Klima et al.:

Examination of Microcystin Neurotoxicity Using Central and Peripheral Human Neurons

Supplementary Data

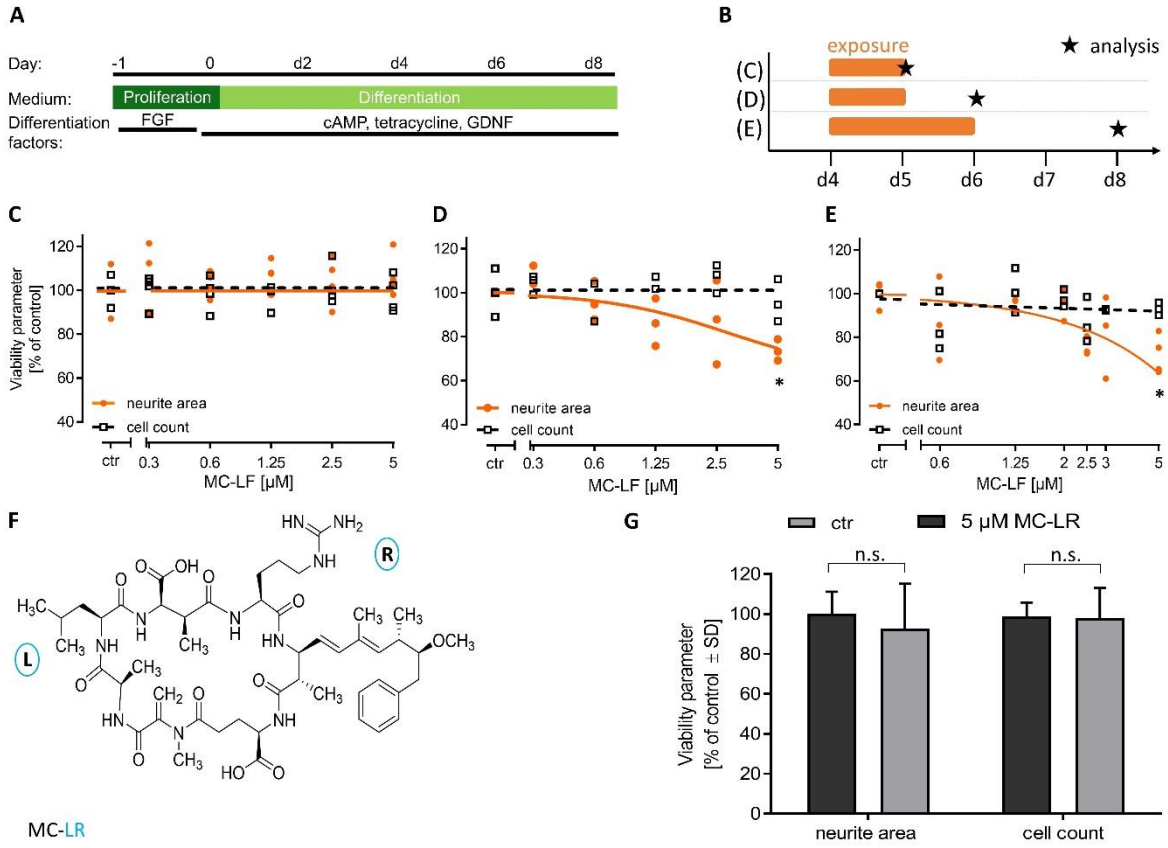

Fig. S1: Time dependent effects of MC on LUHMES
LUHMES are a homogenous cell population that can be differentiated into postmitotic neurons within six days. They were used here as model system for central human neurons. (A) Schematic overview of LUHMES cultivation and differentiation. (B) Schematic depiction of MC-LF exposure scenarios and washout periods in LUHMES. (C) Effect of MC-LF on neurite area (orange) and cell count (black) after a 24 h treatment (d4 until d5) (D), 24 h treatment (d4 until d5) followed by a 24-h washout period (E) or 48 h treatment (d4 until d6) followed by a 48-h washout period. Data points are from three separate experiments, *p < 0.05. (F) Chemical structure of MC-LR. (G) Effect of 5 µM MC-LR on neurite area and cell count after 48 h treatment (d4 until d6). Data are means ± SD; n = 3.

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ALTEX 38(1), SUPPLEMENTARY DATA

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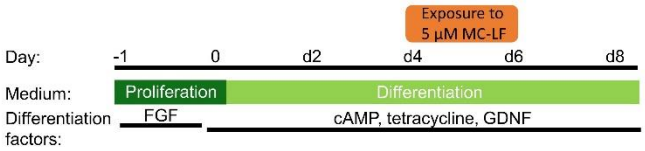
A



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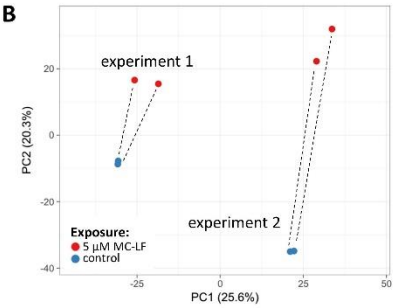

C

most upregulated GOs (biological process)	adjusted p-value
translational initiation	0.000006
nuclear-transcribed mRNA catabolic process nonsense-mediated decay	0.000014
ribonucleoprotein complex biogenesis	0.000110
SRP-dependent co-translational protein targeting to membrane	0.000217
Translation	0.000256
cotranslational protein targeting to membrane	0.000281
peptide biosynthetic process	0.000341
nuclear-transcribed mRNA catabolic process	0.000374
protein targeting to ER	0.000411
establishment of protein localization to endoplasmic reticulum	0.000493

D

downregulated GOs (biological process)	adjusted p-value
mitotic nuclear division	0.000007
mitotic cell cycle process	0.000237
DNA packaging	0.000256
nuclear division	0.000460
organelle fission	0.001165
DNA conformation change	0.006639
mitotic sister chromatid segregation	0.009819
telencephalon regionalization	0.012837
sister chromatid segregation	0.029134
DNA replication-independent nucleosome assembly	0.035066

E

DEGs of GO „heterocycle catabolic process“	Log2 fold change	Short explanation
RABGGTB	0.41	Rab Geranylgeranyltransferase
RPL6	0.67	Ribosomal protein
UBB	0.53	Ubiquitin
UCHL1	0.29	Ubiquitin hydrolase
GSTO1	1.16	Glutathione S-Transferase
PPP1R17	0.78	Inhibits phosphatase activities in PP1 and PP2A
RPL13A	0.86	Ribosomal protein
RPS27L	0.87	Ribosomal protein

F

DEGs of GO „mitotic nuclear division“	Log2 fold change	Short explanation
NCAN	-0.56	Neurocan
DUSP4	-0.64	Phosphatase, inactivating MAPK
EMX2	-0.87	Neurodevelopmental transcription factor
SNCA	-0.58	α-synuclein
SNRPA	-1.17	Small nuclear ribonucleoprotein (splicing)
TCF4	-1.08	Neurodevelopmental transcription factor
TYMS	-1.25	Thymidylate synthetase
HTATSF1	-0.57	transcription factor of transcriptional elongation
CENPK	-0.82	Subunit of the centromere complex
MRPS6	-0.51	Mitochondrial ribosomal protein

Fig. S2: Overrepresented gene ontologies (oGOs) in MC-LF-treated LUHMES
(A) Schematic overview of LUHMES cultivation and differentiation including the exposure period to 5 µM MC-LF for 48 h from d4 until d6. (B) Samples were obtained on d6 from untreated LUHMES (blue) and LUHMES treated with 5 µM MC-LF for 48 h from d4 until d6 and RNA expression profiles were obtained. The PCA was generated with ClustVis. The dotted lines connect the data points treated for 48 h with 5 µM MC-LF of two different biological replicates and their corresponding controls. Data are from two independent biological experiments with two technical replicates each. (C) The gProfiler analysis tool was used to identify overrepresented GOs (oGOs) among the DEGs. The 10 most upregulated biological processes in this analysis are listed with their adjusted p-values. (D) The 10 most downregulated biological processes in this analysis are listed with their adjusted p-values. All adjusted p-values included into the analysis are ≤ 0.05. (E and F) As examples for gene sets within oGO, those for “heterocycle catabolic process” and “mitotic nuclear division” are shown here. All tables indicate the gene symbol, the regulation (Log2 fold change) by MC-LF, and a short comment on the function or the gene name.

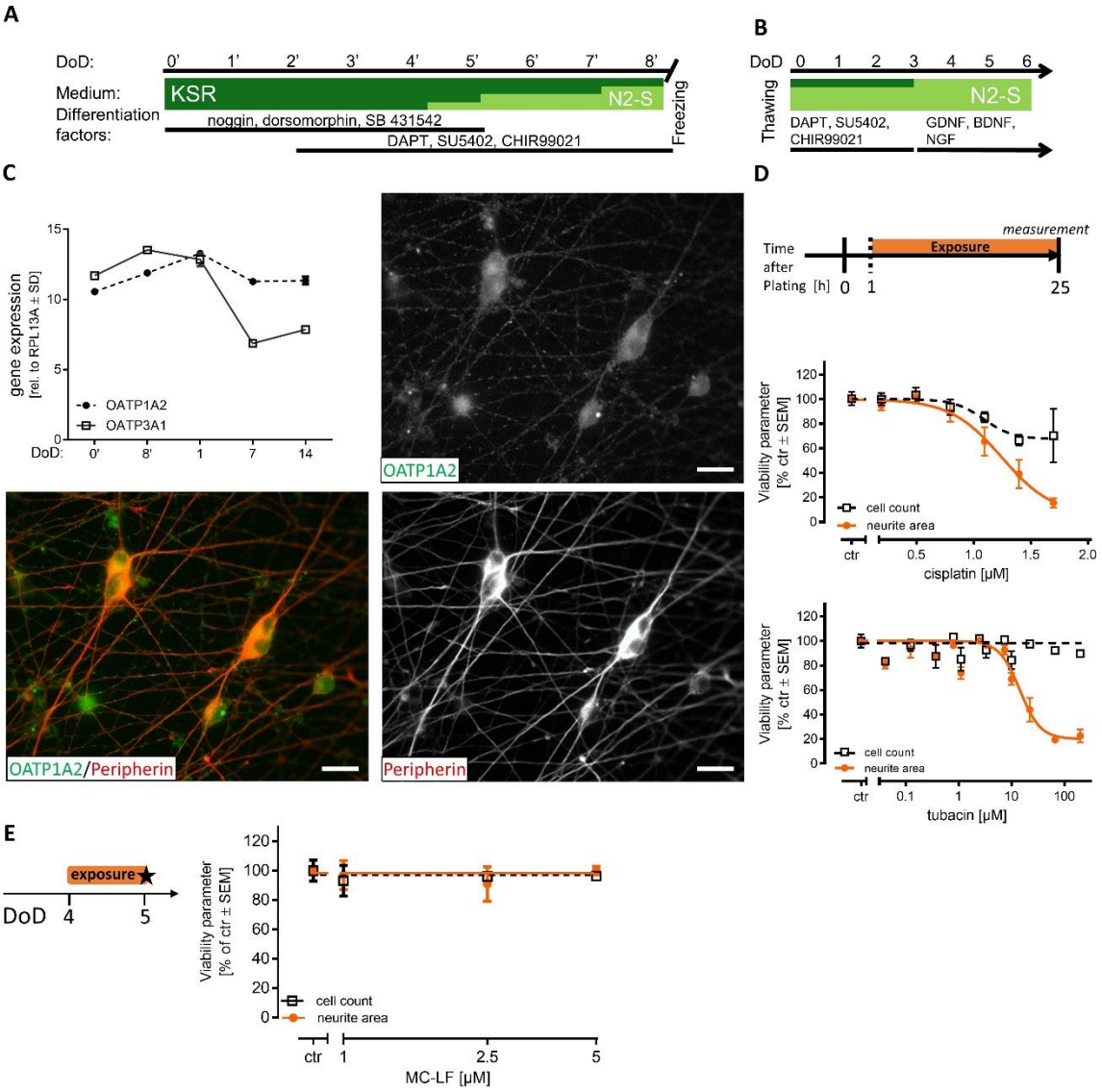

Fig. S3: Characterization of peripheral neurons for toxicity evaluation
(A) Peripheral neurons are used as complementary test system and are generated from pluripotent stem cells according to an established protocol, which includes a freezing step, allowing differentiation of large cell batches and thawing of cells for individual experiments. (B) After thawing, the cells are cultured and treated for various periods. (C) Time course of OATP1A2 (circle) and OATP3A1 (square) expression levels in peripheral neurons determined with RT-qPCR (n = 2). Cells were differentiated on coverslips and fixed on DoD7. Double-immunofluorescence images were obtained for peripheral neurons (using an antibody against peripherin) and for OATP1A2. The individual channels are shown in b/w for maximum clarity, the composite image is shown with OATP1A2 in green and peripherin in red. Scale bar is 20 µm. (D) Schematic depiction of well-established PeriTox assay with treatment of outgrowing neurites with various substances from DoD0 until DoD1. Effect of cisplatin and tubacin on neurite area (orange) and cell count (black) after 24 h treatment from DoD0 until DoD1. Data are means ± SEM; n = 3. (E) Effect of MC-LF on neurite area (orange) and cell count (black) after treatment for 24 h (DoD4 until DoD5) Data are means ± SEM; n = 3.

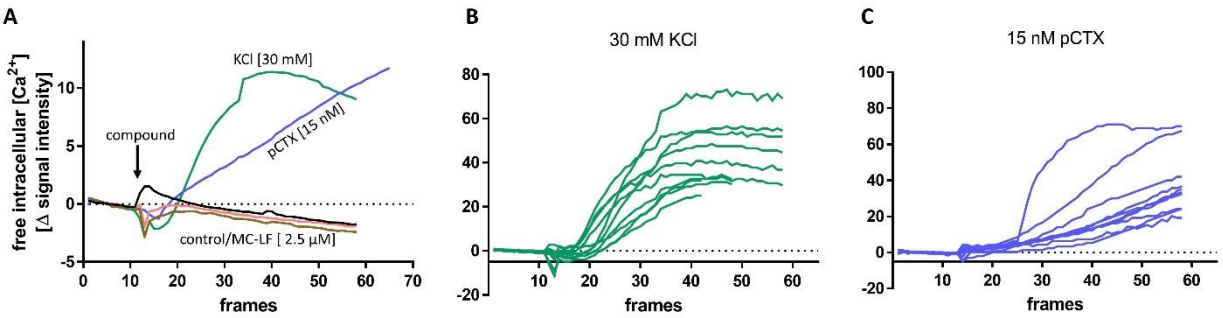

Fig. S4: Ca^{2+} signaling as functional endpoint for toxicity evaluation
Peripheral neurons can be cultivated for longer time periods and are therefore suitable as a test system for electrically active neurons. Cells were cultured for 23 days and Ca^{2+} signaling was analyzed. (A) Cells were treated with solvent control 0.3% DMSO (brown), buffer control (black), positive control 30 mM KCl (green), 15 nM pCTX (blue), or 2.5 µM MC-LF (red). The substance application was performed by a pipettor, images were taken by an automated microscope for 45 s, exported as .avi and analyzed by CaFFEE. The free intracellular Ca^{2+} is shown as the average Δ signal intensity t_{peak} - $t_{baseline}$. n = 1 (B) The free intracellular Ca^{2+} of ten different cells is shown with their individual Δ signal intensity after the application of 30 mM KCl. (C) The free intracellular Ca^{2+} of ten different cells is shown with their individual Δ signal intensity after the application of 15 nM pCTX.