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Electrophysiology with iPSC-derived neurons

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We use this protocol and it's working. This protocol was originally provided by Olga Kopach.

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Abstract

This protocol describes the method to perform patch-clamp recordings of iPSC-derived neurons.

1 Patch-clamp recordings of iPSC-derived neurons are performed using an infrared differential interference contrast (DIC) imaging system on an Olympus BX51WI upright microscope (Olympus, Japan) coupled with a Multipatch 700B amplifier under the control of pClamp 10.2 software package (Molecular Devices, USA)

2 For the recordings, a neuronal culture or co-culture is plated on glass coverslips, placed in a recording chamber mounted on the microscope stage and constantly perfused with a physiological buffer medium.

3 Whole-cell recordings are performed using glass pipettes with a resistance of 3.5-6 MΩ when filled with the intracellular solution.

Note

Perfusion medium (in mM):
126 NaCl, 3 KCl, 2 MgSO4, 2 CaCl2, 26 NaHCO3, 1.25 NaH2PO4, 10 D-glucose
The medium is continuously bubbled with. [M] 95 % volume O2 and
[M] 5 % volume CO2
(pH 7.4) and maintained at 30 °C - 33 °C .

4 Whole-cell recordings are performed using glass pipettes with a resistance of 3.5-6 MΩ when filled with the intracellular solution.

Note

Intracellular solution (in mM):
126 K-gluconate, 4 KCl, 4 MgCl2, 2 BAPTA, 4 Mg-ATP, 0.4 Na-ATP (pH 7.2 , osmolarity ~295 mOsmol)

5 In the whole-cell (immediately after membrane breakthrough), iPSC-derived neurons are recorded
for the resting membrane potential (Vrest), membrane capacitance (Cm), the membrane time constant (τm), and input resistance (Rin)

6 To induce neuronal firing, a series of sub- and supra-threshold rectangular current pulses are applied with a stepwise-increased stimulus intensity at the Vhold set at −60 mV to −70 mV.

Note

The second protocol tested is a slow-ramp current injection, ramped up with a 100–200 pA/s slope.

7 The analysis of the AP waveform was performed for the first AP only to quantify the threshold value, the spike amplitude, overshoot, the spike width (duration at half-maximal amplitude), the rates of depolarisation and re-polarisation phase.