Set-up neuron-glia co-cultures for functional studies on multiple electrode arrays platforms

Francesca Izzi[1] **and Paolo Cesare**[1]

[1]Neuro Microphysiological Systems Group, Natural and Medical Sciences Institute at the University of Tübingen 72770 Reutlingen, Germany

Natural and Medical Sciences Institute at the University of Tübingen
Markwiesenstrasse 55
72770 Reutlingen, Germany
contact@nmi.de

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Studying the nervous system has always been a challenging task. The classical electrophysiological techniques, such as patch-clamp, are time and energy consuming, and lead to low throughput experiments.
By using a high-density multiple electrode arrays (MEA) system, we are able to record the electrical activity of high density cultures of both pure sensory neurons and non-purified neurons with relative glial cells, in the aim of exploiting the mechanisms that lead to the development of neuropathic pain. Surprisingly, cultures of pure neurons show no electrical activity in response to the application of different agonists, suggesting a role for glial cells in the development of pain abnormalities. The implementation of neurons-glia co-cultures and the functional characterization of their interaction mechanisms could then lead to the identification of novel targets and biomarkers involved in neuropathic pain.

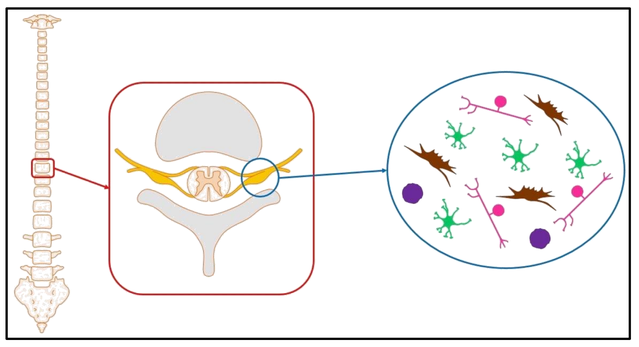

Figure 1: Anatomy of dorsal root ganglion.

Methods

Mice between p13 and p20 were euthanized with CO_2, and the vertebral column was removed. Each column was cut along its main axis, and for each half dorsal root ganglia (DRG) were removed. DRG were dissociated enzymatically, and dead cells and debris were removed by gradient centrifugation. Pure sensory neurons were obtained using the Neuron Isolation Kit (Miltenyi Biotec). Non-purified and purified sensory neurons were plated either as separated cultures or in a combination of both (keeping the same number of neurons) on micro electrode arrays (MEA) and glass coverslips coated with polyethyleneimine (PEI) 0.075 g/ml, and laminin 20 µg/ml, supplied with modified Neurobasal-A medium and kept at 37°C with 5% CO_2. After 1, 3, and 6 days *in vitro*, the electrical activity of each MEA was recorded using MEA2100 system (Multichannel System), and calcium imaging was performed.

Results

Neurons were stimulated with capsaicin 1µM and electrically with different voltages. Calcium imaging experiment was performed using Axio Observer (Carl Zeiss GmbH) and Cal-520 1µM dye (AAT Bioquest).

- Non-purified sensory neurons show electrical activity in response to capsaicin after few hours *in vitro*.
- Purified sensory neurons shows electrical activity in response to capsaicin after few hours *in vitro* (even if not as much as in the co-culture); from 2 days *in vitro*, the activity is not present anymore.
- Purified sensory neurons in MFC-MEA show electrical activity in response to electrical stimulation, with a delay of approximately 150mV compared to non-purified sensory neurons.
- There is a correlation between the presence of non-neuronal cells and the neuronal excitability.

MEA recording

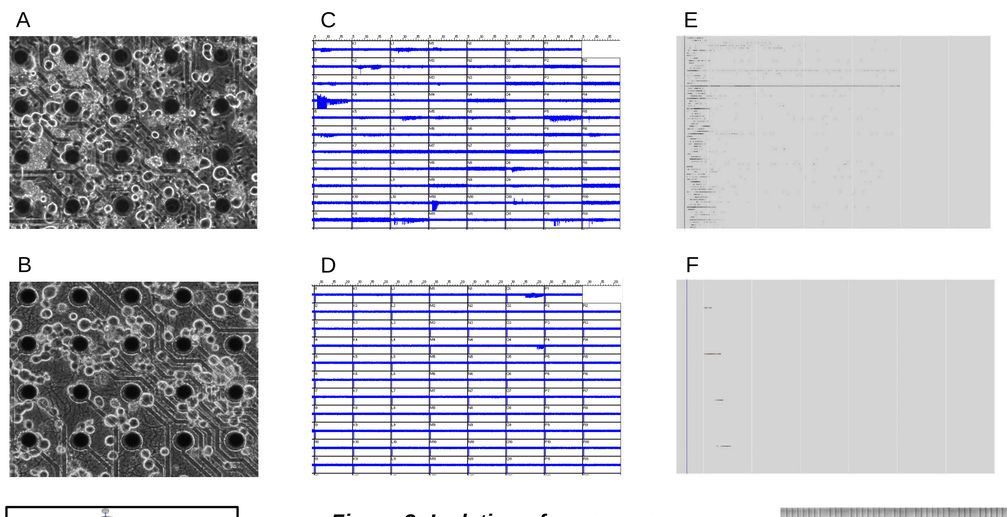

Figure 2: Effect of capsaicin on non-purified and purified DRG neurons. *(A) Non-purified DRG. (B) Purified DRG neurons.*
Application of 1µM capsaicin to DRG neurons lead to the opening of TRPV1 receptor, which allows the entrance of Ca^{2+} and Na^{+} in the cells. This lead to the depolarization of the membrane and the subsequent generation of action potentials, which can be noticed by the number of responding electrodes per MEA (C, E). When capsaicin is applied to a culture of purified DRG neurons (D, F), the response is only observed in a smaller fraction of cells, compared to non-purified neurons.

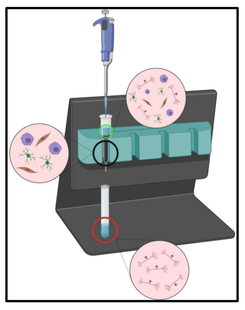

Figure 3: Isolation of pure sensory neurons through magnetically activated cell sorting (MACS) technology. *Non-neuronal cells are labelled with anti-non-neuronal cells antibodies linked with biotin, and with anti-biotin magnetic beads afterwards. Non-neuronal cells are ´magnetically retained in the column, and unlabelled neurons are collected in the tube*

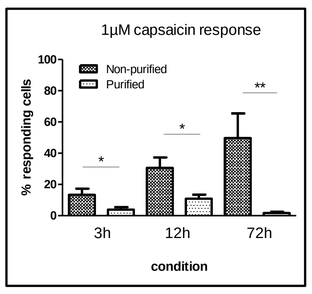

Figure 4: Comparison between non-purified and purified DRG neurons activity in response to capsaicin application. *Percentages of responding DRG per MEA to capsaicin application in non-purified and purified sensory neurons after few hours, 1, and 3 days in vitro.*

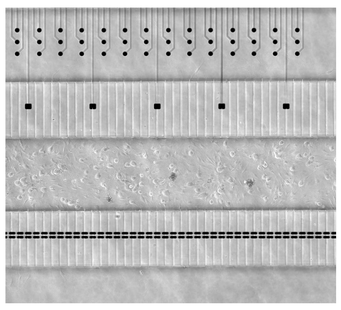

Figure 5: Non-purified DRG in MFC-MEA. *Dendrites in the lower microchannels are stimulated electrically, and the electrical activity evoked by the stimulus is recorded in the electrodes located in the upper microchannels.*

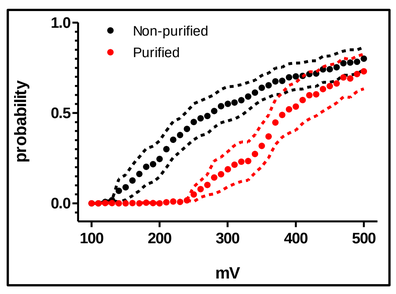

Figure 6: Comparison between non-purified and purified DRG neurons activity in response to electrical stimulation in MFC-MEA. *Probability of evoking a response in purified and non-purified DRG in MFC-MEA in response to different voltages application.*

Role of non-neuronal cells in the excitability of sensory neurons

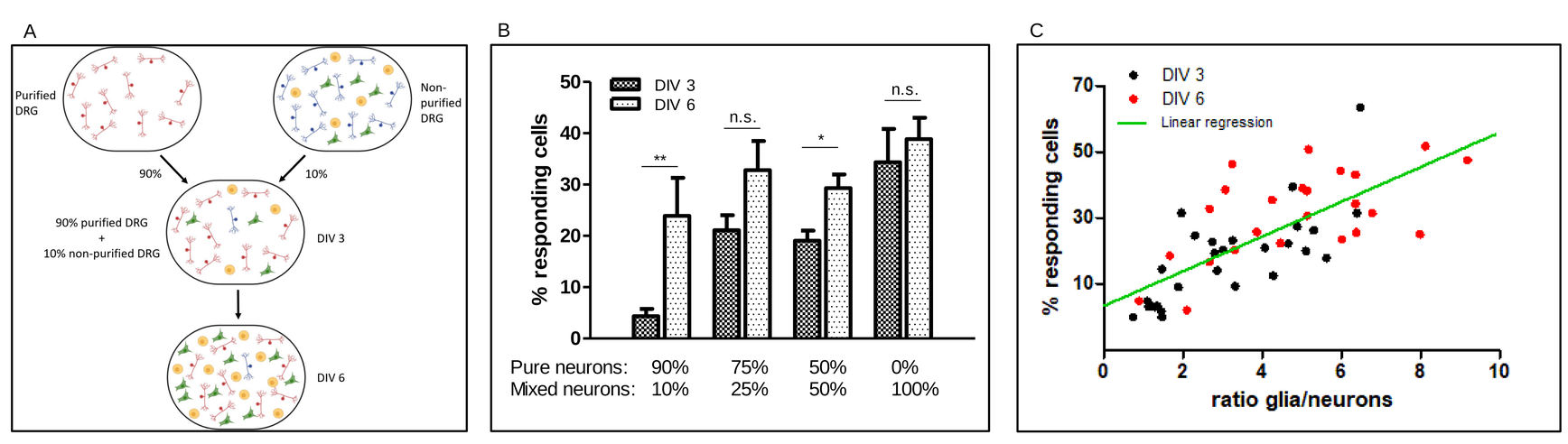

Figure 7: Role of non-neuronal cells in excitability of sensory neurons. *By mixing purified and non-purified DRG, we generated cultures with different number of non-neuronal cells, but keeping the same number of neurons (A). With this, we confirmed the contribution of the non-neuronal component in DRG excitability, and this excitability depends both on time in culture (B) and percentage of non-neuronal cells (C).*

Summary and Outlook

It has been demonstrated that sensory neurons possess electrical activity in response to capsaicin already after few hours *in vitro*; however, in the case of purified neurons, this activity is largely reduced after 1 day *in vitro*. This cannot be explained by a lack in the capsaicin receptor TRPV1, whose presence has been proved by calcium-imaging experiment.
The ability of purified neurons to be activated by capsaicin during the first 24 hours only after plating suggests their dependence on non-neuronal cells for the expression of proteins which may be required to preserve their excitability.
Electrical stimulation on microfluidics devices showed that there is a shift in the threshold necessary to evoke action potentials in purified sensory neurons.
All these data combined lead to the hypothesis that non-neuronal cells, such as Schwann cells and satellite glial cells, play an active role in establishing the excitability of sensory neurons under physiological and pathological conditions, such as chronic pain. Future studies, which include transcriptomics analysis and combination of MEA recording and calcium imaging at the same time, will be addressed in order to identify proteins that are dysregulated in purified sensory neurons, and their relationship with non-neuronal cells.

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