PWD99

Forward Programmed Human Sensory Neurons for Modeling Genetic Pain Disorders

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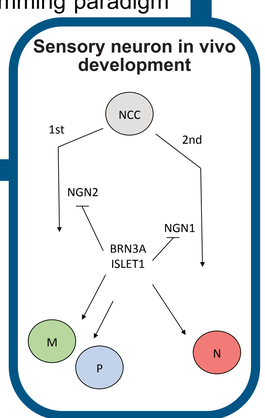
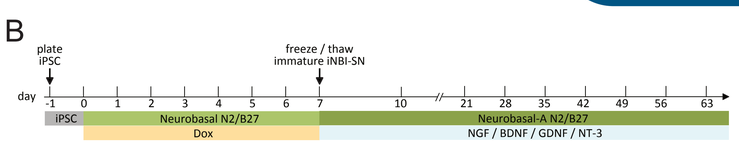
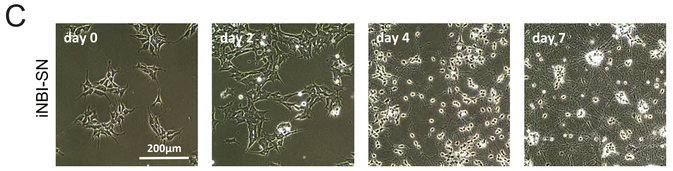
1. Introduction

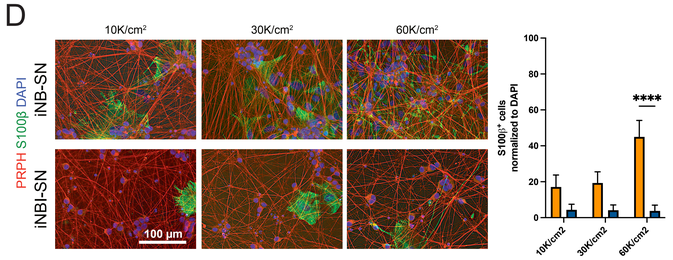
- Human in vitro models of the peripheral nervous system and sensory neurons are becoming increasingly important as more differences between human and rodent sensory neurons in terms of gene and protein expression, ion channel functionality and subtype specification are discovered.
- Conventional differentiation protocols are prone to batch-to-batch variation and need optimization for new iPSC-lines.
- Therefore we implemented a novel forward programming paradigm to overcome this probelm:
  We generated efficently and robust functional human sensory neurons via overexpression of fate specifying transcription factors.



2. Forward Programming of Human Sensory Neurons

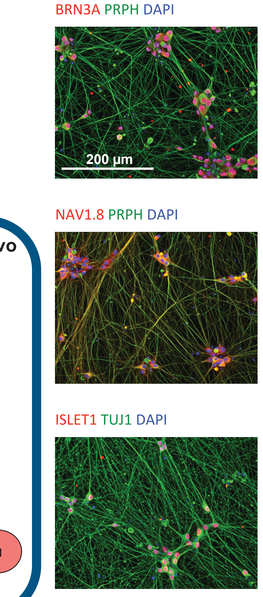


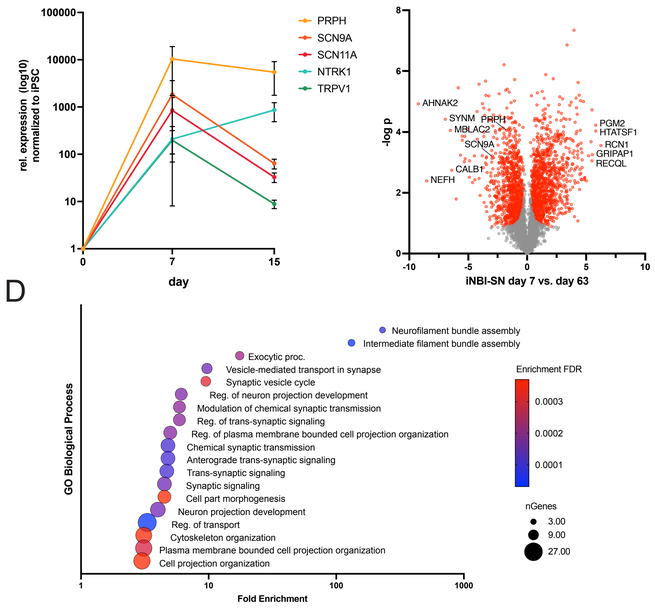




(A) Schematic representation of the AAVS1 targeting vector for doxycycline-inducible gene expression. **(B)** Forward programming paradigm for the generation of functional sensory neurons (iNBI-SN). Following transgene induction of *NGN1*, *BRN3A* and *ISLET1* by doxycycline, the cells enter a sensory neuron fate and can be cryopreserved. Further propagation in NGF, BDNF, GDNF and NT-3 is used for full maturation. **(C)** Phase contrast images of the typical morphological changes of iNBI-SN during differentiation. Already after four days of transgene overexpression cells start to acquire a neuron-like morphology and after seven days a neuronal network becomes apperent. **(D)** Representative images of iNB-SN and iNBI-SN cultures at day 28 of culture stained for the neuronal marker PRPH and the glia marker S100β. N=6 independent experiments.

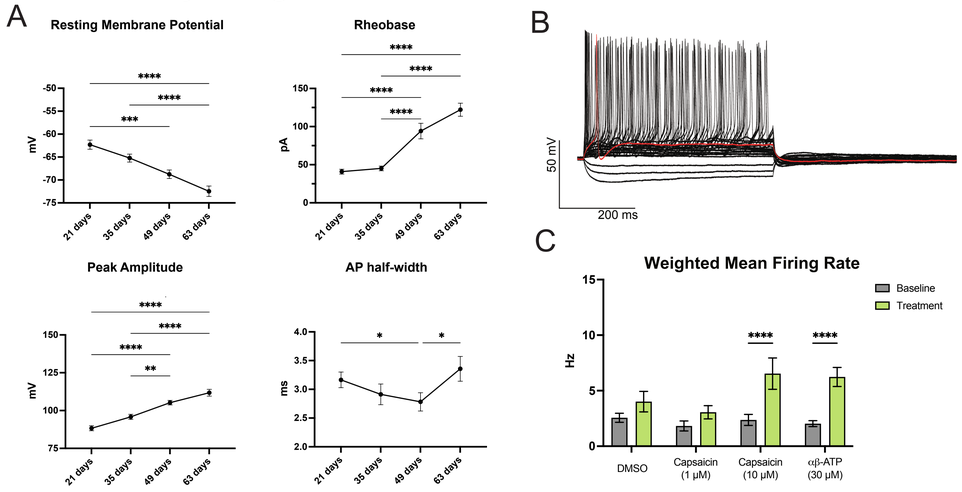
3. Gene and Protein Expression During iNBI-SN Differentiation





(A) Immuncytochemical stainings of sensory neuron markers BRN3A, PRPH, Nav1.8 and ISLET1 as well as the pan-neuronal marker β-tubulin (TUJ1) in iNBI-SN cultures matured for eight weeks. **(B)** Quantitative real-time PCR analysis of gene expression during iNBI-SN differentiation for the sensory neuron and nociceptive neuron marker genes *PRPH*, *SCN9A*, *SCN11A*, *NTRK1* and *TRPV1*. **(C)** Vulcano plot comparing protein expression levels after 7 and 63 days of differentiation. Negative values represent higher expression levels in day 63 samples. Signi cantly regulated proteins are highlighted in red, top ve up- and downregulated proteins, as well as SCN9A and PRPH are labeled with respective names. **(D)** Gene Ontology enrichment analysis for biological processes based on the top 100 upregulated proteins in day 63 iNBI-SN.

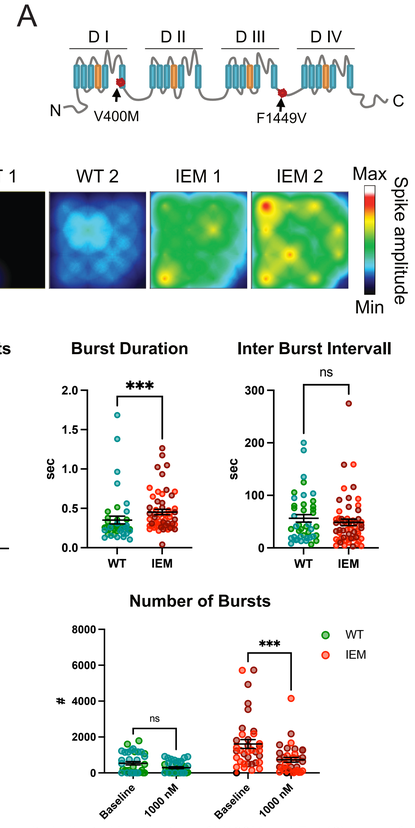
4. Electrophysiological Properties of iNBI-SN



(A) Forward programmed sensory neurons were analyzed with respect to resting membrane potential, rheobase, action potential peak amplitude and action potential half-width. N = 39-40 cells per time point. **(B)** Exemplary trace of induced action potential firing in iNBI-SN after eight weeks of maturation, red trace indicates AP at rheobase. **(C)** Activation of iNBI-SNs by application of 1 and 10 µM Capsaicin and 30 µM αβ-ATP was assessed by multi-electrode array measurements (weighted mean firing rate) N=4-11.

5. Disease Modeling and Drug Testing: IEM

- Inherited Erythromelalgia (IEM) is a rare genetic chronic, extreme pain condition cause by mutation in the sodium ion channel Nav1.7 (SCN9A) with a prevalence of up to 1 in 100,000 people



(A) Schematic overview of Nav1.7 channel structure with indicated localization of V400M and F1449V gain-of-function mutations identified in IEM-patient derived iPSC-lines IEM 1 and IEM 2 respectively. **(B)** Multi-electrode array recordings were performed using the Meastro Edge system (Axion BioSystems). **(C)** Heat map of spontaneous activity (spike amplitude) across representative wells. **(D)** Weighted mean firing rate, number of bursts and burst duration was found to be significantly increased in IEM patient-derived iNBI-SN compared to healthy controls, while inter burst intervall was not found to be altered during 15 minutes multi-electrode array recordings. N=17-24 Wells. Each dot represents a single well. Lighter and darker colors indicate different donors. **(E)** Application of 1000 nM PF-05089771, a specific inhibitor of Nav1.7 significantly reduces weighted mean firing rate and number of bursts in IEM patient-derived iNBI-SN. N=17-18 Wells. Each dot represents a single well. Lighter and darker colors indicate different donors.

7. Conclusions

- Overexpression of fate specifying transcription factors 'NGN1-BRN3A-ISLET1' in iPSCs generates fast and efficiently human sensory neurons.,
- iNBI-SN are electrophysiologically active and functional TRPV1 and P2X3 receptors can be detected by multi-electrode array measurements after eight weeks of maturation.
- iNBI-SN can recapitulate the phenotype of genetic chronic pain disorders, such as inherited erythromelalgia (IEM).
- **Human forward programmed sensory neurons are suitable for disease modeling, drug testing and assay development.**

8. Conflict of Interest Statement

PR was awarded an Axion BioSystems Travel Award. OB is co-founder and shareholder of LIFE & BRAIN GmbH. All other authors declaire no conflict of interest.

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