Exploring the pathophysiology of spinal microglia in chronic pain condition

Introduction:
Recent progress in pain research points to an important role of microglia in the development of chronic pain. Microglia are macrophage-like cells that regulate homeostasis in the central nervous system. They react strongly after surgical incision and nerve injury, in parallel to the development of chronic pain. This is characterized by increased proliferation, morphological changes, and release of proinflammatory and algesic cytokines.

Our past work has shown that blocking this microglia reactivity, by targeting specific peripheral nerves significantly prevents the development of injury-induced chronic pain1,2. We discovered that microglia modify their membrane potential in early timepoints after nerve injury, mainly related to modulation of Kir potassium channels3. The objectives of this application are to unravel the links between membrane potential of spinal microglia and microglial phenotype / pain hypersensitivity and finally, how to target membrane potential therapeutically. The central hypothesis is that membrane potential is an early and key determinant of microglia reactivity leading to spinal neuroinflammatory dysfunction in pathological pain conditions.

Aim of the project:
1) The first aim is to describe the role of microglia membrane potential modification in a neuropathic pain model, to explore the causing determinants and inversely to determine the consequences of modifying the membrane potential on microglial phenotype.
2) The second aim is to discover the contributing part of early abnormal peripheral activity to microglia reactivity. Mainly we will investigate which primary afferent activity, type of fiber (C-, Adelta- or Abeta-fibers), duration, or intensity is necessary to induce microglial reactivity in the spinal cord.

Experimental approach:
The first aim will be addressed by using patch-clamp technique on these non-excitible cells, in ex-vivo slice or cell preparations to search the determinants of membrane potential to be targeted pharmacologically. We will mimic microglial membrane potential changes in vivo with chemogenetic/optogenetic tools (mice line with DREADDs and opsins specifically expressed in microglia, promoter CX3CR1) and analyze the impact on microglia phenotype and response to neurotransmitters, pain hypersensitivity and neuronal output.

For the second aim, the activity of different primary afferents will be modulated, without injury, by using electrical stimulation, optogenetic and chemogenetic tools. Microglial morphological and phenotypic changes will be investigated within the spinal cord by immunohistochemistry and patch clamp recordings on slices.

The following techniques will be used:
- Electrical, optogenetic and chemogenetic stimulations on anesthetized animals
- Patch-clamp (voltage-clamp, current-clamp) on dissociated 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).

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 preventative analgesia in post-operative and chronic pain setting in human.

References: