Exploring the pathophysiology of spinal microglia in chronic pain condition

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Introduction:
Recent progress in pain research points to an important role of immune cells (microglia in the spinal cord and macrophages in the dorsal root ganglion) in chronic pain. Both microglia and macrophages react strongly after surgical incision and nerve injury, in parallel to the development of pain behavior. This is characterized by increased proliferation, morphological changes, and release of proinflammatory and algesic cytokines.
Our past work has shown that blocking microglia reactivity, by targeting specific peripheral nerves significantly prevents the development of injury-induced chronic pain1,2. We discovered that microglia and macrophages modify their membrane potential in early timepoints after nerve injury, mainly related to modulation of Kir potassium channels3,4. The objectives of this application are to unravel the links between peripheral electrical activity and the changes in membrane potential and phenotype of macrophages/microglia. The central hypothesis is that membrane potential is an early and key determinant of immune cells reactivity leading to neuroinflammatory dysfunction in pathological pain conditions. As an early determinant it could be an interesting target.
In parallel to the lab work, samples will be collected in the clinic5 to be tested in the lab and compared to results obtained in mice.

Aim of the project:
1) The first aim is to describe the role secreted content of specific neurons on macrophages/microglia in a neuropathic pain model, to explore the causing determinants of neuro-immune communication.
The specific neurons will be injured/non-injured or different subtypes (nociceptive or non-nociceptive)
2) The second aim is to discover how immune cells excite neurons backwards.
3) Compare results to patient's samples of cohort followed in the pain center

Experimental approach:

In the first aim, will be modulate the activity of different primary afferents, without injury, by using electrical stimulation, optogenetic and chemogenetic tools.
Macrophage/microglial morphological and phenotypic changes will be investigated by immunohistochemistry and patch clamp recordings on cells/slices on these non-excitable cells.
For the second aim, we will challenge neurons with the secreted content of immune cells from ex-vivo preparations.
The following techniques will be used:
- Electrical, optogenetic and chemogenetic stimulations on anesthetized animals
- Patch-clamp (voltage-clamp, current-clamp) on dissociated macrophages/microglia and on spinal cord slices

- Tissue collection (perfusion, dissection) and preparation
- Immunohistochemistry
- Image collection (fluorescent and confocal microscope) and analysis (ImageJ, Zen, Prism, Adobe Illustrator softwares).
- Extracellular vesicle collection and miRNA sequencing

Significance:
The proposed work is significant and innovative because key elements of neuroimmune interaction will be found that hopefully should contribute to novel approaches of preventive analgesia in post-operative and chronic pain setting in human.

References:

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