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# Epifluorescence Time-Lapse Imaging of Hippocampal Neurons

In 1 collection

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**We use this protocol and it's working**

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## Abstract

This protocol outlines the detailed steps for epifluorescence time-lapse imaging of hippocampal neurons, enabling the study of neuronal responses to various pharmacological and metabolic conditions.

## Guidelines

The protocol needs prior approval by the users' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee.

## Materials

**Materials:**

- **Tyrode's solution:** 119 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 50 mM HEPES (pH 7.4), 0-5 mM glucose, 10 mM CNQX ( 6-Cyano-7-nitroquinoxaline-2,3-dione **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**C127 ), 50 mM APV ( DL-2-Amino-5-phosphonopentanoic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A5282 )

- **BSA-complexed palmitate:** 150 µM for feeding media and 100 µM for perfusion (prepared as previously described in Fatty acid induction protocol)

- **Pharmacological inhibitors:**

1. 5 µM KLH45 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**SML1998
2. 40 µM Atglistatin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**SML1075
3. 2 µM Oligomycin from Streptomyces diastatochromogenes **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**O4876
4. 20 µM Etomoxir **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**E1905
5. Tetrodotoxin: 2 µM (Abcam, ab120055)

**Equipments:**

- Microscope: Custom-modified Zeiss Axiovert 200
- Camera: Andor iXon DU-897U-CS0-BVF
- Objective: Zeiss 40X 1.3 NA Fluar with temperature control (37°C)
- Lasers: OBIS solid-state 488 nm, 561nm and 635 nm
- Perfusion system: Laminar flow with temperature control (37°C) at objective
- Platinum-iridium electrodes
- Field stimulation setup capable of generating ~10 V/cm

## Preparation of Neurons

1 Culture hippocampal neurons on glass coverslips under standard conditions following previously described protocol.

2 Transfect neurons with desired constructs using calcium phosphate method previously described, and allow expression for 7 days.

## Preparation of Perfusion Solution

3 Prepare Tyrode's solution as described in materials.

4 Add [M] 100 micromolar (µM) BSA-complexed palmitate to the Tyrode's solution if required for fatty acid studies.

5 For pharmacological experiments, add desired amount of inhibitors as described in material section.

## Feeding with Fatty Acids

4h

6 Supplement neuronal feeding media with [M] 150 micromolar (µM) BSA-complexed palmitate.

7 Incubate neurons with the supplemented media for 04:00:00 .

4h

## JF dye labelling

30m

8 For imaging of Halo tagged proteins, add 100 nM JF635 or JF585 dyes to feeding media and return to incubator for 00:30:00 .

30m

9 Transfer the coverslip to Tyrode's solution to wash unbound dyes.

## Mounting Neurons for Imaging

10 Mount the coverslip with neurons onto the laminar flow perfusion system.

11 Ensure the perfusion system and objective are maintained at 37 °C.

## Perfusion and Inhibitor Treatment

5m

12 Perfuse neurons with the appropriate Tyrode's solution for 00:05:00 before imaging or stimulation. 5m

13 Ensure inhibitors are present during perfusion if required.

## Electrical Stimulation

14 Connect platinum-iridium electrodes with the stimulator.

15 Generate action potentials by applying brief 1 ms electrical pulses.

16 Ensure the field potential is maintained at ~10 V/cm.

## Image Acquisition

17 Acquire time-lapse images using the Andor iXon camera.

18 Record images at the desired time intervals for the duration of the experiment.

19 Monitor temperature and flow conditions throughout the imaging session.

Note

- Ensure all inhibitors are freshly prepared.
- Use hippocampal neurons between 7 to 14 days of transfection.
- Maintain consistent temperature ( 37 °C ) during all imaging and perfusion steps.
- Protect light-sensitive compounds (BSA-complexed palmitate) from prolonged light exposure.
- Ensure that the neurons are healthy and responsive during electrical stimulation.