Electrophysiological characterization of human dopaminergic neurons derived from LUHMES cells

Udo Kraushaar [1], Dominik Loser [1-4], Timm Danker [2], Clemens Möller [3], Marcel Leist [4]

[1] NMI Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, GER; [2] NMI-TT GmbH, Reutlingen, GER; [3] Life Sciences Faculty, Albstadt-Sigmaringen University, Sigmaringen, GER; [4] Doerenkamp-Zbinden Chair for in vitro Toxicology and Biomedicine, University of Konstanz, Konstanz, GER

NMI - Natural and Medical Sciences Institute at the University of Tübingen
Markwiesenstrasse 55
72770 Reutlingen, Germany

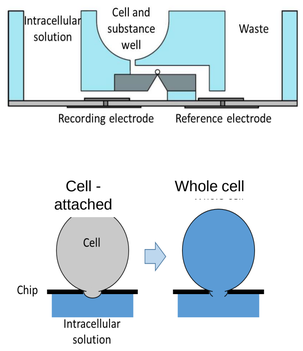
E-Mail: udo.kraushaar@nmi.de

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 basesd on animal primary cells lack predictivity due to mostly weak correlation between animal and human data i. 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.

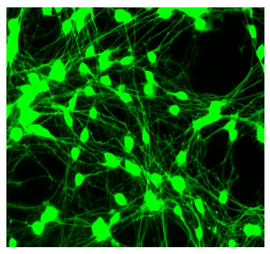
Materials & Methods

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



Materials & Methods

The LUHMES cells were differentiated for 2 days in T75 flasks, coated with PLO (1 mg/ml) and Fibronection (1 mg/ml). The cells were further differentiated in a density of 20k per well in 384-well plates (Greiner Bio-One, GER), coated with 0.1% PEI. The Ca^{2+} imaging recordings were performed on day 9 of the differentiation with the HTS Functional Drug Screening System FDSS/µCELL (Hamamatsu Photonics, JP) combined with the Ca^{2+} -sensitive dye Cal-520™ AM (AAT Bioquest, US) at 37°C.



LUHMES cells stained with Cal-520™ on day 9 of the differentiation.

Results

Biophysical characterization of voltage-gated sodium (Na_v) channels using automated patch clamp

- Activation properties

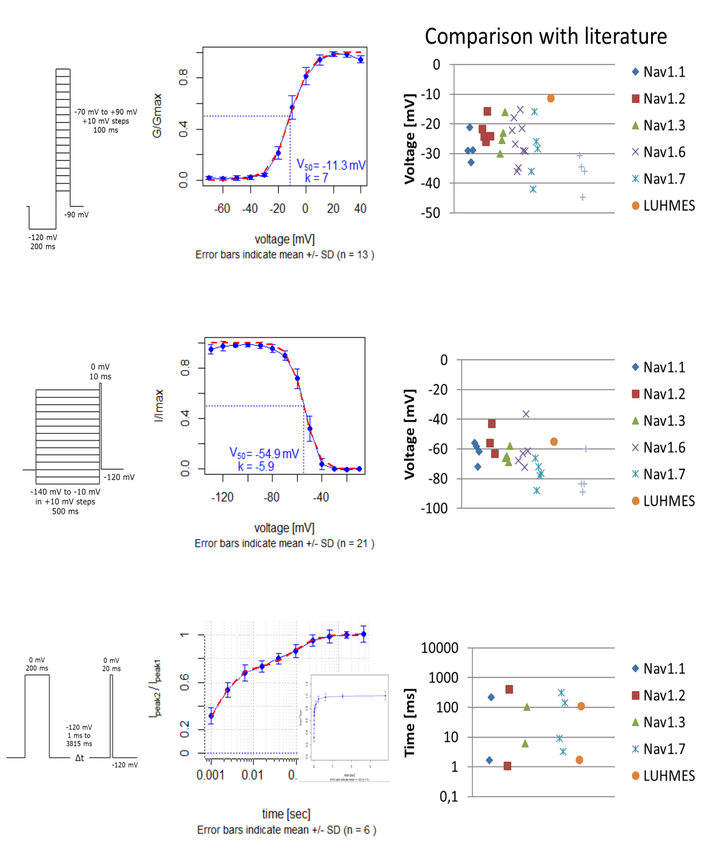
  Evaluation of the normalized conductance (G/G_{max}) by estimating the half-maximal activation voltage (V_{50}) of -11.3 mV using a Boltzmann fit.

- Inactivation properties

  Analysis of the steady-state inactivation of the Na_v channels by calculating I/Imax. The Boltzmann fit determines a half-inactivation voltage (V_{50}) of -54.9 mV.

- Recovery from inactivation

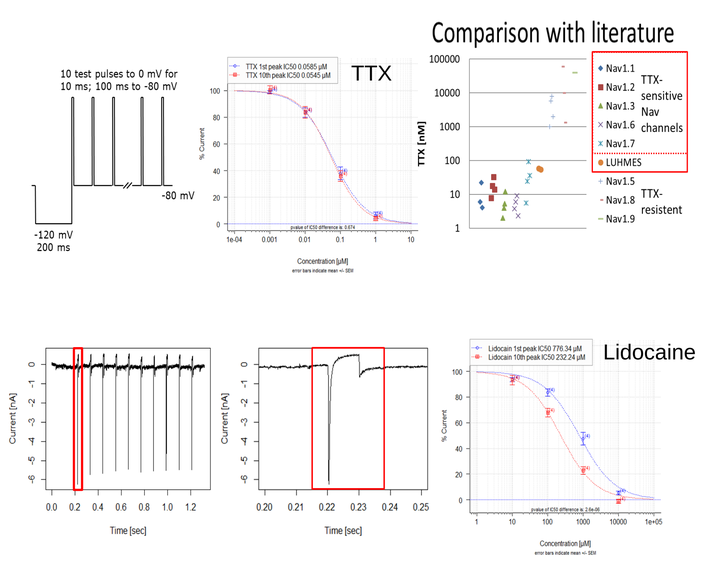
  A bi-exponential fit was used to describe the obtained data. This resulted in two time constants of 1.71 ms & 112 ms.



Pharmacological characterization of Na_v channels using automated patch clamp

- Effect of Tetrodotoxin (TTX) on Na_v channel currents to distinguish TTX-sensitive and TTX-resistant Na_v channels. The IC_{50} values suggest that only TTX-sensitive Na_v channel are present and TTX shows no use-dependent effect.

- Effect of Lidocaine (local anesthetic) which is a known use-dependent Na_v channel inhibitor. The IC_{50} values of the first and the last peak are significantly different.

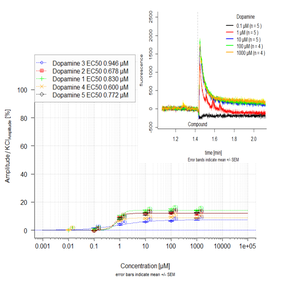


Results

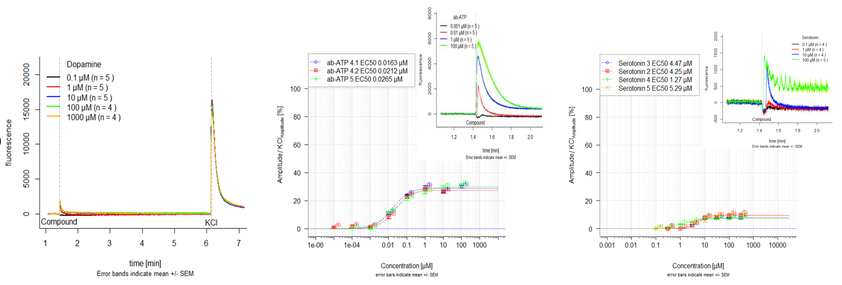
Characterization of neurotransmitter receptors using HTS Ca^{2+} imaging

- Concentration-dependent effects of agonists of different neurotransmitter receptor were examined on various differentiations. The EC_{50} values show a high consistency over several differentiations.

Receptor	Ca^{2+} imaging	mRNA analysis
Dopamine		
Serotonin		
GABA		
Glutamate		
Nicotinic ACh		
Muscarinic ACh		
Adrenergic		
Glycine		
Histamine		
Purinergic		
Adenosine		



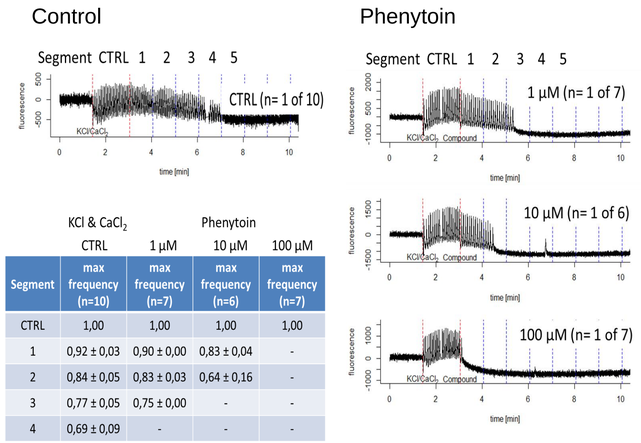
- Recording protocol:
  - 1.5 min control phase
  - Compound application
  - 4.5 min incubation
  - KCl (30 mM) applicatio



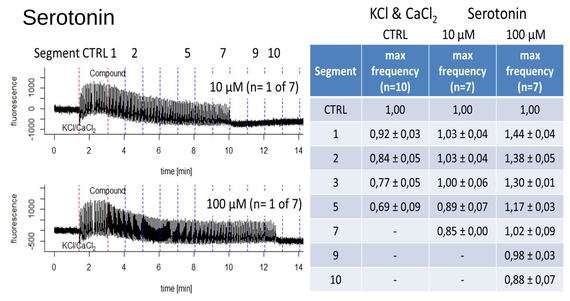
Investigated of neuronal network activity using HTS Ca^{2+} imaging

The oscillation frequency of the Ca^{2+} oscillations was examined with a FFT-based analysis.

- Hyperpolarization induced Ca^{2+} oscillations due to a synchronous activity of the neuronal network. Phenytoin (anticonvulsant drug) blocks Na_v channel currents and exhibits a concentration-dependent inhibition of the Ca^{2+} oscillations. Serotonin increased the oscillation duration and frequency.
- Serotonin (100 µM) induced Ca^{2+} oscillations were blocked by NNC 55-0396 (T-type Ca^{2+} channel blocker).

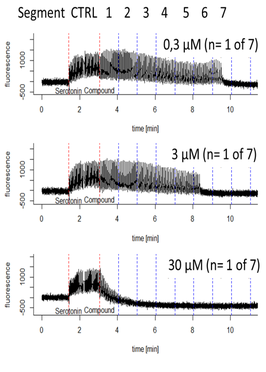


KCl & $CaCl_2$	Phenytoin		
CTRL	1 µM	10 µM	100 µM
Segment	max frequency (n=10)	max frequency (n=7)	max frequency (n=6)	max frequency (n=7)
CTRL	1,00	1,00	1,00	1,00
1	0,92 ± 0,03	0,90 ± 0,00	0,83 ± 0,04	-
2	0,84 ± 0,05	0,83 ± 0,03	0,64 ± 0,16	-
3	0,77 ± 0,05	0,75 ± 0,00	-	-
4	0,69 ± 0,09	-	-	-



KCl & $CaCl_2$	Serotonin	
CTRL	10 µM	100 µM
Segment	max frequency (n=10)	max frequency (n=7)	max frequency (n=7)
CTRL	1,00	1,00	1,00
1	0,92 ± 0,03	1,03 ± 0,04	1,44 ± 0,04
2	0,84 ± 0,05	1,03 ± 0,04	1,38 ± 0,05
3	0,77 ± 0,05	1,00 ± 0,06	1,30 ± 0,01
5	0,69 ± 0,09	0,89 ± 0,07	1,17 ± 0,03
7	-	0,85 ± 0,00	1,02 ± 0,09
9	-	-	0,98 ± 0,03
10	-	-	0,88 ± 0,07

NNC 55-0396



Serotonin (100 µM)	NNC 55-0396		
CTRL	0,3 µM	3 µM	30 µM
Segment	max frequency (n=10)	max frequency (n=7)	max frequency (n=7)	max frequency (n=7)
CTRL	1,00	1,00	1,00	1,00
1	0,81 ± 0,05	0,78 ± 0,03	0,78 ± 0,02	0,81 ± 0,05
2	0,79 ± 0,04	0,74 ± 0,03	0,75 ± 0,03	-
3	0,75 ± 0,04	0,70 ± 0,03	0,71 ± 0,02	-
5	0,68 ± 0,04	0,63 ± 0,03	0,64 ± 0,05	-
6	0,62 ± 0,06	0,60 ± 0,04	0,59 ± 0,06	-
7	0,59 ± 0,02	0,57 ± 0,03	-	-

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.

Acknowledgment for funding:

KPK-InViTe Kooperatives Promotionskolleg Konstanz / Sigmaringen

Baden-Württemberg
MINISTERIUM FÜR WISSENSCHAFT, FORSCHUNG UND KUNST