Altered control of glutamate transmission by glial cells is one of the key pathogenic mechanisms of amyotrophic lateral sclerosis. Glutamate-mediated excitotoxicity in the cerebral cortex, the brainstem and the spinal cord causes the degeneration of motoneurons which in turn results in muscle weakness in early stages and paralysis at later stages. The impaired uptake of glutamate by astrocytes has been largely documented in the literature and our group has developed research projects aimed at further characterizing this glial dysfunction (1). As the disease also involves neuroinflammation, we have considered the influence of inflammatory mediators on the expression and activity of glial glutamate transporter subtypes.

Using a transgenic rat model of amyotrophic lateral sclerosis, we have previously demonstrated that the progression of the disease is accompanied by a differential regulation of the glutamate transporters isoforms GLT-1a and GLT-1b in several brain structures. Our recent data indicate that these isoforms are differentially regulated by inflammatory mediators (2) (Figure 1). The shorter splice variant GLT-1b contains a PDZ domain known to interact with the scaffold protein PICK1 and we have shown that this protein is also regulated in the context of amyotrophic lateral sclerosis (3,4).

Our group also considers neurotransmitter receptors present on glial cells as putative molecular targets to pharmacologically modulate the expression and activity of astrocytic glutamate transporters in the context of the disease. Thus, we have herein demonstrated that a lipophilic analogue of the vasoactive intestinal peptide can promote the expression of GLT-1 and improve the survival of transgenic rats developing amyotrophic lateral sclerosis. Considering that this peptide exerts modulatory influences on inflammation and that the animal treatment preserves spinal motoneurons (Figure 2), these observations support the proposed link between neuroinflammation and excitotoxicity in neurodegenerative diseases (Goursaud et al., submitted).

**Fig.1:** Influence of TNF-α on GLT-1a proteins expression in cortical astrocytes from wild-type or hSOD1<sup>G93A</sup> rats. Expression of the transporter was examined by Western blot on samples from cell cultures maintained in control conditions or treated with TNF-α (20 ng/mL) for 72 h.

**Fig.2:** The vasoactive intestinal peptide SNV administrated in a rodent model of amyotrophic lateral sclerosis extents the survival of the transgenic animals (A) and preserves motoneurons number in the lumbar spinal cord (B).
REGULATION OF IMMUNE RESPONSES FOLLOWING NERVE LESIONS AND ITS RELEVANCE FOR THE TRANSITION FROM ACUTE TO CHRONIC PAIN

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Within this research theme we, together with our clinical colleagues from the Clinique universitaires Saint-Luc and (inter)national collaborators, aim to understand why postoperative pain can become chronic in some cases (10-50% of surgical patients) but spontaneously resolves in other cases. On the basis of clinical studies and animal models we focus our efforts at the immune system which is activated upon surgery. In particular, we study the immune system in the context of so-called vulnerability factors, which are thought to trigger a dysregulation of immune responses following surgery (5).

We and others have previously shown that nerve trauma in peripheral tissues, which occurs in many types of clinical surgical interventions, elicits immune responses in the central nervous system that are very persistent over time (6) (Figure 3). On the basis of studies on genetically modified rats, we obtained evidence suggesting that an imbalance/amplification of immune responses in the spinal cord resulted, on the long-term, in altered immune markers and higher pain scores (7) (Figure 4). These original data demonstrate the importance of controlling the immune response after surgery, not only to reduce early pain, but also to prevent chronic pain after tissue healing.

Work in progress concerns: (i) validating new animal models to investigate the molecular regulation of immune responses in relation to pain chronification or pain resolution, and (ii) pharmacological and cell-based immune modulatory interventions to attenuate or prevent chronic pain.

Fig. 3: Reactive microgliosis in the spinal dorsal horn persists for many weeks and even months after nerve lesion (upper two panels: Iba-1 immunoreactive microglia/macrophages in the ipsilateral and contralateral dorsal horn with hypertrophied ‘reactive’ cells and multi-ramified ‘surveilling’ cells, respectively; lower panel: quantified Iba-1 signals).
REGULATION OF G-PROTEIN COUPLED RECEPTORS SIGNALLING BY INFLAMMATORY MEDIATORS

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G-protein coupled receptors constitute the largest family of pharmacological targets, in particular in neurosciences. Our group has contributed to develop the concept that the complexity of cell signalling associated with these receptors is larger than initially proposed. One given receptor can couple to several G-proteins and these couplings are influenced by the activating ligand as well as by the cell and ionic environments (8). We here investigate whether the inflammatory environment may alter the functional response to drugs acting on these receptors. Both in vitro, on cultures of glial cells exposed to inflammatory mediators and in vitro, in the spinal cord of animals with peripheral nerve lesions, we characterize the changes in the expression of the receptor or in the availability of signalling partners.

Our recent studies have demonstrated that the expression of the glial metabotropic glutamate receptors mGlu3 and mGlu5 (that act as sensors of the extracellular glutamate) is strongly regulated in response to soluble inflammatory triggers (9). As these regulations are associated with major changes in the associated signalling in astrocytes, our ongoing studies aim at delineating the physiological consequences on the neuroprotective activities supported by these glial cells.

Similarly, we also study the regulation of signalling pathways associated with the CB1 cannabinoid receptor. While presence of this receptor on astrocytes was recently demonstrated (10), we have evidenced regulation of its expression and other signalling partners in the spinal cord of animals with peripheral nerve lesions. Our work aims at characterizing the consequence of these regulations on the analgesic properties of CB1 receptor ligands.

Fig. 4: hSOD1<sup>G93A</sup> transgenic rats with amplified immune responses to nerve lesion (filled squares in upper panel) show a more severe pain hypersensitivity (i.e. reduction in paw withdrawal latency to radiant heat source) than wild type rats with a nerve lesion (filled circles in upper panel). Only transgenic rats of the peripheral nerve lesion group showed increased expression of the immune markers Nox-2, toll-like receptor-4 and interleukin 1-β at three weeks after nerve injury (i.e. partial sciatic nerve ligation).
EQUIPMENT

- Radioligand binding platform for pharmacological screening (24 and 96 sample harvesters)
- Scintillation counters (vials and microplate readers)
- Fluorescence microscope station for dynamic measures in living cells
- Real-time PCR
- Immunohistochemistry and biochemistry
- Animal neurosurgery platform with assisted mechanical ventilation
- Locomotor behaviour assessment platform
- Pain behaviour (algesimetry) assessment platform
- Cell culture facilities

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