PERSPECTIVE

Beyond peripheral nerve injury: spinal gliopathy and maladaptive synaptic plasticity

Who drew the borders between central and peripheral nervous system? A complex question but a simple answer.

Human anatomy and clinical neurology need to differentiate peripheral nerves from central nervous system (CNS), peripheral or central disorders, respectively. However, there are no anatomical and functional clefts between peripheral axons and central synapses. There is a direct continuity *from the periphery to the center*, from the receptor to the sensory neuron, from the spinal motor neuron to the muscle, just the neuromuscular junction.

Thus, it is conceivable that the "periphery" could modulate the "center" and *vice versa* in response to external stimuli or stressors (injury, toxic, degeneration).

Experimental models demonstrated a significant reshaping of the spinal circuitry following peripheral nerve injury (PNI): both chronic constriction injury (CCI) and spared nerve injury (SNI) animal models have been shown to perturb spinal homeostasis and induce a condition called maladaptive synaptic plasticity.

Glial cells play a key role in this process, actively modify the spinal circuitry and are involved in 1) formation of structural and functional synapses, 2) release of glial transmitters that in turn influence the balance of neural networks, 3) metabolic supply to neurons and 4) counteract the internal and external stressors. Therefore, the view of "embedded elements among neurons, holding them together" (*brain glue*) (Virchow, 1846) has been challenged and actually the concept of tetrapartite synapses (including pre- and post-synaptic neurons, astrocytes/microglia and extracellular matrix) represents the fundamental of the system biology in the CNS (Papa et al., 2014).

In the past years, we have focused on mechanisms of glial-induced maladaptive synaptic plasticity and how to restore spinal homeostasis through glial-targeted pathways.

Changes underlying maladaptive synaptic plasticity start from the periphery. An increased sprouting of primary afferent fibers (C and A-δ), morphological alterations of nerve myelination and dorsal root ganglia morphology were found after CCI, establishing a precise correlation between changes of spinal plasticity and peripheral sensitization (Cirillo et al., 2010). In the spinal cord, moreover, PNI induced activation of glial cells, a process that is defined as *reactive gliosis*. Glial reaction is characterized by 1) astrocytic hypertrophy and increased expression of the cytoskeletal marker, the glial fibrillary acidic protein (GFAP) and 2) activation of microglia, the resident immune cells of CNS responsible for release of inflammatory mediators that sustain the cascade-activation of other glial cells.

The neuroinflammatory reaction is paralleled by the significant reduction of the glial glutamate transporters (gGTs) expression, resulting in a net increase of the free glutamate in the synaptic cleft. This is responsible for a consequent spillover outside the synapse, producing activation of both ionotropic and metabotropic neuronal and glial glutamate receptors, and finally determining receptors' sensitization, changes of synaptic function and maladaptive synaptic organization (**Figure 1**).

On the neuronal side, the increased expression of presynaptic neuronal glutamate transporters and the plastic response of GABAergic neurons, through active remodeling and branching of GABAergic axons, represent the strategy to face the increase of the free glutamate and mitigate the excitatory glutamate transmission and excitotoxicity. Furthermore, the failure of the main glial anti-oxidant system, the xCT antiporter, affects neuronal resources against excitotoxicity due to depletion of glutathione, the main source of protection from oxidative damage (Cirillo et al., 2011).

Thus, it is clear that PNI induces a remodeling of the somatosensory signaling pathways at different levels, from the periphery to the spinal cord and furthermore to several sovraspinal pathways. We reported a significant remodeling of glial and neuronal glutamate transporters in sovraspinal brain regions involved in pain/sensory processing (medial prefrontal cortex, amygdala, thalamus and periaqueductal grey) after SNI (Marcello et al., 2013).

Understanding the role played by glial activation in mechanisms of maladaptive plasticity will open new perspectives in targeting *reactive gliosis* by novel molecules.

Evidence has highlighted the role of neurotrophins (NTs) and their receptors (TrkA, TrkB, p75NTR) in the remodeling of neural networks following PNI. NTs are secreted as pro-NTs (pro-NGF, pro-BDNF, pro-NT3) and then cleaved in the active form in the extracellular space through a well-regulated protease system. In particular, extracellular matrix metalloproteinases (MMPs) are responsible for NGF degradation and tissue plasminogen activator (tPA) – plasmin system for the conversion of pro-NTs in the active forms (Cirillo et al., 2012). We demonstrated that modulation of these systems was able to restore spinal homeostasis following PNI. Our results showed that *reactive gliosis*is is paralleled by a significant reduction of endogenous NGF (eNGF) content and a consequent compensatory increased expression of NTs' receptors.

Intrathecal (i.t.) administration of nerve growth factor (NGF) or its mimetic peptide BB14® restored the homeostasis of afferent peripheral nerve fibers, nociceptive ganglionic and spinal neurons (Colangelo et al., 2008). Interestingly, NGF/BB14® treatment reduced

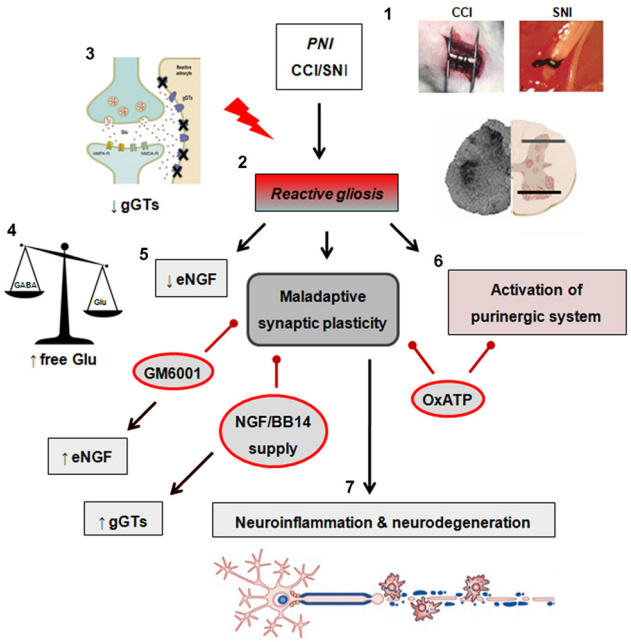

Figure 1 Schematic representation of the cascade of events leading to spinal maladaptive synaptic plasticity following peripheral nerve injury.
(1) Chronic constriction injury (CCI) and spared nerve injury (SNI) of the sciatic nerve induce (2) reactive gliosis both in the dorsal and ventral horn of the spinal cord, (3) reduction of the glial glutamate transporters (gGTs), (4) failure of glutamate (Glu) uptake system that accumulates in the synaptic cleft and spills over, inducing sensitization of spinal neurons and leading to excitotoxicity. Finally (5) a reduction of endogenous NGF (eNGF) content and (6) activation of purinergic system contribute to the establishment of the maladaptive synaptic plasticity that boosts a (7) neuroinflammatory and neurodegenerative process, that ultimately lead to neuronal/axonal degeneration. I.t. administration of GM6001, an inhibitor of matrix metalloproteinases responsible for NGF degradation, or NGF or NGF synthetic peptide BB14®, or oxidized ATP (OxATP), an antagonist of the P2X receptors, recover glial reaction, increase gGTs expression and eNGF content, facing maladaptive synaptic plasticity, counteracting the neuroinflammatory and neurodegenerative process. PNI: Peripheral nerve injury; Glu: glutamate; eNGF: endogenous nerve growth factor; BB14®: NGF-like synthetic peptide; OxATP: oxidized ATP. —● indicate inhibition.

glial reaction in the dorsal horn of the spinal cord and rescued neuropathic behavior, counteracting the long-lasting changes in the spinal cord neuro-glial network. These treatments, moreover, were found to restore the eNGF levels in the spinal cord, both directly (following NGF/BB14® supply) or after inhibition of the NGF degrading system through i.t. administration of GM6001, a MMPs inhibitor.

Recently, we proposed a different experimental approach to counteract glial response after PNI. Evidence from experimental models highlights the activation of the ATP/ADP purinergic system as a key-modulating pathway for sensory perception. In particular, ATP plays a key role in mechanisms of inflammation and neuropathic pain and its receptors (P2XRs) are expressed in glial cells and over-expressed in condition of *reactive gliosis*. Based on these concepts, P2XRs represent potential targets for limiting neuroinflammatory and glial response and P2XRs antagonists could represent a reasonable strategy to limit the *reactive gliosis* and the consequent maladaptive plasticity. Accordingly, we demonstrated that systemic administration of oxidized ATP (OxATP), a non-selective antagonist of P2XRs, reduced spinal *reactive gliosis*, restoring neuroglial structural and functional homeostasis (Cirillo et al., 2015).

To further dissect mechanisms of the spinal reaction, we analyzed the remodeling of the ventral horn components following the SNI. After axotomy of the peripheral nerve fibers, spinal motor neurons become denervated and the consequent Wallerian degeneration determines dramatic changes in the neuroglial framework. The SNI/CCI models give the opportunity to perturb the spinal circuitry "from the periphery", preserving the functional anatomy of the motor neuron circuitry and representing a valid strategy to study the pathophysiology of motor neurons diseases.

SNI, indeed, induced activation of microglia and astrocytic in the ventral horn with a parallel reduction of gGTs and eNGF. I.t. NGF/BB14® administration restored synaptic circuitry showing a neuroprotective activity of eNGF, reducing astrocytic response. In the ventral horn, the behavior of the two glial populations was dramatically divergent following NGF/BB14® treatment (De Luca et al., 2016). In fact, these treatments had no effects on microglial activation, allowing us to hypothesize that a combined inflammatory and initial neurodegenerative process could be the reason for a stronger and self-perpetuating microglial reaction that represents the first step for degeneration of motor neurons after peripheral axotomy. Motor neuron degeneration, in fact, occurs after retrograde Wallerian degeneration of axotomized peripheral motor fibers. The consequent alteration of glutamate homeostasis, resulting from failure of astrocytic reuptake system, the persistent neuroinflammatory reaction and the reduction of neurotrophic support in the ventral horn creates the environmental condition to boost neurodegeneration (**Figure 1**). These alterations, in fact, have been reported in both animal models and patients affected by motor neuron diseases.

Our data, moreover, suggests that the loop neuron/microglia should be targeted to switch off the neurodegenerative process. This is very different to the *reactive gliosis* phenomena reported in the dorsal horn, in which the activated microglia represents an initial step, which resolves after a week following the nerve injury.

Based on the concept of the tetrapartite synapse, we further analyzed the role of the extracellular matrix (ECM) remodeling following PNI. Targeting the system of the MMPs, a family of enzymes essentially involved in the plasticity of the CNS, could be a valid therapeutic strategy to prevent maladaptive plasticity in the ventral horn. MMPs, in fact, are essential for brain development and synapse function, NTs content, modulation of the ECM and synapse morphology. Activation of MMPs (as occurs after PNI) promotes neuroinflammatory response, allowing cellular migration (essentially the microglial cells) and release of proinflammatory cytokines (*e.g.*, interleukin 1β).

For these reasons, inhibition of MMPs could represent a disease-modifying strategy to prevent maladaptive changes in the CNS. In our recent work (Cirillo et al., 2016), we evaluated the morpho-functional changes of the tetrasynapse components surrounding the axotomized motor neurons, focusing the relation between ECM modulators (inhibition of MMPs) and neurotrophins content. Glial-induced maladaptive plasticity in the ventral horn after SNI was rescued by both i.t. administration of GM6001, and NGF like peptide BB14®. These results suggest that modulation of ECM remodeling, glial reaction and neurotrophic supply represent fundamentals for motor neuron vitality supporting synaptic homeostasis following PNI. Although mechanisms of MMPs activity in neuroinflammatory and degenerative disorders remain unclear, we support the essential role of MMPs in the maladaptive plasticity of the spinal ventral horn following SNI, and its target role in designing efficient therapeutic strategies.

In conclusion, models of PNI induce spinal maladaptive plasticity that could be considered both a basis for the neurodegenerative process and a pre-requisite condition for chronic neuropathic pain. Recently, the report of Grace et al. (2016) has shown that opioid analgesic treatment increases the magnitude and/or duration of neuropathic pain. This should prompt us to change our perspective in a system biology approach, respecting the evolutionary complexity of the CNS.

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