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Electrophysiology

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ABSTRACT

Electrophysiology of iPSC-derived mDA neurons

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1 Visualized patch-clamp recordings from cell cultures were performed using an infrared differential interference contrast imaging system and a Multipatch 700B amplifier controlled by pClamp 10.2 software package (Molecular Devices, USA).

1.1 For the recordings, a neuronal culture on a glass coverslip was placed in a recording chamber mounted on the stage of an Olympus BX51WI upright

microscope (Olympus, Japan).

1.2 The perfusion solution contained the following (in mM): 119 NaCl, 2.5 KCl, 1.3 Na_2SO_4, 2.5 $CaCl_2$, 26.2 $NaHCO_3$, 1 NaH_2PO_4, 2 $CaCl_2$, 2 $MgCl_2$, 10 glucose (or 22 in some recordings) and was continuously bubbled with 95% O_2 and 5% CO_2, at a pH of 7.4.

1.3 Whole-cell recordings were performed at **32-34 °C** ; the patch-clamp pipette resistance was 3-7 MΩ depending on particular experimental conditions.

1.4 Series resistance was monitored throughout experiments using a +5 mV step command, cells with very high series resistance (above 25 MΩ) or unstable holding current were rejected.

1.5 The intracellular pipette solution for voltage-clamp experiments contained (in mM): 120.5 CsCl, 10 KOH-HEPES, 2 EGTA, 8 NaCl, 5 QX-314 Br^- salt, 2 Na-ATP, 0.3 Na-GTP.

1.6 For current-clamp experiments, the intracellular solution contained (in mM): 126 K-gluconate, 4 NaCl, 5 HEPES, 15 glucose, 1 K_2SO_4×7 H_2O, 2 BAPTA, 3 Na-ATP. The pH was adjusted to 7.2 and osmolarity adjusted to 295 mOsm.

1.7 To isolate response of NMDA receptors we added to a perfusion solution: 50 mM picrotoxin, 20 mM NBQX, 1 mM strychnine, 1 mM CGP-55845, 100 mM MCPG, with zero Mg^{2+}.

1.8 To isolate response of $GABA_A$receptors, we added 50 mM APV, 20 mM NBQX, 1 mM strychnine, 1 mM CGP-55845, 100 mM MCPG. All chemicals were purchased from Tocris Bioscience.

2 In the whole-cell (immediately after membrane breakthrough), iPSC-derived neurons were recorded for the resting membrane potential (V_{rest}), membrane capacitance (C_m), the membrane time constant (τ_m), and input resistance (R_{in}), measured from the hyperpolarizing squire current pulse steps in current mode

2.1 To assess the firing capability of the cells, a series of sub- and supra-threshold rectangular current pulses were applied to elicit neuronal firing, with a stepwise-increased stimulus intensity (an increment of 5–10 pA).

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2.2 The V_{rest} was set at −60 mV to −70 mV, by injecting a hyperpolarizing bias current where required.

2.3 The analysis of the AP waveform was performed for the first AP only.

2.4 The parameters of individual APs recorded were: the spike amplitude (measured from the threshold to the peak), the threshold value, overshoot and the spike width (duration at half-maximal amplitude), the rates of depolarisation and repolarisation phases.

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