Human iPSC-derived PNS mono- & co-culture systems for pain and sensory neuron research and drug discovery

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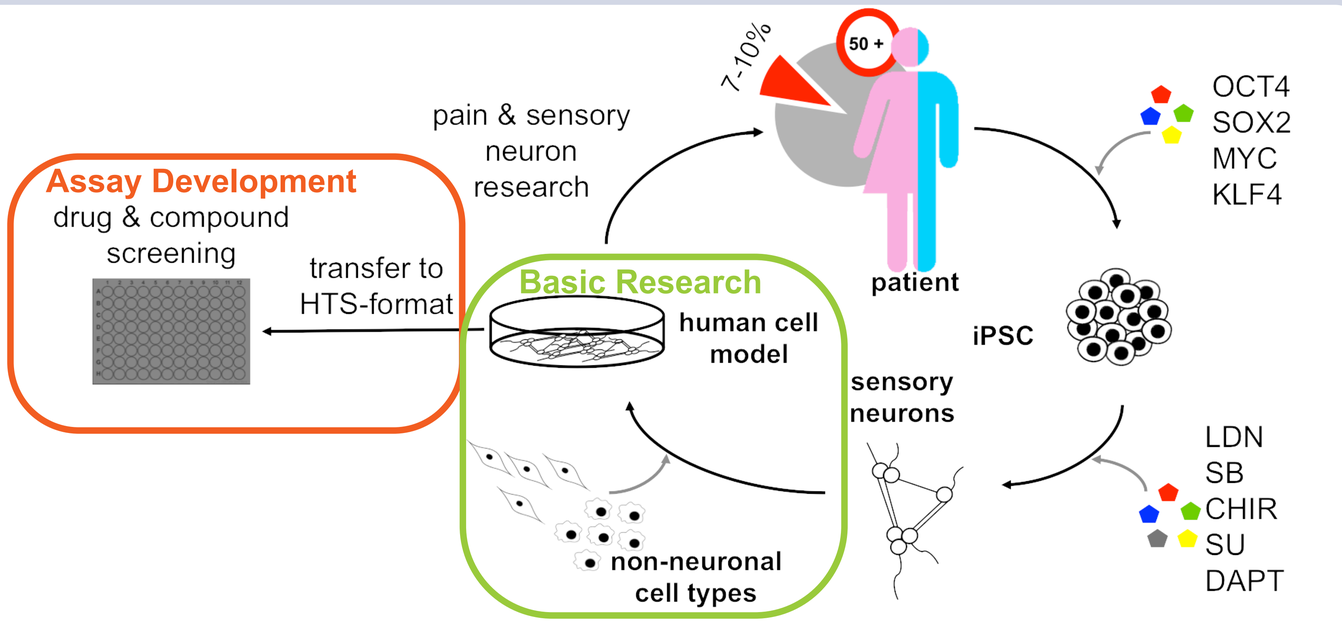
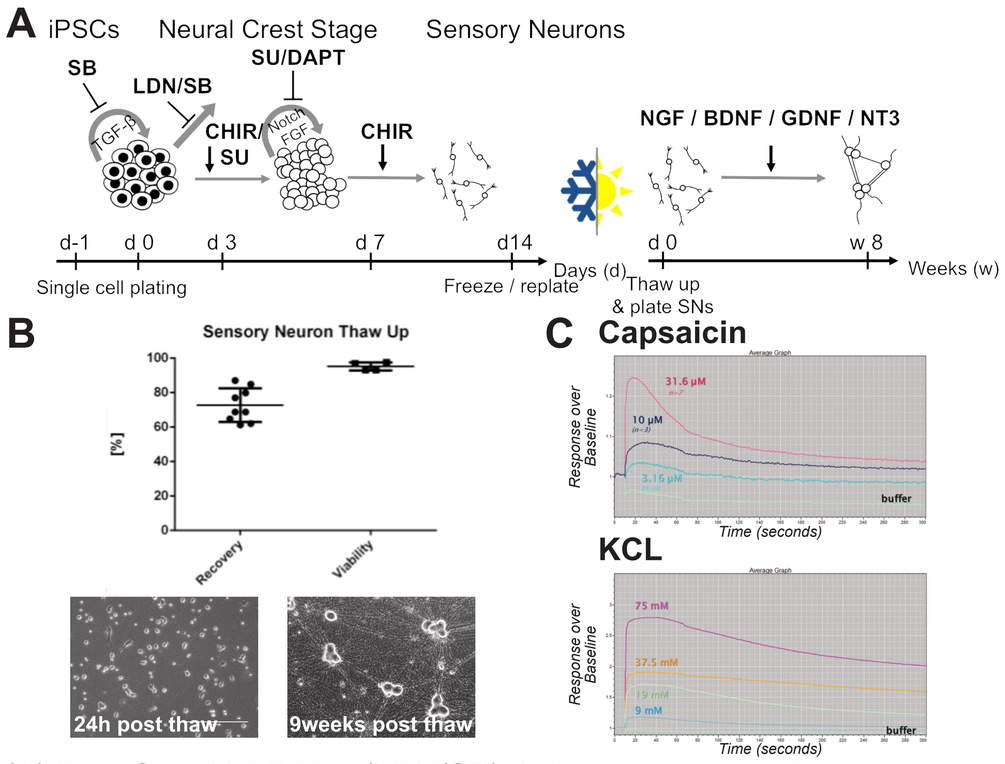
1. Introduction

- 7-10% of the population suffer from chronic neuropathic pain.
- Current medication is often non-efficacious with serious side effects.
- Translation from rodent data to human patients is problematic, suitable human cellular models are missing.
- Sensory neuron cell bodies cluster together with **non-neuronal cells** (glia, immune cells) in the dorsal root ganglion, from where signals are transmitted from the PNS to the CNS.
- We aim to develop:
  i) an in vitro model of neuron-glia interactions
  ii) HTS compatible cell systems



2. Large scale differentiation and cryo-preservation of iPSC-SN

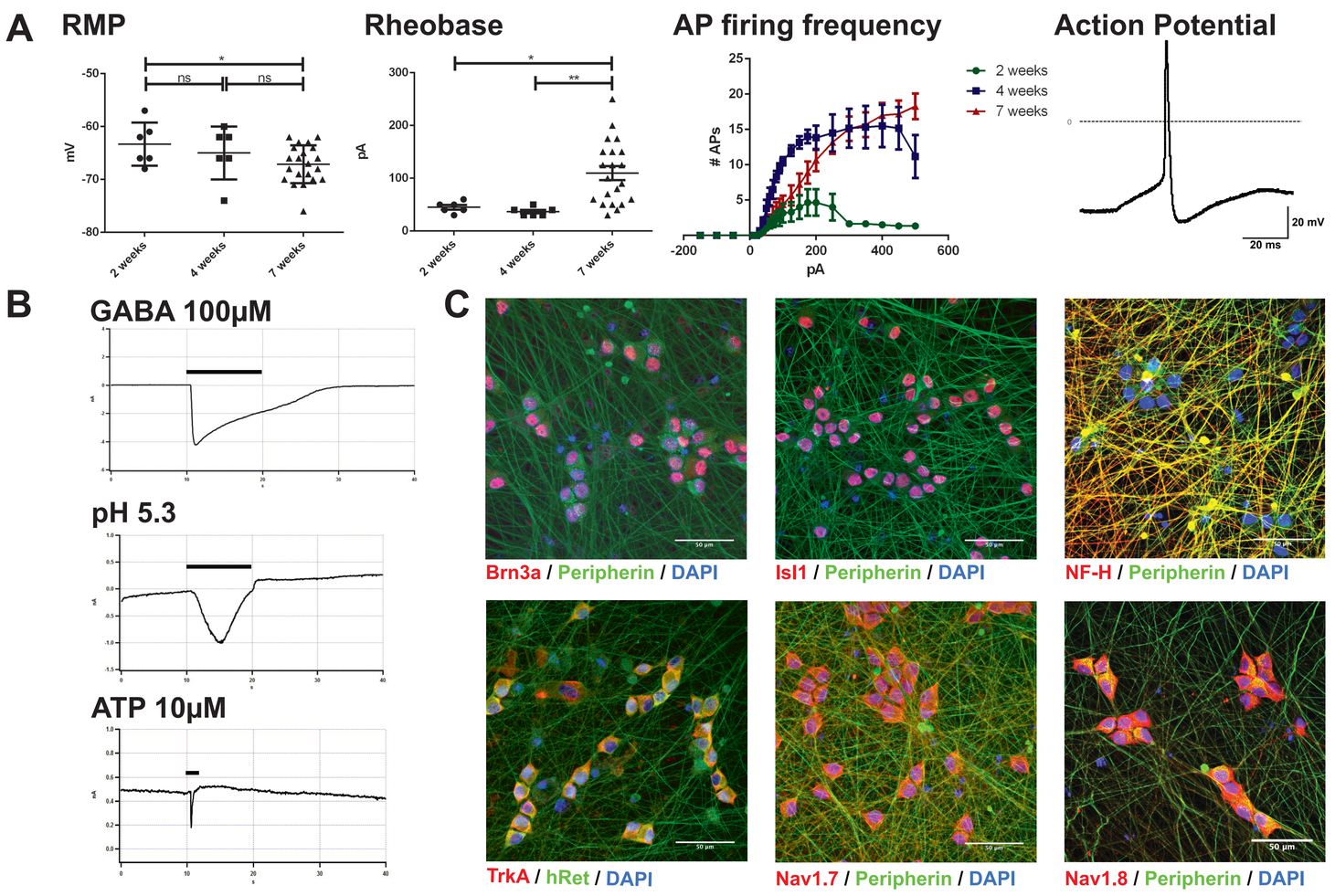


(A) Dual Smad inhibition (LDN/SB) induces neuroectoderm formation, CHIR, SU and DAPT guides differentiation towards sensory neurons
- differentiation is scalable from 6-Well to T175-Flasks

(B) iPSC-SN can be cryopreserved at day 14 of differentiation. iPSC-SN show a high viability and good recovery after thawing.

(C) Cryopreserved iPSC-SN can be employed for FLIPR-assays in an HTS-format. iPSC-SN show specific reactivity to pain relevant stimuli on a population level like Capsaicin (TrpV1) and KCl (CaV) in a concentration dependend manner.

3. Characterization of iPSC-SN

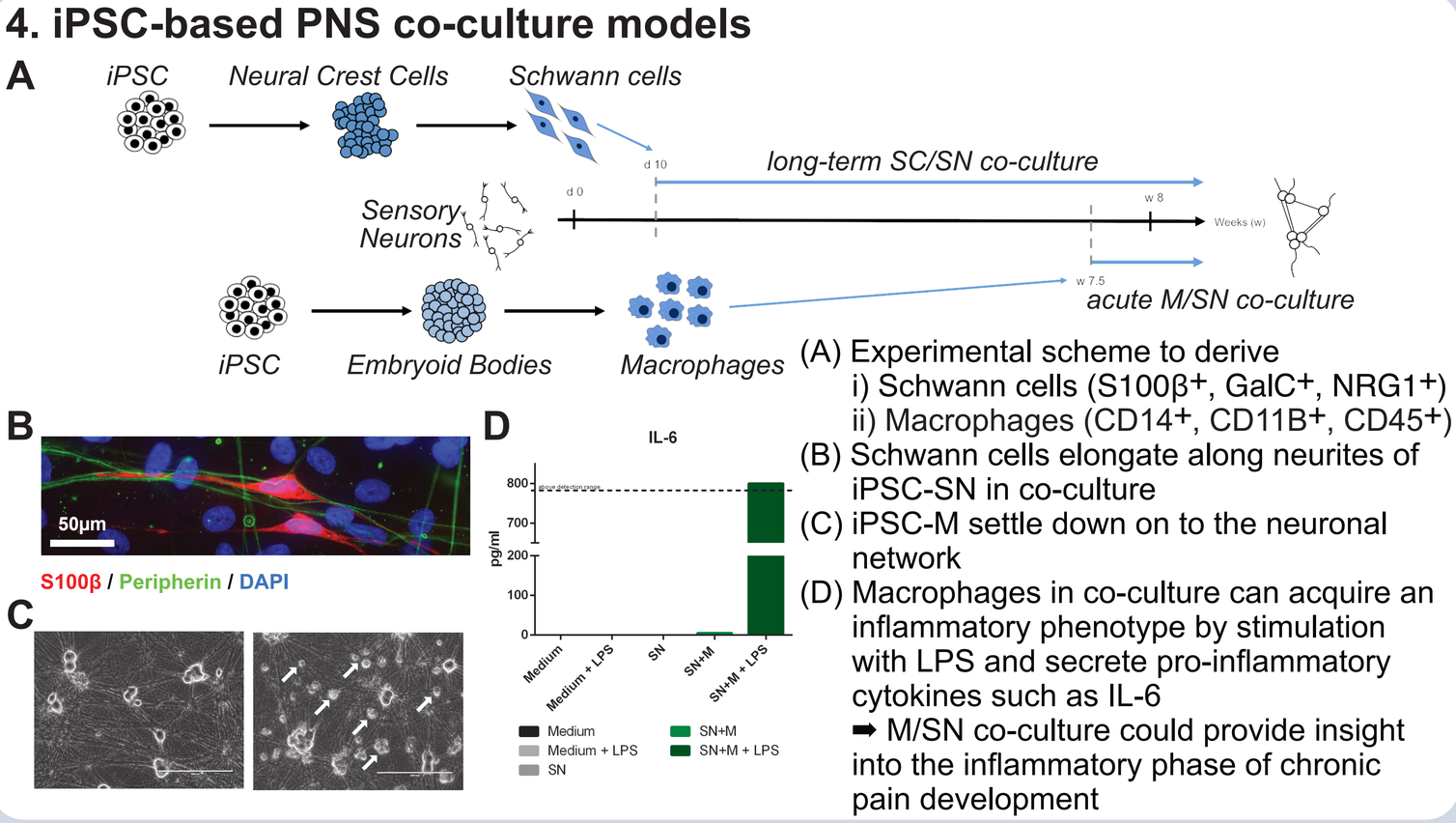


(A) RMP, Rheobase and AP firing frequency change over time-course of maturation.

(B) iPSC-SN show specific responses to the activation of ligand-gated ion channels (stimuli: GABA, ATP and a pH-shift) on a single cell level in manual patch clamp.

(C) Homogeneous expression pattern of key sensory neuron markers after 3 weeks of maturation.

4. iPSC-based PNS co-culture models



(A) Experimental scheme to derive
i) Schwann cells (S100β+, GalC+, NRG1+)
ii) Macrophages (CD14+, CD11B+, CD45+)

(B) Schwann cells elongate along neurites of iPSC-SN in co-culture

(C) iPSC-M settle down on to the neuronal network

(D) Macrophages in co-culture can acquire an inflammatory phenotype by stimulation with LPS and secrete pro-inflammatory cytokines such as IL-6
➡ M/SN co-culture could provide insight into the inflammatory phase of chronic pain development

5. Summary & Outlook

- Establishment of robust, large scale differentiation of cryopreservable sensory neurons from iPSCs
- In-depth characterization of iPSC-derived sensory neurons
  - iPSC-SN express key sensory neural markers e.g. Brn3a
  - Characteristic sensory neuron functionality could be detected by manual patch clamp and FLIPR calcium assays
- Establishment of Schwann cell/ sensory neuron and Macrophage/ sensory neuron co-culture paradigms are expected to provide insight into non cell-autonomous effects underlying chronic pain
- Next steps:
  - Evaluate effect of SC/SN co-culture on SN maturation
  - Evaluate effect of pro-in ammatory cytokines on iPSC-SNs

6. Acknowledgement

This work was co-funded by the Ministry of Innovation, Science and Research of the State of North Rhine-Westphalia, the European Union (EFRE-0800407 & EFRE-0800408) and the Innovative Medicines Initiative 2 Joint Undertaking (IMI2 JU) under grant agreement No 116072. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation program and the European Federation of Pharmaceutical Industries and Associations (EFPIA).

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