In cooperation with

Electrophysiological characterization of human induced pluripotent stem cell-derived dopaminergic neurons using manual and automated patch clamp systems

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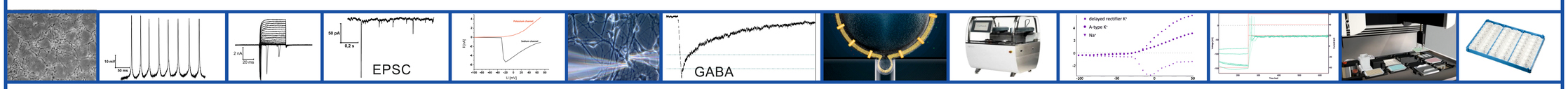


Introduction

We present a basic electrophysiological characterization of the Dopa.4U, human induced pluripotentent stem cell-derived dopaminergic neurons of Axiogenesis (Cologne, Germany). That included examing the voltage-gated ion channels as well as ligand-gated GABA receptors on the manual patch clamp and the automated QPatch system (Sophion - Biolin Scientific, Ballerup, Denmark).

Our results suggest the feasibility of an automated electrophysiological characterization of suspended neuronal cells.

Materials & Methods

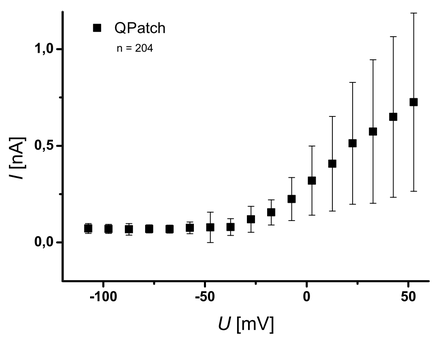
The Dopa.4U were cultured on Geltrex® or Matrigel® coated culture dishes for at least 9 days. Because of using suspended cells for measurments with automated system, the following results consider and compare attempts of detached Dopa.4U. Of three different harvesting-compounds tested, Accutase® (Sigma Aldrich) seemed to allow for the mildest treatment. All manual experiments were executed using the triple EPC 10 amplifier (HEKA Elektronik, Germany).

Results

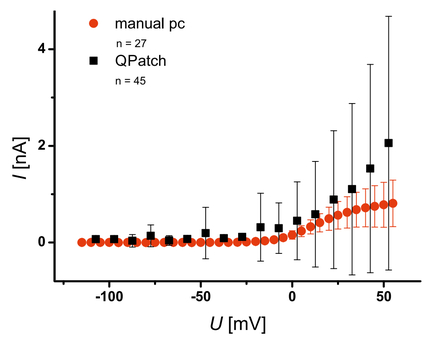
In manual patch clamp experiments, two voltage-gated ion channel types (K_V and Na_V) were characterized with the potassium-channel blocker 4-Aminopyridine (4-AP) and the sodium-channel blocker Tetrodotoxin (TTX) leading to IC_{50} values of 1 mM and 1 nM, respectively. With the automated QPatch system, a gigaseal rate of up to 90 % and a success rate of up to 70 % have been achieved. Detailed analysis of the voltage gated ion channels revealed 83 % cells with rapidly inactivating A-type potassium channels, 43 % cells with sodium channels, 18 % cells with delayed rectifying potassium channels, and 21 % cells with both, A-type and delayed rectifying potassium channels. Pharmacological investigations with different channel blockers provided stable results for the A-type potassium channel with 4-AP (IC_{50} = 0.4 mM ±1.3 mM) and Tetraethylammonium (TEA; IC_{50} = 0.1 mM ±0,04 mM), as well as for the sodium channel with TTX (IC_{50} = 2.9 nM ±0,01 nM). First experiments have shown that the QPatch system allows for reproducing induced action potentials, which were obtained with the manual system in the current clamp mode.

Current-Voltage-Curves

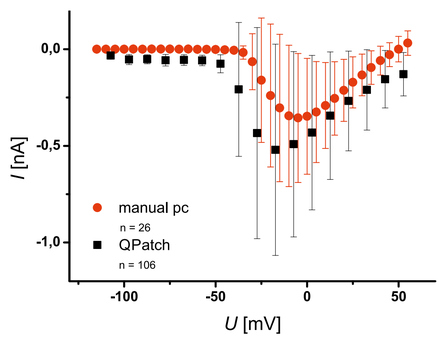
A-type potassium channel



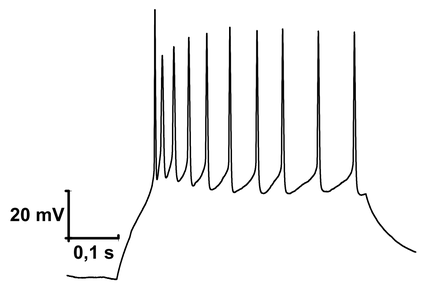
Delayed rectifying potassium channels



Voltage gated sodium channel

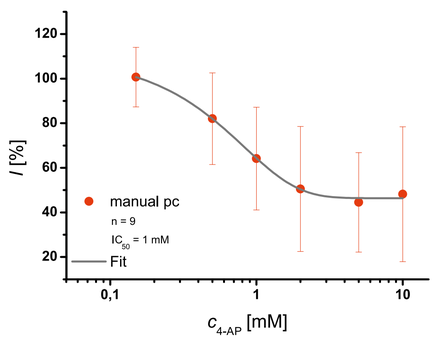


Induced action potentials (manual)

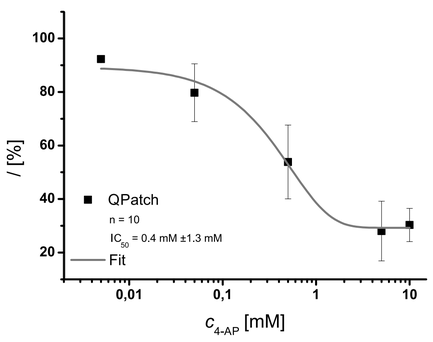


Dose-Response-Curves

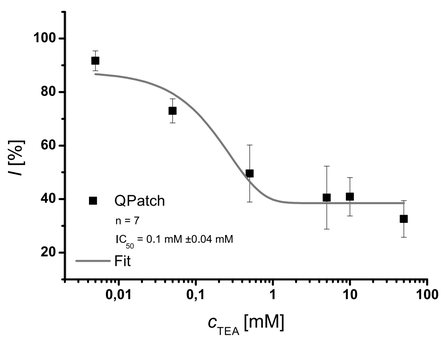
4-AP/ delayed K_v



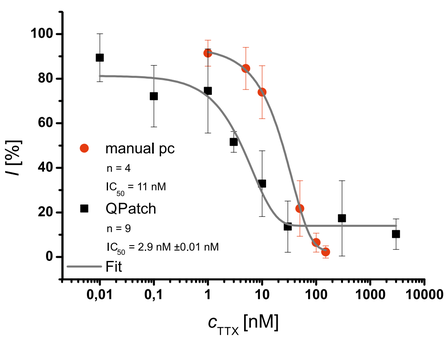
4-AP/ A-type K_v



TEA/ A-type K_v



TTX/ Na_v



Summary

	Gigaseal rate	Success rate	A-type K_V	Delayed K_V	Na_V	GABA currents	Induced AP
manual	≤ 97%	≤ 96%	-	U_{open} = -20mV ±8mV → 4-AP (with adherent cells) IC_{50} = 1mM	U_{open} = -35mV ±8mV → TTX (with adherent cells) IC_{50} = 11nM	only in adherent cells	possible
automated	≤ 90%	≤ 70%	U_{open} = -32mV ±7mV → 4-AP IC_{50} = 0,4mM ±1,3mM → TEA IC_{50} = 0,1mM ±0,04mM	U_{open} = -27mV ±14mV	U_{open} = -37mV ±8mV → TTX IC_{50} = 2,9nM ±0,01nM		possible

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