

This study was designed to improve our knowledge regarding mechanisms of nociceptive signaling at spinal cord level. One of the forms of spinal cord synaptic transmission modulation is central sensitization, a manifestation of synaptic plasticity at spinal cord level, which was found to be present at many chronic pain syndromes. This study deals mainly with a development of calcium imaging technique with a final goal to study mechanisms of central sensitization *in vitro* on population of dorsal horn neurons.

We have analyzed synaptically evoked intracellular Ca changes as a result of dorsal root stimulation in a superficial dorsal horn area in spinal cord slices and found two types of Ca responses: one synchronized with electrical stimulation and a second one, delayed response due to Ca release from internal stores. The delayed Ca release was not previously shown to be present in these neurons and it was not dependent on activation of ionotropic glutamatergic receptors, suggesting involvement of metabotropic receptor pathway. The presence of this delayed type of Ca response could have a significant role in the induction of some types of chronic pain syndromes, since intracellular calcium increase is thought to be a key trigger point in spinal cord neurons sensitization.

An important role in neuronal calcium homeostasis play calcium buffers. We have focused on their possible role in pathological pain states using experimental model of arthritis to study changes in expression of Ca buffer parvalbumin in the spinal cord and Ca bufferes parvalbumin, calbindin, and calretinin in retrogradely labeled spinothalamic tract neurons. In our experiments arthritis induced significant downregulation of parvalbumin in the ipsilateral dorsal horn neuropil. Some of the results also suggested that these changes could occur in GABAergic neurons, located in this dorsal horn area.

Using the spinal cord slices calcium imaging we have developed an *in vitro* model of dorsal horn neurons sensitization. Bulk loading of the slice with fluorescent probe enabled us to study intracellular calcium changes in a population of dorsal horn neurons. This was of great advantage compared to the patch clamp technique that enables to study just one or two neurons simultaneously. Capsaicin was used to activate TRPV1 receptors present on primary afferent fibers and to induce sensitization of dorsal horn neurons, recorded as an increase of intracellular Ca transient induced by control dorsal root stimulation. Using this model together with transgenic labeling of specific neuronal populations could be useful in the future, especially for pharmacological testing of new analgesic drugs.