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Micro electrode arrays to investigate neuron-glia crosstalk in neuropathic pain in-vitro models

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Neuropathic Pain (NP) is frequently associated with peripheral nerve injury or disease. Experimental models of neuropathic pain showed that non-neuronal cells play a very active role in the development of sensory abnormalities, but the exact mechanisms haven't been clarified yet.
By using a high-density multiple electrode array (MEA) system, we are able to record the electrical activity of both pure sensory neurons and co-cultures of neurons-glia. Surprisingly, cultures of pure neurons show no electrical activity in response to capsaicin application, suggesting a role for non-neuronal 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 NP.

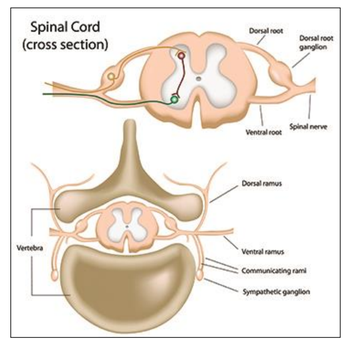

Figure 1: Anatomy of dorsal root ganglion. "DRG Stimulation - Pain has finally met its match" – Ainsworth institute of pain management, New York.

MEA recording

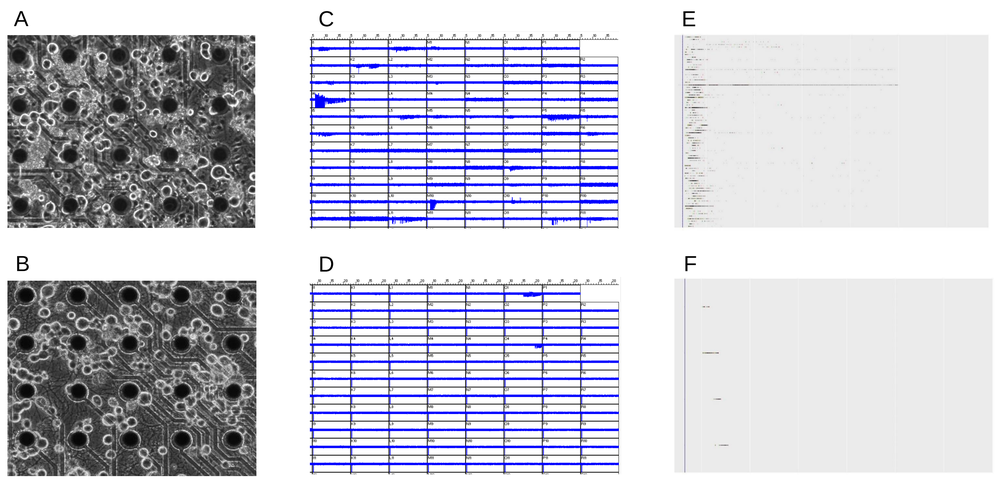

Figure 2: Effect of capsaicin on non-purified and purified DRG neurons. *(A) Non-purified DRG. (B) Purified DRG neurons.*
Application of 1µM capsaicin to DRG neurons lead to the opening of TRPV1 receptor, which allows the entrance of Ca^{2+} and Na^{+} in the cells. This lead to the depolarization of 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.

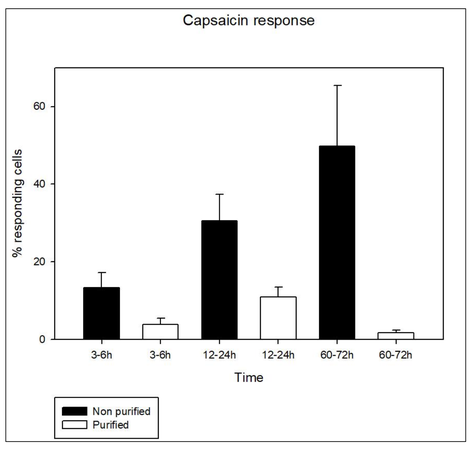

Figure 3: Comparison between non-purified and purified DRG neurons activity in response to capsaicin application. *Percentages of responding DRG per MEA to capsaicin application in non-purified and purified sensory neurons after few hours, 1, and 3 days in vitro.*

Calcium imaging

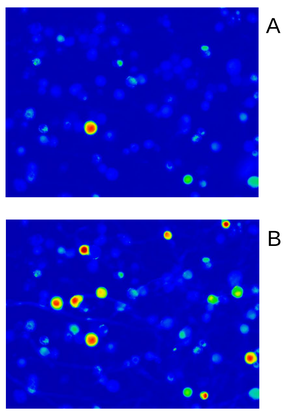

Figure 4: Calcium imaging in purified DRG neurons. *The presence of the capsaicin receptor TRPV1 in purified neurons is confirmed by calcium imaging experiment. Application of 1µM of capsaicin to purified sensory neurons (A) leads to opening of the calcium permeable TRPV1 channel, causing a large increase in intracellular calcium (B).*

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 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 2-4 days *in vitro*, the electrical activity of each MEA was recorded using MEA2100 system (Multichannel System), and calcium imaging was performed.
Perforated patch-clamp was performed after 2 days *in vitro*; membrane pores were generated with 40mg/ml amphotericin B.

Results

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

- Non-purified 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.
- Calcium imaging on the purified neurons showed the entrance of calcium in the cells, proving that the capsaicin receptor TRPV1 is still present.
- Perforated patch-clamp demonstrated that purified neurons membrane depolarizes in response to capsaicin, and the ability of firing action potentials is comparable to that from non-purified neurons.

Patch-clamp

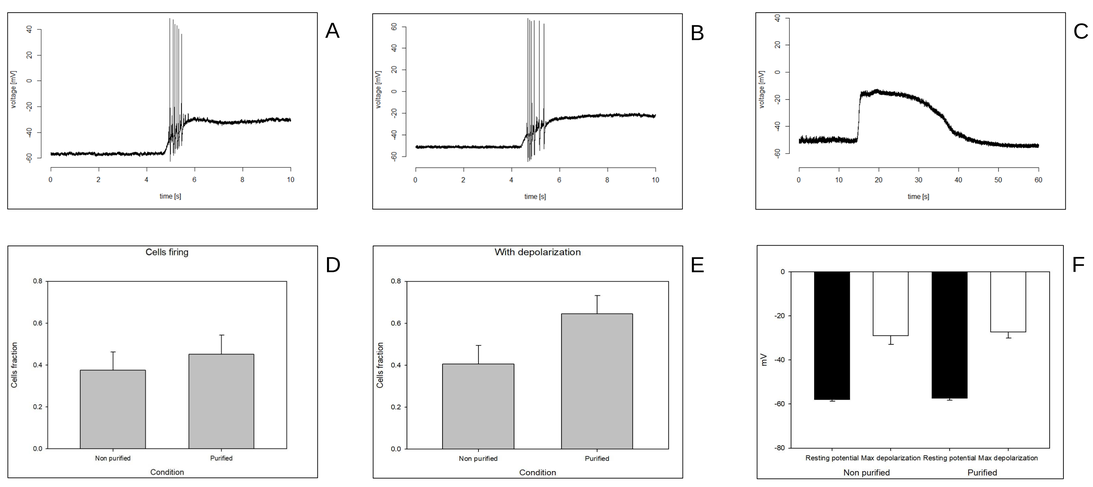

Figure 5: Perforated patch-clamp on non-purified and purified DRG neurons. *1µm capsaicin leads to membrane depolarization, following action potentials firing in both non-purified (A) and purified (B) neurons. In some purified neurons, no firing was observed after membrane depolarization (C). (D) Percentages of DRG neurons responding to capsaicin. (E) Percentage of DRG neurons able to fire action potentials. No difference was noticed in terms of resting membrane potential and depolarization amplitude between non-purified and purified neurons (F).*

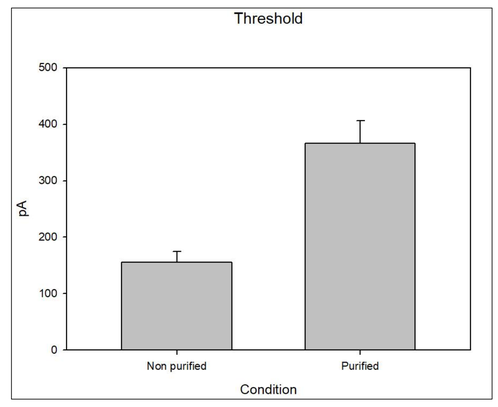

Figure 6: Current injected in CC-mode necessary to evoke an action potential. *Purified DRG neurons require an amount of current higher than the non-purified in order to evoke an action potential.*

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.
Perforated patch-clamp experiments showed no major differences in membrane depolarization between purified and non-purified neurons. However, although perforated patch-clamp is considered less invasive than the traditional patch-clamp, the small pores generated from amphotericin may still alter the intracellular concentrations of ions important for neuronal excitability.

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. 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 conditioned medium experiments and the use of permeable membrane for cell culture, 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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