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SUR Project: Exploring the pathophysiology of spinal microglia in chronic pain condition

Introduction:
Pain research points to an important role of microglia in the development of chronic pain. Microglia are macrophage-like cells that not only regulate homeostasis in the central nervous system, but react strongly after surgical incision and peripheral 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 preventing this microglia reactivity, by targeting specific peripheral nerves significantly prevents the development of injury-induced chronic pain[1,2]. We discovered that microglia modify their membrane potential in early timepoints after nerve injury, mainly related to modulation of Kir potassium channels which influences their proliferation[3]. The objectives of this application are to unravel the links between membrane potential of spinal microglia and their phenotype and pain hypersensitivity. 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. The final goal is to modulate membrane potential of microglia therapeutically in pain conditions.

Aims of the project:
1) The first aim is to describe the role of microglia membrane potential modification in a neuropathic pain model by exploring 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 SUR project proposed will be focused on the Aim that allows the best match by summer 2023 for a short project.

In the first aim we will use patch-clamp technique on these non-excitable cells, in ex-vivo spinal cord slices or cell preparations to search the determinants of membrane potential that can be targeted pharmacologically. We will mimic microglial membrane potential changes in vivo using chemogenetic/optogenetic tools (genetically-modified mice lines with DREADDs and opsins specifically expressed in microglia) and analyze the impact on microglia phenotype and response to neurotransmitters, pain hypersensitivity.
For the second aim, we will modulate the activity of specific primary afferents 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. Key elements of neuroimmune interaction will be discovered that hopefully should contribute to novel approaches of preventing/treating pain in human.

References:

1 Wen, Y. R. *et al.* Nerve conduction blockade in the sciatic nerve prevents but does not reverse the activation of p38 mitogen-activated protein kinase in spinal microglia in the rat spared nerve injury model. *Anesthesiology* **107**, 312-321, doi:10.1097/01.anes.0000270759.11086.e7 (2007).

2 Suter, M. R., Berta, T., Gao, Y. J., Decosterd, I. & Ji, R. R. Large A-fiber activity is required for microglial proliferation and p38 MAPK activation in the spinal cord: different effects of resiniferatoxin and bupivacaine on spinal microglial changes after spared nerve injury. *Molecular pain* **5**, 53, doi:10.1186/1744-8069-5-53 (2009).

3 Gattlen, C. *et al.* The inhibition of Kir2.1 potassium channels depolarizes spinal microglial cells, reduces their proliferation, and attenuates neuropathic pain. *Glia* **68**, 2119-2135, doi:10.1002/glia.23831 (2020).