A role for the primary cilium in network properties of neurons

Emma Dyke[1], Chantal Bijnagte-Schoenmaker[1], Rachel Mijdam[3], Lisa Rahm[4], Marie Le Bihan[1,2], Ummi Ciptasari[1,2], Jindřiška Leischner Fialová[5], Brooke Latour[1], Dirk Schubert[6], Ronald Roepman[1], Nael Nadif Kasri[1, 2]

1) Department of Human Genetics, Radboudumc, Nijmegen, The Netherlands; 2) Donders Institute for Brain, Cognition and Behaviour, Nijmegen, The Netherlands; 3) Radboudumc, Neurology, Nijmegen, The Netherlands; 4) Radboudumc, Donders Institute for Brain, Cognition, and Behaviour, Human Genetics, Nijmegen, The Netherlands; 5) Department of Biology, University of Copenhagen, Copenhagen, Denmark; 6) Donders Institute for Brain, Cognition and Behavior, Department of Cognitive Neuroscience, Nijmegen, The Netherlands.

SCIIS STUDYING CILIARY SIGNALLING IN DEVELOPMENT & DISEASE MSCA INNOVATIVE TRAINING NETWORK

Radboudumc

Marie Curie Actions

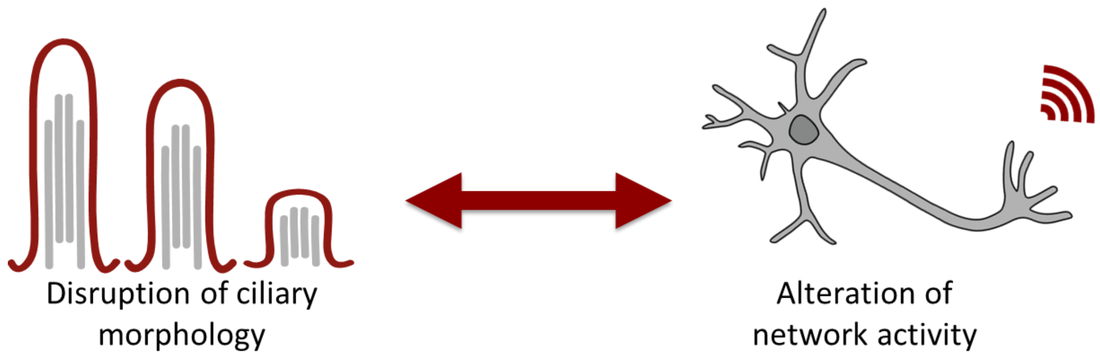
Introduction

The **primary cilium** – often referred to as the cells' antenna - is a small organelle involved in a diverse array of signalling pathways. Dysfunction of the cilium results in a spectrum of developmental disorders including the neurodevelopmental disorder, ***Joubert syndrome***. Up to 90% of neurons in the mammalian cerebral cortex are ciliated, yet the function of this organelle in excitatory neurotransmission has not been fully established.

We seek to:

- Further characterise the role of the primary cilium in establishing a neuronal network,
- Generate a model in which to study the role *Joubert syndrome*-related proteins play in network formation and maturation.

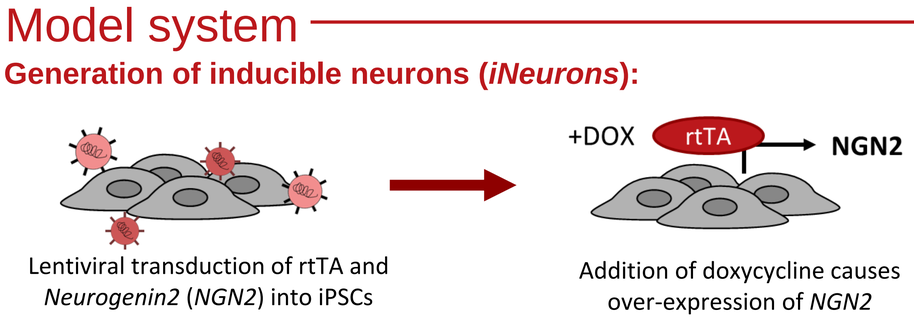
Discussion and concluding remarks



The primary cilium is linked to the excitatory properties of *iNeurons* with morphological changes preceding and following abnormal network activity.

Model system

Generation of inducible neurons (*iNeurons*):



Lentiviral transduction of rtTA and *Neurogenin2* (*NGN2*) into iPSCs

Addition of doxycycline causes over-expression of *NGN2*

Over-expression of *NGN2* induces excitatory neuronal cell fate

iNeurons used for…

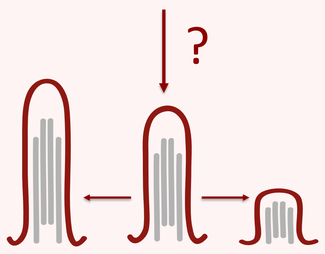
Study of ciliary morphology in response to different stimuli

Study of network activity on *micro electrode arrays* in response to ciliary dysfunction using Maestro Pro MEA system (Axion BioSystems)

Cilia respond to changes in network properties

The primary cilia of *iNeurons* show bidirectional changes in volume in response to decrease or increase in activity:

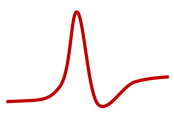
Manipulation of network activity:



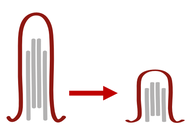
Recent work has shown that knockdown of ciliary protein expression alters the intrinsic excitability of cultured neurons. We sought to investigate if manipulation of network state could affect ciliary morphology.

Tetrodotoxin (TTX):

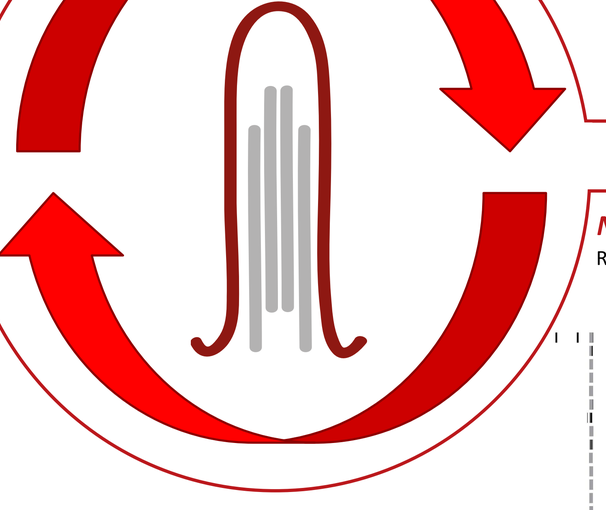
Potassium chloride (KCl):

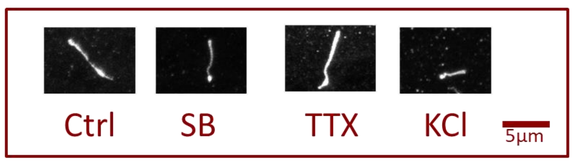


SB399885 (SB):

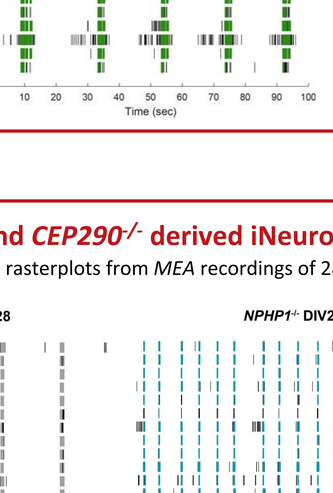


Ciliary volume of treated iNeurons

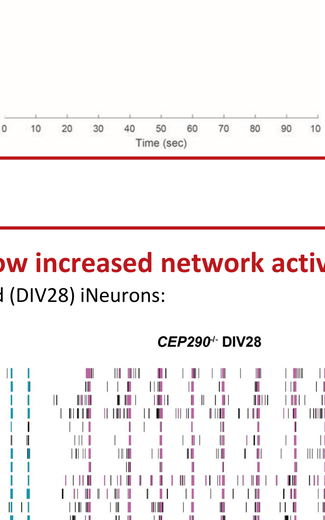




Network burst frequency

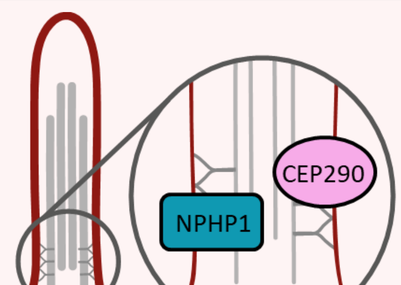


Mean inter burst interval

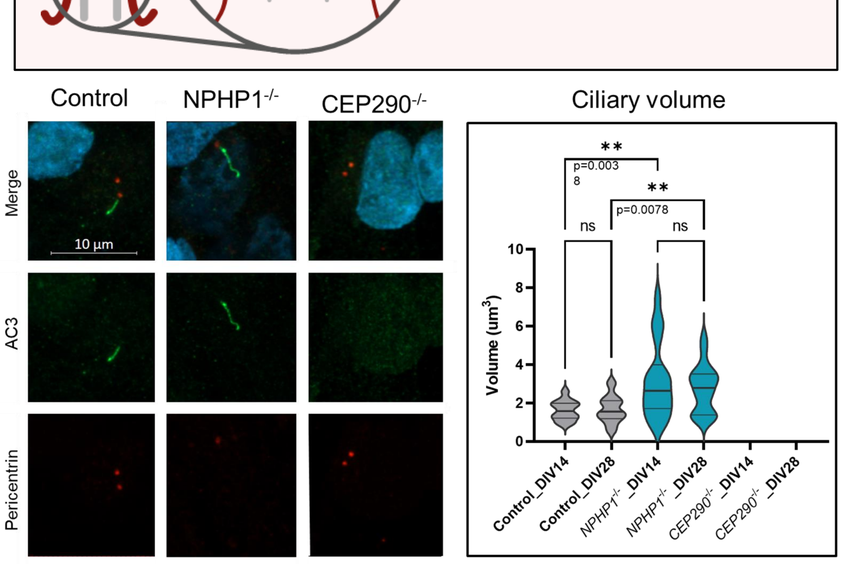


Alteration of ciliation changes activity

Loss of transition zone proteins alters ciliary morphology:



NPHP1 and CEP290 form components of important complexes localised to the transition zone. This part of the cilium is essential for regulating ciliary composition.



Immunostaining of iNeurons derived from *NPHP1*$^{-/-}$ and *CEP290*$^{-/-}$ iPSCs

***NPHP1*$^{-/-}$ and *CEP290*$^{-/-}$ derived iNeurons show increased network activity:**

Representative rasterplots from *MEA* recordings of 28 day old (DIV28) iNeurons:

Loss of transition zone proteins CEP290 and NPHP1 leads to loss of cilia, and increase in ciliary volume respectively. Despite the opposite morphologies the cell lines display similar network dynamics indicating that loss of transition zone proteins alters the basal network activity of *iNeurons*.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 861329