imaging (stored at -20 °C)

	A	B	C
	Drug	[mM]	g/100 mL
	D-Gluconic Acid (K)	135	3.16
	HEPES (K)	10	0.28
	MgCl2	4	400 µL (1M stock)

+ 1 mg/mL ATP , 0.1 mg/mL GTP and 1.5 mg/mL phosphocreatine
pH=7.35, 275 mOsm

Add [M] 10 micromolar (µM) Alexa Fluor 594 to visualize dendritic arbor morphology and [M] 100 micromolar (µM) Fluo-5F, a calcium indicator, to enable the detection of dendritic calcium transients.

Fill a syringe with filter with the internal solution and keep on ice by the rig.

Ex vivo electrophysiology and Ca^{+2} transient 2-photon imaging

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 a healthy cell to patch.

9 Follow the steps indicated in the protocol linked below for whole cell recordings in "current clamp" mode.
Allow the internal solution containing the dyes to dialyze the cell before start your recordings.

Protocol

NAME

Ex vivo electrophysiology

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PREVIEW

10 Turn off IR light source and switch to 2-photon laser scanning mode by sliding mirror to allow 2P laser excitation (wavelength: 810 nm), opening iris to PMTs, and start imaging using Toronado software and Axograph. Turn on PMT and dynode power sources.

11 Select a region of interest in the dendritic arbor to measure the calcium transients using an appropriate zoom in Toronado.

12 Record the peak fluorescent changes of the calcium indicator Fluo-5F under the 2-*photon spot photometry* or *2-photon point scanning* acquisition mode in Toronado, which allows 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 to elicit a single backpropagating action potential (bAP) by injecting a somatic depolarizing current in current-clamp mode and add a 'Photometry' (TTL) pulse on that protocol with the same duration as the photometry pulse set on Toronado.
- Image three to five dendritic spots in the same optical plane to have an average per cell.
- Different drugs can be applied after recording a baseline period.

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.

13 Analyze recorded calcium transients in Axograph.