Transdifferentiation and microfluidic chambers as tools to approach the *in-vivo* sketch of human nociceptors

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Chronic pain affects ~20% of the European population and drugs currently used to treat this condition present limiting side effects [1], which alter the compliance to the treatment. Sensory neurons have unique morphology, with axons that can be up to one meter long [2].

Currently, sensory neurons are extracted from rodent's dorsal root or trigeminal ganglia.

This technique has two main issues:
- ethical limitations
- low representativity of human sensory system

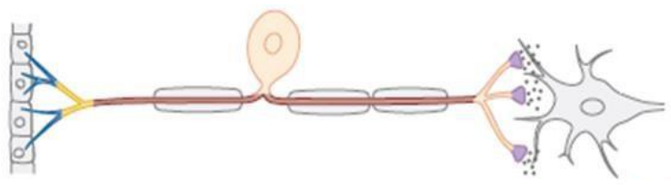

Figure 1. Sensory neurons have pseudounipolar morphology with one long axon that innervates the periphery while it establishes synapsis in the central nervous system.

OBJECTIVES

1. Develop a human-based nociceptor model;
2. Optimize microfluidic chambers culture to investigate peripheral pain signalling.

1. It is possible to convert fibroblasts to sensory neurons

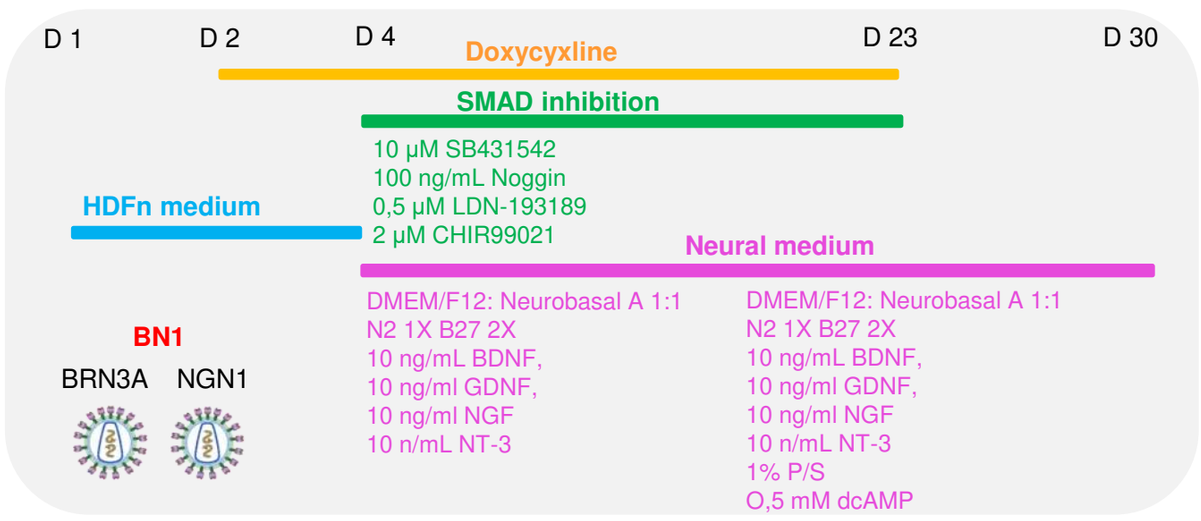

Figure 2. (A) Lentiviral vectors BRN3A and NGN1 (BN1) [3] are used to transduce human dermal fibroblasts (HDFn). **(B)** Cells are treated with **small molecules** inhibitors of SMAD and MEK/ERK and with an activator of the WNT route [4].

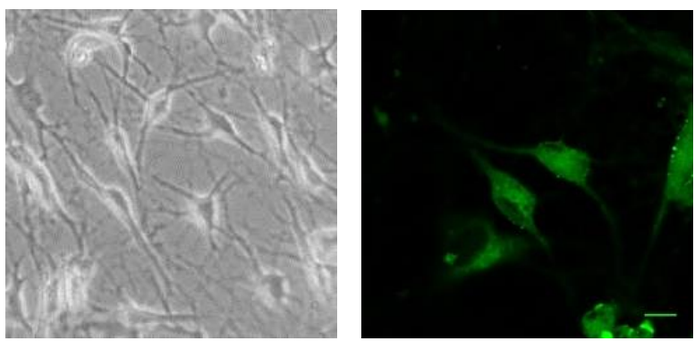

Figure 3. Induced neurons: bright field and loaded with calcium sensitive dye Fluo4. Scale bar 10 µm.

The expression of neuronal markers has been assessed with qPCR and the functionality of induced neurons has been assayed through calcium imaging.

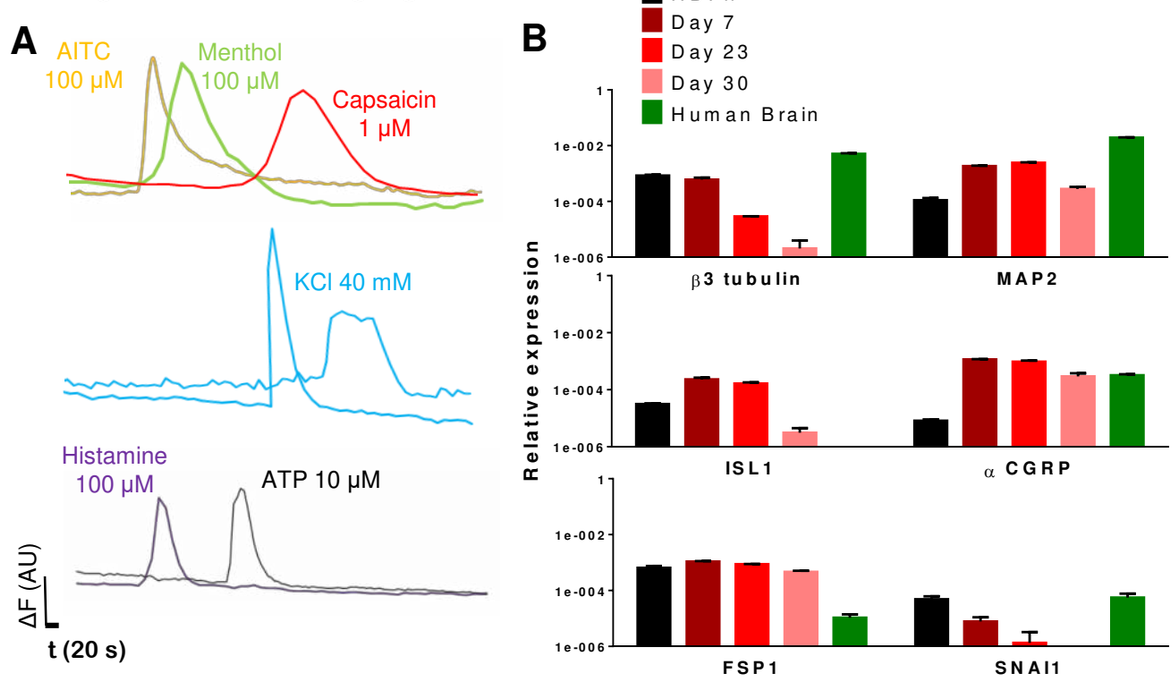

Figure 4. Induced neurons (A) exhibit functional properties and (B) express molecular hallmarks of immature and mature neurons (β3-tubulin, MAP2), of development (ISL1) and of the peptidergic sensory neuron lineage (α-CGRP). Furthermore, induced neurons downregulate fibroblasts markers (FSP1, SNAI1).

2. Microfluidic chambers allow us to study sensory neurons in a physiological sketch

Rodent nociceptors are cultured in microfluidic chambers [5], and their functionality is assessed by calcium imaging and microelectrode arrays (MEA).

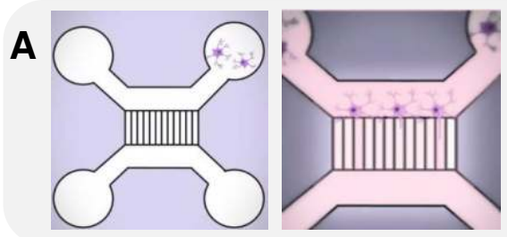

Figure 5. Representation of a microfluidic chamber.
(A) From above **(B)** Side view.

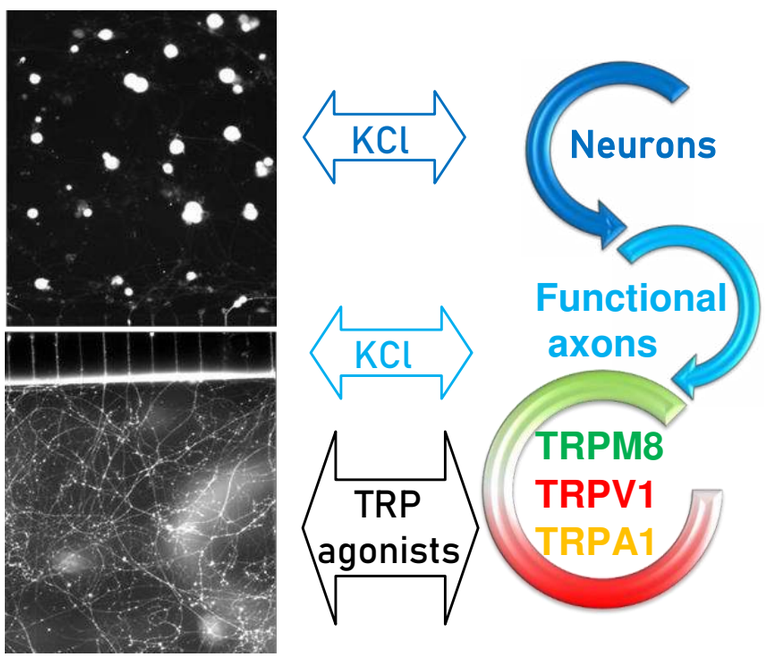

Figure 6. Sensory neurons seeded in a microfluidic chamber. Axons are stimulated with potassium chloride (KCl) and TRP channels agonists. Calcium concentration or voltage changes are measured in the soma.

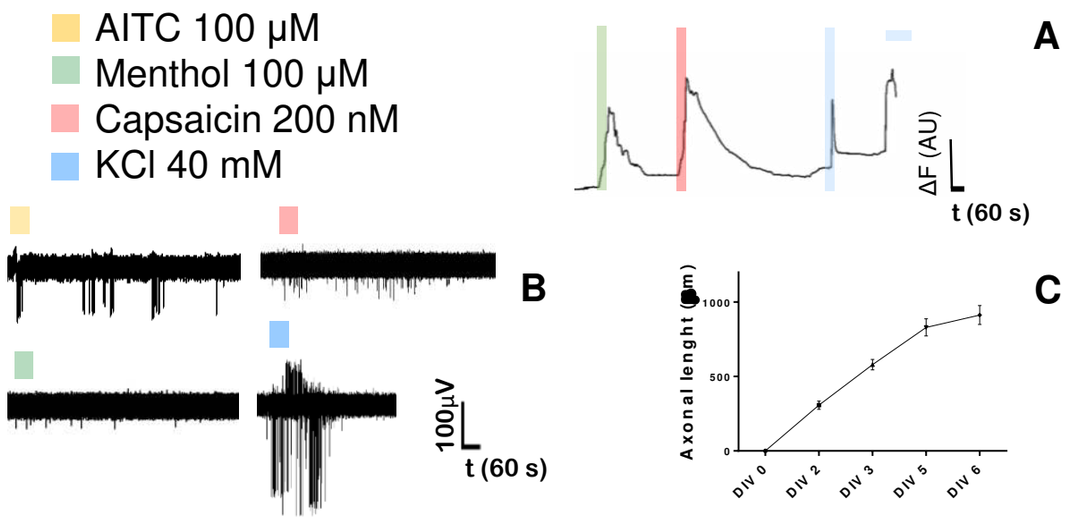

Figure 7. (A) Representative **calcium imaging trace (B)** Representative **MEA recordings**. **(C)** Axonal lenght ± SEM during days in vitro (DIV) (N=3).

CONCLUSIONS

1. Although direct differentiation of fibroblasts to sensory neurons needs further optimization, it is a challenging and valuable method to generate phenocopies of human nociceptors.
2. Culturing sensory neurons in microfluidic chambers allows us to study pain signalling in a pseudo-physiological layout.

[1] Eccleston, C et al., European Pain Management; Oxford University Press: Oxford, UK, 2018.
[2] Kandel, E.R., et al., Principles of neuroscience - Fifth edition, New York: The McGraw Hill Company, 2013.
[3] Blanchard, J.W., Eade, K.T., et al., Selective conversion of fibroblasts into peripheral sensory neurons. Nat Neurosci, 2015;18(1):25-35.
[4] Ladewig, J., et al., Small molecules enable highly efficient neuronal conversion of human fibroblasts. Nat Methods. 2012 Jun;9(6):575-8.
[5] Tsantoulas, C., et al., Probing Functional Properties of Nociceptive Axons Using a Microfluidic Culture System. PLOS ONE, 2013; 8(11): e80722. 8.

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