Analyzing the developmental profile and physiological function of neuronal K_v7 channels

Elif Karabatak, Thomas Budde
University Hospital Münster, Germany
Institute of Physiology I

Introduction

K_v7 (KCNQ or M-) channels generate voltage dependent K^+ currents and represent the molecular basis of the M-current (I_M). The five known subtypes ($K_v7.1$-7.5) are characterized by different tissue expression and physiological properties.[1] In most neurons, native M-channels are composed of $K_v7.2$ and $K_v7.3$ subunits, and are responsible for controlling the neuronal excitability.[2] During physiological as well as pathophysiological conditions (e.g. epilepsy) channel modulators play a significant role.[3] Previous studies from our group point out the importance of M-channels in the thalamocortical system, making them interesting targets for further investigation.[4]

With this project, we thus aim to investigate:
1) The developmental expression profile of $K_v7.2$ and $K_v7.3$ in thalamic neurons. 2) The modulation of these channels by different compounds including PIP_2, cholesterol analogues and novel activators. 3) The properties of I_M in prethalamic/ thalamic neurons in health and disease.
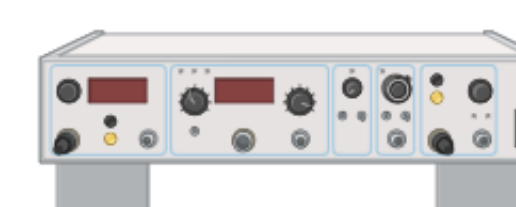
Methods
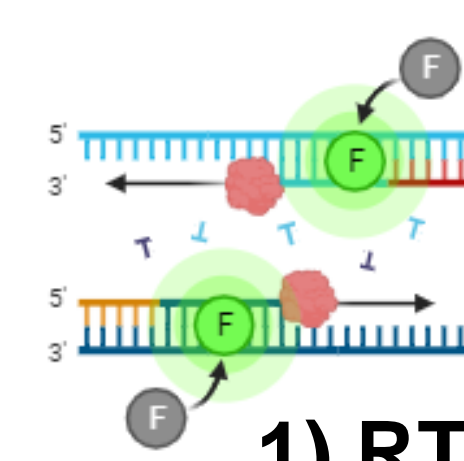
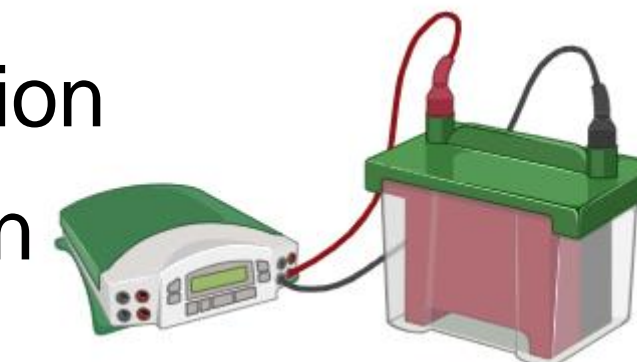**1) RT-qPCR** for the investigation of ion channel expression on mRNA level

2) Western blot for the investigation of ion channel expression on protein level

3) Cell transfection for generating a cell line with functional M-channel

5) Patch-clamp for the examination of electrophysiological ion channel activity
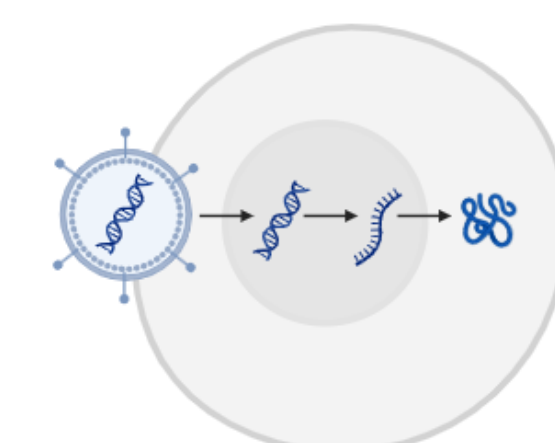
4) Mouse models C57BL/6 (wild-type mice) KCNQ3 KO mice

Focus on $K_v7.2$ and $K_v7.3$ channels

Figures generated with BioRender

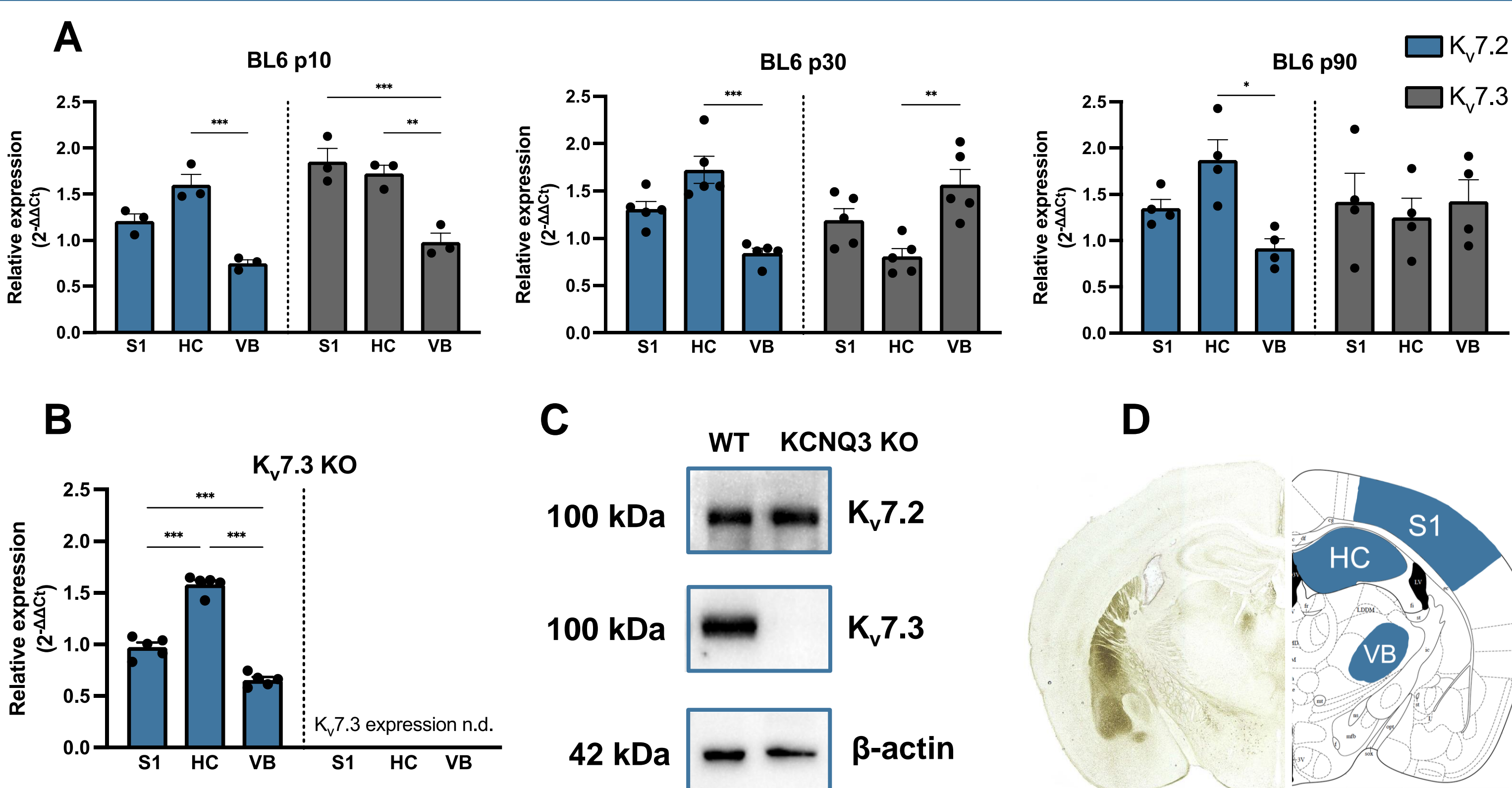## Developmental expression profile of $K_v7.2$ and $K_v7.3$ in mouse brain

Figure 1: $K_v7.2$ and $K_v7.3$ gene and protein expression in mice during development.
S1: Somatosensory cortex. HC: Hippocampus. VB: Ventrobasal complex. **A)** Relative gene expression in C57BL/6JOlaHsd mice. Early infant mice (*n*=3): p10, adolescent mice (*n*=4): p30, adult mice (*n*=4): p90. **B)** Relative gene expression in KCNQ3 KO mice (*n*=5): p90 and older. One-way Anova test was applied. Error bars represented as ± SEM. *p = 0.033, **p = 0.01, ***p < 0.001. **C)** Representative data for western blotting. $K_v7.2$ and $K_v7.3$ in mouse brain: p30. **D)** Regions of interest in coronal brain slices (Modified from mouse brain atlas, Paxinos and Franklin).

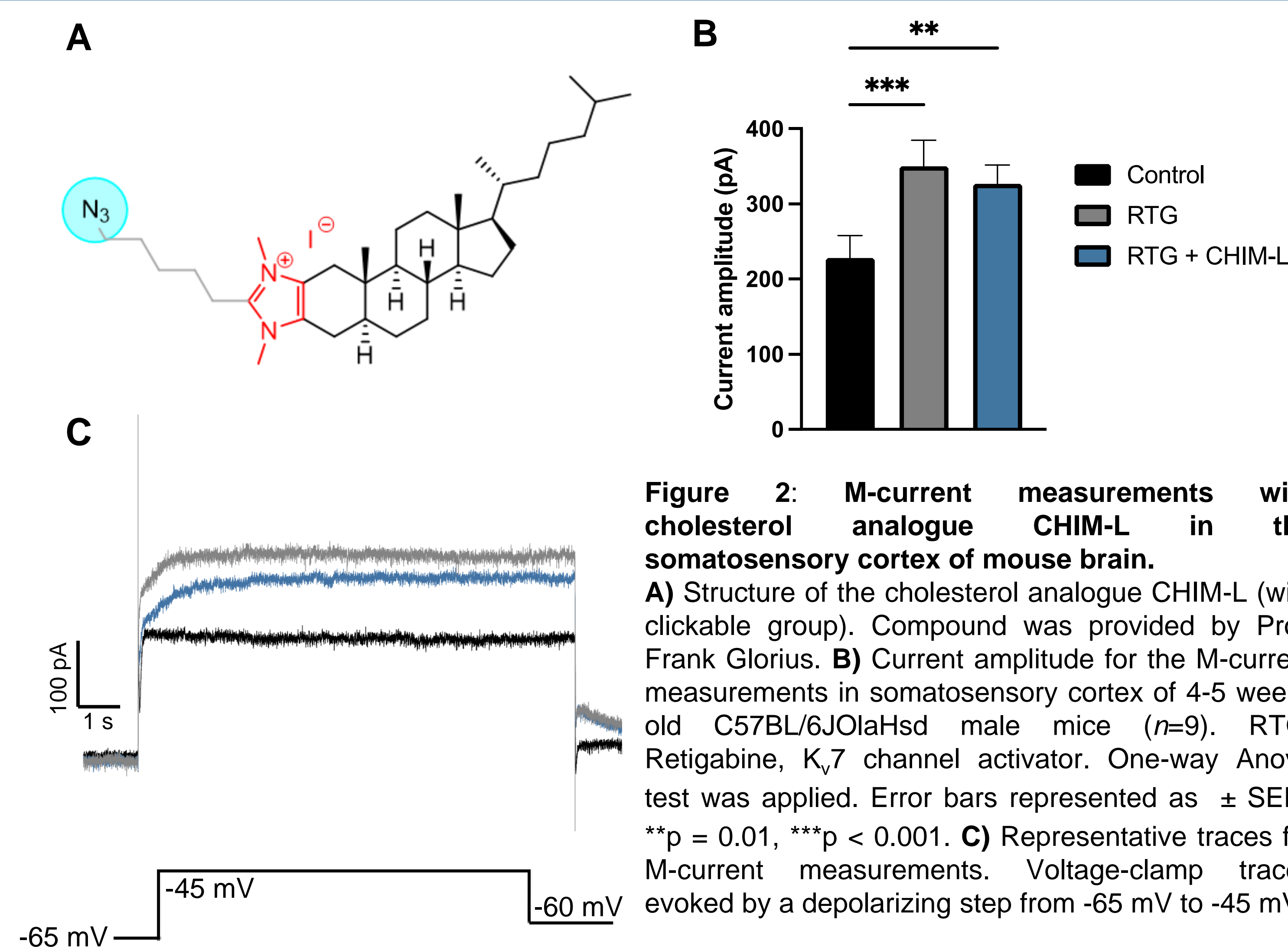## K_v7 modulation with the cholesterol analogue CHIM-L

Figure 2: M-current measurements with cholesterol analogue CHIM-L in the somatosensory cortex of mouse brain.
A) Structure of the cholesterol analogue CHIM-L (with clickable group). Compound was provided by Prof. Frank Glorius. **B)** Current amplitude for the M-current measurements in somatosensory cortex of 4-5 weeks old C57BL/6JOlaHsd male mice (*n*=9). RTG: Retigabine, K_v7 channel activator. One-way Anova test was applied. Error bars represented as ± SEM. **p = 0.01, ***p < 0.001. **C)** Representative traces for M-current measurements. Voltage-clamp traces evoked by a depolarizing step from -65 mV to -45 mV.
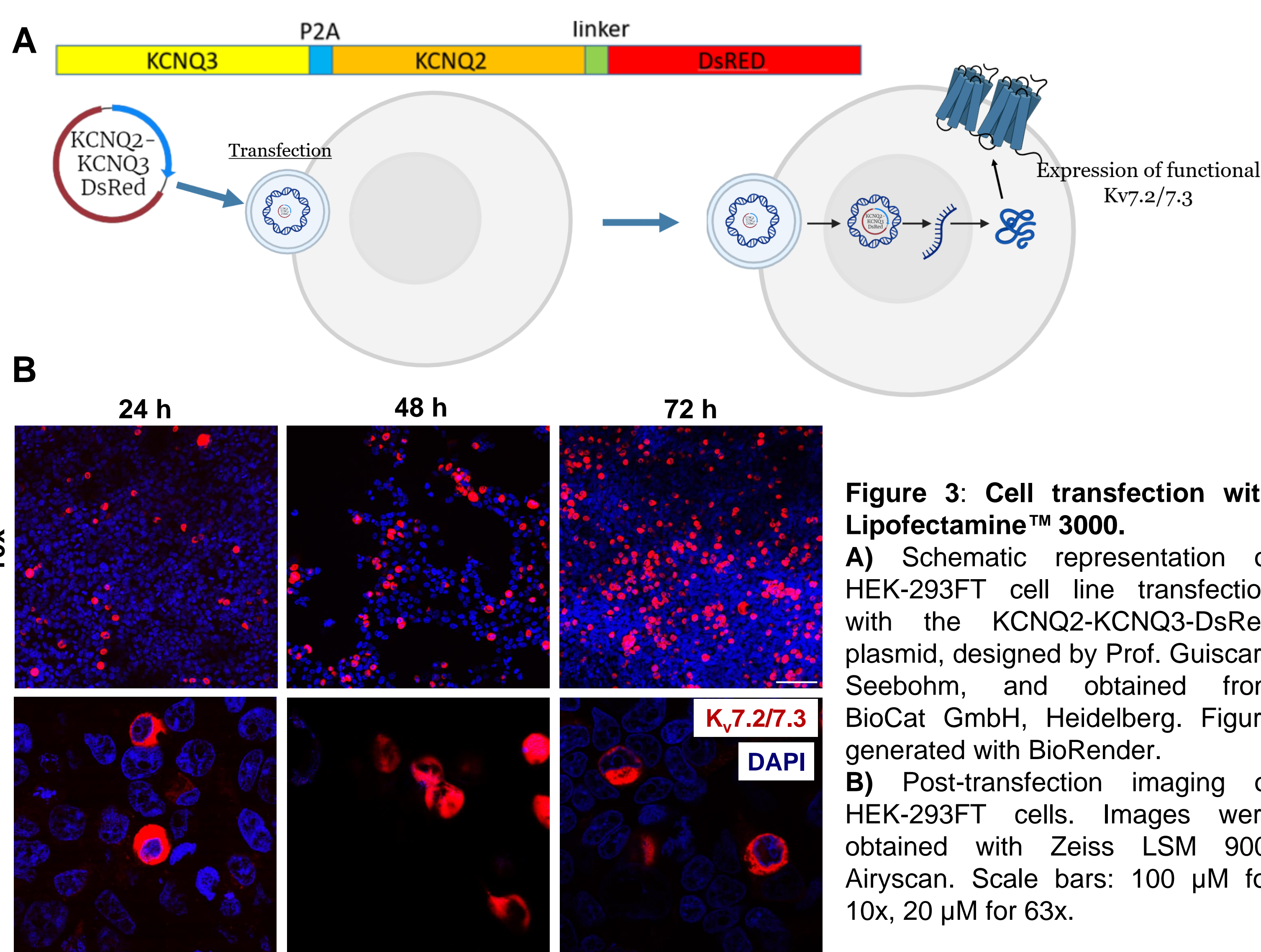
Cell transfection

Figure 3: Cell transfection with Lipofectamine™ 3000.
A) Schematic representation of HEK-293FT cell line transfection with the KCNQ2-KCNQ3-DsRed plasmid, designed by Prof. Guiscard Seebohm, and obtained from BioCat GmbH, Heidelberg. Figure generated with BioRender. **B)** Post-transfection imaging of HEK-293FT cells. Images were obtained with Zeiss LSM 900, Airyscan. Scale bars: 100 μM for 10x, 20 μM for 63x.

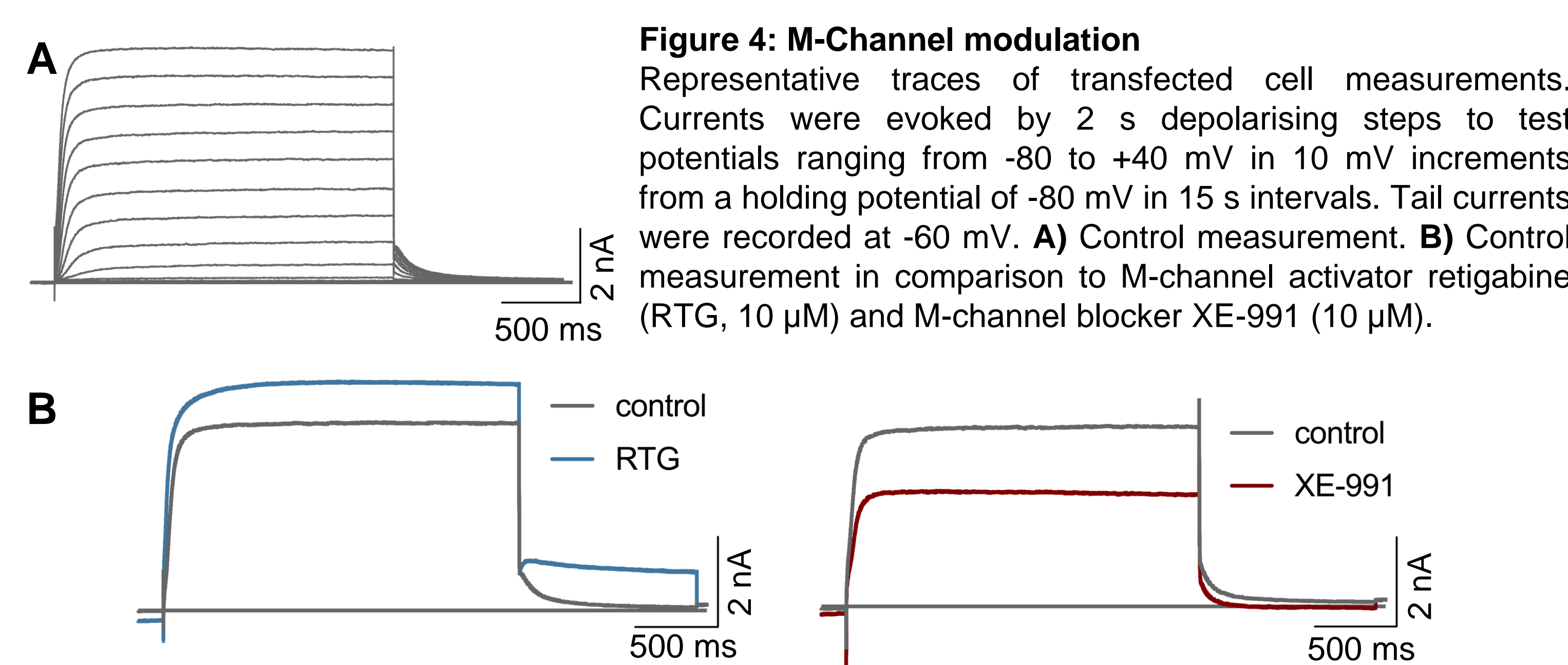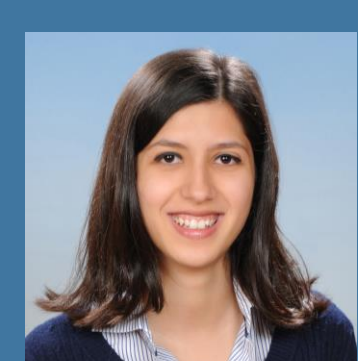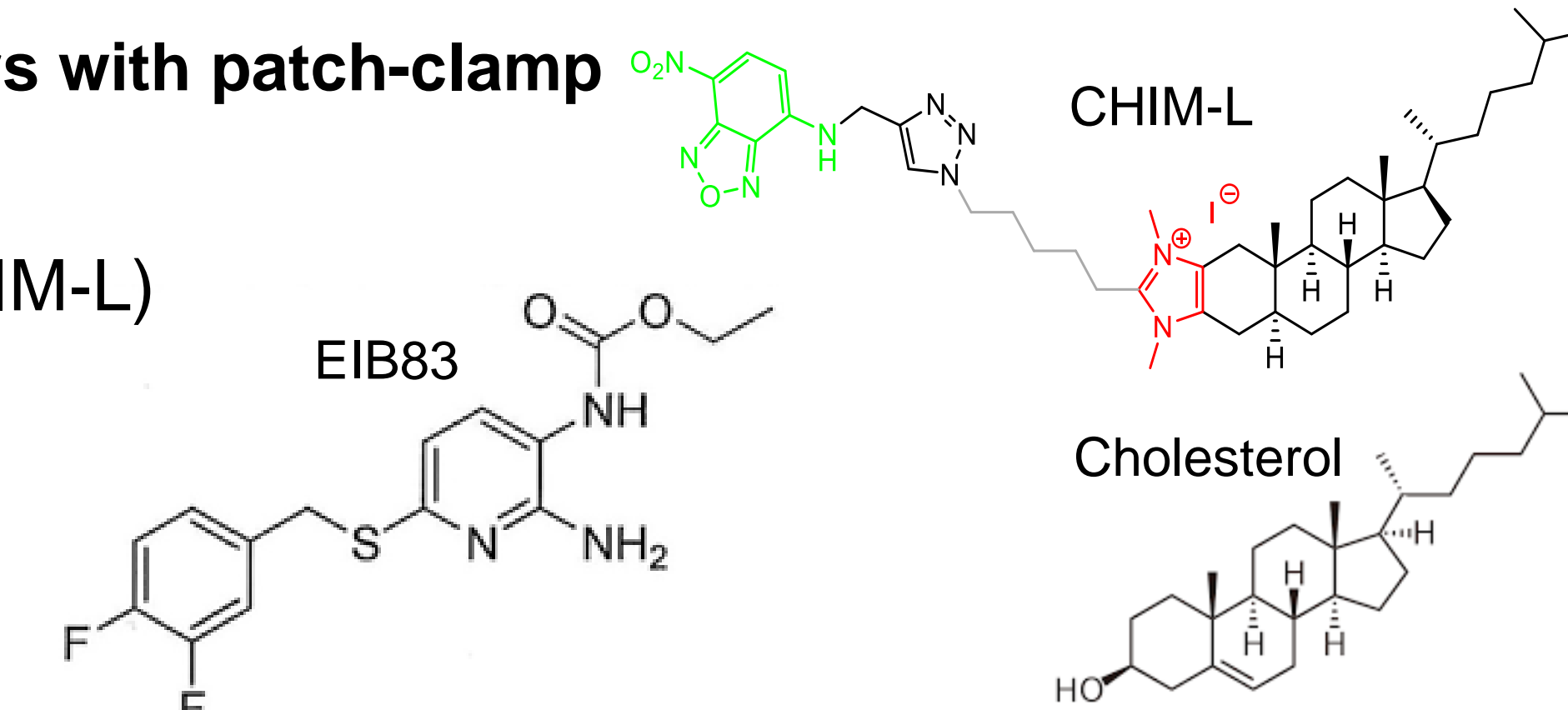## Functionality test of transfected cells

Figure 4: M-Channel modulation
Representative traces of transfected cell measurements. Currents were evoked by 2 s depolarising steps to test potentials ranging from -80 to +40 mV in 10 mV increments from a holding potential of -80 mV in 15 s intervals. Tail currents were recorded at -60 mV. **A)** Control measurement. **B)** Control measurement in comparison to M-channel activator retigabine (RTG, 10 μM) and M-channel blocker XE-991 (10 μM).

Future prospects

Test of M-channel modulators with patch-clamp
- Natural cholesterol
- Cholesterol analogues (CHIM-L)
- PIP_2 analogues
- Retigabine analogues

Elif Karabatak
PhD Candidate
Member of Physiology 1 since 09/2023
E-Mail: elif.karabatak@ukmuenster.de

References:
[1] Dirkx *et al.*, Front. Physiol., **2020**, S. 1240.
[2] Brown *et al.*, Br. J. Pharmacol., **2009**, 156(8), 1185-1195.
[3] Borgini *et al.*, RSC Med. Chem., **2021**, 12.4, 483-537.
[4] Cerina *et al.*, Br. J. Pharmacol., **2015**, 172.12, 3126-3140.