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Live imaging of synaptic vesicle dynamics in human iPSC-derived Dopamine Neurons (DaNs) using synapto-pHluorin

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Kaitlyn Cramb[1,2,3,4], Ira Milosevic[5], Richard Wade-Martins[1,2,3,4]

[1]Department of Physiology, Anatomy and Genetics, University of Oxford, Sherrington Building, Sherrington Rd, Oxford, OX1 3PT, UK;
[2]Kavli Institute for Neuroscience Discovery, University of Oxford, Dorothy Crowfoot Hodgkin Building, South Park Road, Oxford OX1 3QU, UK;
[3]Oxford Parkinson's Disease Centre, University of Oxford, Oxford OX1 3PT, UK;
[4]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815, USA;
[5]Nuffield Department of Medicine (NDM), University of Oxford, Henry Wellcome Building for Molecular Physiology, Old Road, Oxford, OX3 8BN, UK

Cláudia C. Mendes
University of Oxford

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We use this protocol and it's working

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Abstract

This protocol utilizes synaptic vesicle-specific pH-sensitive synapto-pHluorin in a lentiviral vector to measure synaptic vesicle dynamics in iPSC-derived dopamine neurons following their differentiation using an expanded Krik's protocol as described in **Williamson, M.G. & Madureira, M. et al. (2023)**. This protocol employs electrical or chemical stimulation in concert with live imaging to measure various aspects of synaptic vesicle dynamics including vesicle pool size and recycling.

Materials

Reagents:

- HEK293-T LentiX (Takara Bio UK, CAT#632180)
- FBS (Merck Life Science, F7524-500 mL)
- Lipofectamine 3000 (Thermofisher)
- ViralBoost (Alstem)
- Lenti-X concentrator (Takara Bioscience)

Equipment:

- Opera Phenix™ Plus high-content imaging system (Revvity; CAT# HH14001000) - https://www.revvity.com/gb-en/product/opera-phenix-plus-system-hh14001000
- Nikon eclipse Ti microscope
- Hammatsu EM-CCD camera

SOLUTIONS:

Preparing Classic Tyrodes:

1. Make up in ddH_2O:

- 124 mM NaCl
- 1 mM KCl
- 25 mM Hepes
- 2 mM CaCl2
- 1 mM MgCl2
- 6 g/L glucose
- pH to 7.4

Preparing High Stimulation Tyrodes:

1. Make up in ddH_2O:

- 10 mM NaCl
- 112 mM KCl
- 25 mM Hepes
- 4 mM CaCl2
- 2 mM MgCl2
- 6 g/L glucose
- pH 7.4

Preparing Ammonium Tyrodes:

1. Make up in ddH_2O:

- 69 mM NaCl
- 2.5 mM KCl
- 25 mM Hepes
- 2 mM CaCl2
- 2 mM MgCl2

- 6 g/L glucose
- 50 mM ammonium chloride
- pH to 7.4

Safety warnings

Dispose of all materials as per local guidelines.

***** Extra precaution should be taken when handling and disposing of material that has come into contact with lentivirus *****

Ethics statement

The protocols.io team notes that research involving animals and humans must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board.

Before start

Prior to use, produce or purchase appropriate lentiviral construct.

Produce iPSC-derived dopamine neurons according to the following **Protocol: Differentiation of human Dopamine Neurons (DaNs) from induced pluripotent stem cells (iPSCs)**.

Lentiviral Production and Infection of Dopamine Neurons (DaNs)

1 Thaw HEK293-T LentiX in high glucose 1x DMEM with Glutamax and 10% FBS.

2 Grow until ~90% confluence and then when ready, and prior to transfection, coat 15 cm dishes with poly-L-lysine for 1 hour at 37°C.

3 Aspirate coating and let dry.

4 Transfect HEK cells with construct along with psPAX6, pMD2G and pAdVantage using Lipofectamine 3000.

Note

This should be done later in the day, and the next step done early the next morning.

5 The following morning, add ViralBoost as per manufacturer's instructions along with fresh media.

6 Collect virus two to three days following transfection into a falcon tube and spin for 10 minutes at 500g.

7 Concentrate using Lenti-X concentrator as per manufacturer's instructions for 48 hours at 4°C.

7.1 Spin at 1500g for 45 minutes at 4°C.

7.2 Aspirate supernatant and resuspend pellet in 1:100 (300 µL for 30 mL) of PBS.

7.3 Aliquot virus and store aliquots at -80°C.

Note

This can be done either simultaneous as replating or immediately following.

Concentration of virus to be used is virus and batch-dependant and requires prior virus titration.

Live Imaging of synapto-pHluorins

8 Plate coverslip into a pre-warmed bath of Classic Tyrodes Solution (see **Materials**; 500 µL for a 18mm coverslip is sufficient) on a suitable microscope.

9 Begin live imaging and allow for 30 seconds to 1 minute of recording to first occur to establish baseline fluorescence.

10 Provide electrical or chemical stimulation using 500 µL of High Stimulation Tyrodes Solution with high KCl (see **Materials**).

Note

Prepare tyrodes buffer at 2x KCl concentration such that the classic tyrodes solution does not need to be first removed before adding on the additional solution. This allows for as little disruption to the cells being imaged as possible.

11 Obtain maximum signal by addition of Ammonium Tyrodes Solution (see **Materials**).

Image Analysis

12 Using ImageJ analyse individual region of interests (ROIs) along the neuronal processes determined based on the increase in fluorescence when ammonium was added (maximum signal).

13 Measure fluorescent intensity changes over time of >30 ROIs over time per recording.