Electrophysiological characterization of human dopaminergic neurons derived from LUHMES cells

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Introduction
The loss of dopaminergic neurons in the substantia nigra plays an important role in the development of the Parkinson’s disease. The symptoms of this disease typically occur after around 80% of these neurons degenerated. This cell decay can be caused or promoted by genetic defects or environmental factors including chemical compounds like pesticides. For a proper testing of neurotoxic effects on these neurons as well as for the development of neuroprotective drugs, assays based on animal primary cells lack pre-
dictivity due to mostly weak correlation between animal and human data. Therefore, models based on human neuronal cells have a high potential to overcome the limitations of animal models. One interesting neuronal cell line is the LUHMES (Lund human mesencephalic) line, which consists of immortalized fetal human mesencephalic cells that can be differentiated into fully post-mitotic dopaminergic neurons in 6 days (Scholz et al., 2011). We here describe functional properties of these cells as a fundament for the development of LUHMES-based pharmacological assays.

Materials & Methods
The LUHMES cells were differentiated for 2 days in T75 flasks, coated with PDL (1 mg/ml) and Fibronectin (1 mg/ml). The cells were further differentiated in a density of 200 per well in 384-well plates (Greiner Bio-One, GER), coated with 0.1% PEl. The Ca2+ imaging recordings were performed on day 9 of the differentiation with the HTS Functional Drug Screening System FDSu/jCELL (Hamamatsu Photonics, JP) combined with the Ca2+-sensitive dye Cal-520TM AM (AAT Bioquest, US) at 37°C.

Results
Biophysical characterization of voltage-gated sodium (Na+) channels using automated patch clamp

- Activation properties
  - Evaluation of the normalized conductance (G/Gmax) by estimating the half-maximal activation voltage (V1/2) of -11.3 mV using a Boltzmann fit.
- Inactivation properties
  - Analysis of the steady-state inactivation of the Na+ channels by calculating V1/2. The Boltzmann fit determines a half-inactivation voltage (V1/2) of -54.9 mV.
- Recovery from inactivation
  - A bi-exponential fit was used to describe the obtained data. This resulted in two time constants of 1.71 ms & 112 ms.

Pharmacological characterization of Na+ channels using automated patch clamp

- Effect of Tetrodotoxin (TTX) on Na+ channel currents to distinguish TTX-sensitive and TTX-resistant Na+ channels. The IC50 values suggest that only TTX-sensitive Na+ channel are present and TTX shows no use-
dependent effect.
- Effect of Lidocaine (local anesthetic) which is a known use-dependent Na+ channel inhibitor. The IC50 values of the first and the last peak are significantly different.

Conclusion
The results show that we were able to differentiate the cells derived from LUHMES cells with neuronal electrophysiological characteristics with low batch-to-batch variations. Addressing these neurons with the calcium imaging system could offer a great opportunity for a high-throughput assessment of the neurotoxic potential of novel drug candidates on a neuronal network in the future.


Materials & Methods

The LUHMES cells were cultivated for 9 days in differentiation medium in T75 or T175 flasks, coated with PDL (1 mg/ml) and Fibronectin (1 mg/ml). The recordings were performed with the patch clamp automated QPatch (Sophion Bioscience, DK) with 16X QPlates which enables parallel recordings of 16 independent experiments. For the experiments the cells were coated with 0.05% trypsin and resuspended in a concentration of 3-4 x 10^6 cells per ml.

Results

Characterization of neurotransmitter receptors using HTS Ca2+ imaging

- Concentration-dependent effects of agonists of different neurotransmitter receptor were examined on various differentiations. The EC50 values show a high consistency over several differentiations.
- Recording protocol:
  - 1.5 min control phase
  - Compound application
  - 4.5 min incubation
  - KCl (30 mM) application

Investigated of neuronal network activity using HTS Ca2+ imaging

The oscillation frequency of the Ca2+ oscillations was examined with a FFT-based analysis.

- Hyperpolarization induced Ca2+ oscillations due to a synchronous activity of the neuronal network. Phenylthion (anticonvulsant drug) blocks Na+ channel currents and exhibits a concentration-dependent inhibition of the Ca2+ oscillations. Serotonin increased the oscillation duration and frequency.
- Serotonin (100 μM) induced Ca2+ oscillations were blocked by NNC 55-0396 (T-type Ca2+ channel blocker).

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