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Live Imaging of Primary Mouse Neuron Cultures

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol describes live imaging of primary neuron cultures. Included are methods for preparing hippocampal or dopamine neuronal cultures from neonatal mouse brain tissue. The imaging described involves labeling of dopamine neurons with a fluorescent DAT ligand and using virally encoded pHlourin sensors to measure vesicular release of neurotransmitter as the neurons are electrically stimulated. This technique was used in Jain et al., 2023 to compare vesicular release in neurons between various transgenic knockout mouse lines.

Protocol materials

✕ HyClone Fetal Bovine Serum **Fisher Scientific Catalog #SH3039603**

✕ D-()-Glucose solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #G8769**

✕ B27 supplement without retinoic acid (50x) **Gibco - Thermo Fisher Scientific Catalog #17504044**

✕ Neurobasal™ Medium **Gibco - Thermo Fisher Scientific Catalog #21103049**

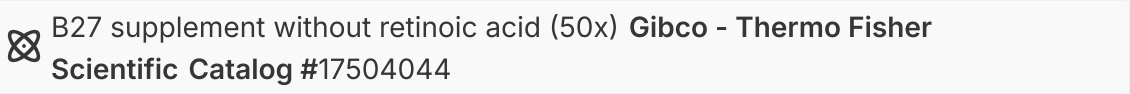
✕ Glutamax (100x) **Gibco - Thermo Fisher Scientific Catalog #35050-061**

✕ Basal Medium Eagle (BME) **Thermo Fisher Catalog #21010046**

✕ GDNF Recombinant Human Protein **Thermo Fisher Catalog #PHC7045**

Preparation of Hippocampal Cultures

- 1 Coat coverslips with Poly-L-lysine
- 2 Collect mouse pups at postnatal day 0
- 2.1 For Jain et al., 2023, cultures were prepared from WT, β 3A KO, β 3B KO, and *mocha* mice
- 3 Decapitate and remove brain, dissect out hippocampi from each hemisphere into Hank's balanced salt solution (HBSS) containing 10 mM HEPES and 20 mM glucose
- 4 Digest with papain (20 units/mL) in papain digestion buffer (HBSS with 20 mM CaCl_2 , 5 mM EDTA, 0.2 mg/mL L-Cysteine, 10 mM HEPES at pH 7.4) for 15 minutes
- 5 Wash hippocampi three times with HBSS containing 10 mM HEPES and 20 mM glucose
- 6 Triturate hippocampi to get a single cell suspension
- 7 Using a hemocytometer, count the density of cells in the suspension
- 8 Plate cells onto the poly-L-lysine-coated coverslip at a density of 350 cells/mm² in Minimal Essential Medium containing:
 - 1X B27 supplement


 - 5% Fetal Bovine Serum (FBS)


 - 21 mM glucose





9 Store cultures in incubator at 37 °C

10 After day 1 *in vitro* (DIV1), replace 3/4 of the medium with Neurobasal medium

Neurobasal™ Medium **Gibco - Thermo Fisher Scientific Catalog #21103049**

containing:

- 1X B27 supplement

B27 supplement without retinoic acid (50x) **Gibco - Thermo Fisher Scientific Catalog #17504044**

- GlutaMAX

Glutamax (100x) **Gibco - Thermo Fisher Scientific Catalog #35050-061**

11 Infect cells at DIV4-5 using lentiviral vectors

11.1 For Jain et al., 2023, viruses encoding VGLUT2-pH, VMAT2-pH, or VGAT-pH were used

12 At DIV 6-7, inhibit glial proliferation by treating cultures with 4 µM cytosine arabinoside (Ara-C)

Preparation of Dopamine Neuron Cultures

13 Coat coverslips with poly-L-lysine and laminin; plate astrocyte monolayer onto coverslips

14 Collect mouse pups at postnatal day 0

15 Decapitate and remove brain, dissect out midbrain (including ventral tegmental area and substantia nigra) from each hemisphere into Hank's balanced salt solution (HBSS) containing 10 mM HEPES and 20 mM glucose

16 Digest with papain (20 units/mL) in papain digestion buffer (HBSS with 20 mM CaCl₂, 5 mM EDTA, 0.2 mg/mL L-Cysteine, 10 mM HEPES at pH 7.4) for 15 minutes

17 Wash digested tissue three times with HBSS containing 10 mM HEPES and 20 mM glucose



- 18 Triturate tissue to get a single cell suspension
- 19 Using a hemocytometer, count the density of cells in the suspension
- 20 Plate cells onto the pre-prepared coverslips at a density of 1000 cells/mm² in medium consisting of 60% Neurobasal Medium
 -  Neurobasal™ Medium **Gibco - Thermo Fisher Scientific Catalog #21103049** , 30 %
Basal Eagle Medium
 -  Basal Medium Eagle (BME) **Thermo Fisher Catalog #21010046** , 10% FBS
 -  HyClone Fetal Bovine Serum **Fisher Scientific Catalog #SH3039603** containing:
 - 1X B27 supplement
 -  B27 supplement without retinoic acid (50x) **Gibco - Thermo Fisher Scientific Catalog #17504044**
 - 2 mM GlutaMAX
 -  Glutamax (100x) **Gibco - Thermo Fisher Scientific Catalog #35050-061**
 - 10 ng/mL glial cell line-derived neurotrophic factor (GDNF)
 -  GDNF Recombinant Human Protein **Thermo Fisher Catalog #PHC7045**
 - 1X penicillin/streptomycin
- 21 At DIV 2-4, infect with lentivirus
- 21.1 For Jain et al., 2023, cells were infected with lentivirus encoding VMAT2-pH or VGLUT2-pH

Live Imaging

- 22 Maintain cultures until ready for imaging. Live imaging of cultures is performed at DIV14-16 for hippocampal cultures and DIV13-15 for dopamine neuron cultures
- 23 Prepare Tyrode's buffer:
 - 119 mM NaCl
 - 25 mM HEPES

- 2 mM CaCl_2
- 2 mM MgCl_2
- 2.5 mM KCl
- 30 mM glucose

Adjust pH to 7.4

- 24 For midbrain cultures, label dopamine neurons, incubate dopamine neuron cultures in Tyrode's buffer (119 mM NaCl, 25 mM HEPES, 2 mM CaCl_2 , 2 mM MgCl_2 , 2.5 mM KCl, and 30 mM
- 24.1 Make Tyrode's +JHC1-64 by adding the fluorescent DAT ligand JHC1-64 at 30 nM to Tyrode's buffer
- 24.2 Incubate dopamine neuron culture in Tyrode's +JHC1-64 for 5 minutes at  Room temperature
- 24.3 Rinse neurons in Tyrode's buffer (no JHC 1-64)
- 25 Add fresh Tyrode's buffer to culture dishes, mount coverslips in a perfusion and stimulation chamber of a TE300 inverted epifluorescence microscope
- 26 Monitor fluorescence:
- For pHlourin, use 470/40 nm excitation and 525/70 nm emission bandpass filters
 - For JHC1-64, use 545/25 nm excitation and 605/70 nm emission bandpass filters
- Use the red channel to identify dopamine neurons then switch to the pHlourin channel for experiments
- 27 Place platinum-iridium stimulating electrodes near the dopamine neuron being observed
- 28 Acquire images, typically at 1 Hz except for Fluo-5F image where images are acquired at 10 Hz
- 29 While acquiring images, evoke action potentials using 1 ms bipolar current pulses through the stimulating electrode at 5-10 V/cm