



Jan 28, 2026

🌐 Whole-cell patch-clamp of human iPSC-derived Dopamine Neurons (DaNs)

DOI

<https://dx.doi.org/10.17504/protocols.io.bp2l6yjdqvqe/v1>



Kaitlyn Cramb^{1,2,3,4}, Dayne Beccano-Kelly^{1,2,3}, Richard Wade-Martins^{1,2,3,4}

¹Department of Physiology, Anatomy and Genetics, University of Oxford, Sherrington Building, Sherrington Rd, Oxford, OX1 3PT, UK;

²Kavli Institute for Neuroscience Discovery, University of Oxford, Dorothy Crowfoot Hodgkin Building, South Park Road, Oxford OX1 3QU, UK;

³Oxford Parkinson's Disease Centre, University of Oxford, Oxford OX1 3PT, UK;

⁴Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815, USA



Cláudia C. Mendes

University of Oxford

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.bp2l6yjdqvqe/v1>



Protocol Citation: Kaitlyn Cramb, Dayne Beccano-Kelly, Richard Wade-Martins 2026. Whole-cell patch-clamp of human iPSC-derived Dopamine Neurons (DaNs). protocols.io <https://dx.doi.org/10.17504/protocols.io.bp2l6yjydvqe/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: June 17, 2025

Last Modified: January 28, 2026

Protocol Integer ID: 220400

Keywords: electrophysiological properties of individual human dopamine neuron, individual human dopamine neuron, human dopamine neuron, studies in human dopamine neuron, derived dopamine neuron, dopamine neuron, individual neuron, human motor neuron, excitatory postsynaptic current, electrophysiological property, cell patch clamp, human ipsc, resting membrane potential, postsynaptic current, potassium current, membrane potential, clamp, ipsc

Funders Acknowledgements:

Aligning Science Across Parkinson's Collaborative Research Network

Grant ID: ASAP-020370, ASAP-025192

Abstract

This protocol measures electrophysiological properties of individual human dopamine neurons using whole-cell patch clamp, based on previously published studies in human dopamine neurons from Beccano-Kelly et al., 2023 as well as human motor neurons from Vahsen et al., 2023. This protocol measures the resting membrane potential, capacitance, voltage-dependent sodium and potassium currents and excitatory postsynaptic currents (EPSCs) of individual neurons differentiated from human iPSCs using a modified Krik's protocol, as reported in Williamson and Madureira et al., 2023.

Materials

SOLUTIONS:

Preparing Extracellular Solution:

1. Prepare in milliQ ddH₂O

- 167 mM NaCl
- 2.4 mM KCl
- 1 mM MgCl₂
- 10 mM glucose
- 10 mM HEPES
- 2 mM CaCl₂

2. Adjust to a pH of 7.4 and 300 mOsm

3. Store at 4°C

Preparing Intracellular Solution:

1. Prepare in milliQ ddH₂O on ice

- 140 mM K-Gluconate
- 6 mM NaCl
- 1 mM EGTA
- 10 mM HEPES
- 4 mM MgATP
- 0.5 mM Na₃GTP

2. Adjust to pH 7.3 and 290 mOsm

3. Store at -20°C

Equipment:

- AHS-LAMP 12V/100W Halogen Lamp (Olympus, Scientifica, CAT# CLD-LAM28)
- Borosilicate glass pipettes (Premium Thin Wall Borosilicate Capillary Glass with Filament, Model G150TF-4) (Multi Channel Systems, CAT# 640805)
- Faraday Cage 36" x 48" (900mm x 1200mm) (Scientifica, CAT# 81-333-06)
- Digidata 1550A digitiser (Molecular Device, Scientifica, CAT# DD1550A0)
- Faraday Cage 36" x 48" (900mm x 1200mm) (Scientifica, CAT# 81-333-06)
- Full Perimeter Enclosure for 36" x 48" x 2" (900mm x 1200mm x 50mm) (Scientifica, CAT# 81-321-06)
- MultiClamp 700B amplifier (Molecular Device, Scientifica, CAT# MultiClamp 700B)
- Plan Fluorite no-cover 60x water immersion objective (LUMPLFLN60XW) (Olympus, Scientifica, CAT# N2667800)
- PLN10X/0.25 Plan Achromat objective with 10x magnification (Olympus, Scientifica, CAT# N1215800)
- SliceScope Pro 6000 with one Control cube and one PatchPad – metric (Scientifica, CAT# SSPro-6000- 00C-P)
- Sutter P-97 Flaming Brown puller (Sutter Instrument Company, WPI, CAT# P-1000)
- U-TR30NIR Trinocular tube (Olympus, Scientifica, CAT# N1511900)



- WI-DICD Condenser (Scientifica, CAT# 38183)

Software:

- Clampfit 10.3 (pCLAMP Software suite, Molecular Devices).
- ClampEx 6 software.

Safety warnings

- ! Dispose all materials as per local guidelines.

Before start

Produce dopamine neurons following **Protocol: Differentiation of human Dopamine Neurons (DaNs) from induced pluripotent stem cells** and plate at 100 000 cells / coverslip of a 13mm coverslip on the final replating day.

Before beginning to patch, prepare fresh internal and extracellular solutions as well as pulling borosilicate glass pipettes (0.86 mm inner diameter; 1.5 mm outer diameter) fresh the day of whole-cell patching (with a resistance ranging from 5-7 M Ω).

Preparation for Whole-Cell Patch-Clamping

- 1 Pre-warm fresh Extracellular Solution (see **Materials**) to 37°C in water bath.
- 2 Ensure all equipment (digitizer, micromanipulator, camera, amplifier etc.) is turned on.
- 3 Collect a coverslip from the incubator and place in a pre-warmed bath of Extracellular Solution.
- 4 Using a 1 mL syringe and needle, take up the internal solution (kept on ice) and filter.
- 5 Fill a freshly pulled borosilicate glass pipette half way, or sufficiently until the electrode can reach the liquid.
- 6 Attach the glass pipette to the pipette holder of the micromanipulator.
- 6.1 Submerge the pipette into the Extracellular Solution.
- 6.2 Open Clampex software and open membrane test.
- 6.3 Switch to bath mode and confirm correct pipette resistance.

Whole-Cell Patch Clamping Individual Dopamine Neurons

- 7 Search for a healthy cell using the microscope.
- 8 Using the microscope and micromanipulator, carefully lower the glass pipette down above the chosen cell until both are visible under the objective but no contact occurs.
- 9 Begin blowing as the tip approaches the cell until contact is made and a slight increase in membrane



- resistance is observed.
- 10 Simultaneously switch to patch mode and begin suction, until 1 G Ω of pipette resistance is observed.
 - 11 Following the formation of a seal, snap-suck to gently rupture the cell membrane and 'break in'.
 - 12 Ensure series resistance (Rs) remains below <30 M Ω and continually check between protocols.
 - 13 Select and run desired recording programs as described in **steps 14 and 15**.

Recording Programs

- 14 Record simultaneously Na⁺ and K⁺ currents in voltage clamp.
- 15 Set the protocol of a series of 400 ms square voltage steps of 10 mV increments from -70 mV to +70 mV.
- 16 Set signal sampling and filter rate at 10 kHz and 2 kHz, respectively.
- 17 Pre-set leak subtraction with 4 sub-sweeps and a settling time of 250 ms.

Analysis

- 18 Perform analysis using Clampfit 10.3.