PERSPECTIVE

The plasticity of plasticity: lesson from remote microglia induced by focal central nervous system injury

The last decades have represented an important season in the re-conceptualization of brain plasticity, especially in extending the concept at events occurring beyond the developmental stage and in demonstrating the profound impact of these changes on so-called spontaneous recovery after central nervous system (CNS) injuries. The study of the cellular, molecular, functional and structural mechanisms involved in brain plasticity has clearly emphasized the multitude of players engaged in this phenomenon, leading to the conceptualization that non-neuronal cells and non-neuronal mechanisms intervene in these changes and orchestrate some of the responses observed (Werner and Stevens, 2015). Among the different non-neuronal cells and non-neuronal substrates of injury-induced plasticity is becoming increasingly recognized that microglia drive a series of intrinsic CNS responses after damage (Sandvig et al., 2018; Bisicchia et al., 2019). Interestingly, the modulatory influence of microglia on brain after injury constitutes a subset of the broader brain plasticity phenomena, defined "plasticity of plasticity" (Banati, 2002). The term well describes the great capacity of microglia in modifying their morphology and their transcriptional identity in response to environment alteration/injury. The responses of microglia, like the other CNS cells, are not linear, compartmentalized, or binary, but are multifaceted, finely tuned by the extrinsic and intrinsic factors such as the nature of the stimulus, the extracellular environment and the molecular repertoire that is involved in the response and the prior state of the cells (Lynch, 2009).

Multiple signals converge on microglia and contribute to their dynamism, which includes morphological changes, altered expression of cell surface markers and inflammation-related genes, increased phagocytic capacity, and greater proliferation and migration at the site of damage (Kettenmann et al., 2011). Although microglia play a critical role in CNS injury and multiple functions of these cells have been clarified in this context, however, we are still far from explaining the exact contribution of microglia to brain injury. In particular, in this field, the plethora of research on CNS trauma-induced microglia activation is focused at the site of injury and immediately adjacent, whereas relatively few studies investigate how regions, functionally connected to but far from the primary site of injury could also suffer from microglia-induced secondary degeneration. After brain or spinal cord focal injury remote damage might result from an axonal lesion or from trans-neuronal effects leading to spread of damage signals along anatomical and functional connections, and it can be either anterograde or retrograde, indicating the direction of the degeneration relative to the prime site of injury (Viscomi and Molinari, 2014). Since the occurrence of such secondary responses beyond the primary lesion site can interfere with the integrity and function of several neuronal networks, remote damage is particularly relevant because it may act as a decisional node in functional recovery (Zhang et al., 2012).

Experimental studies in animals as well as neuroimaging studies in humans have clearly demonstrated that the microglia dynamism after injury is not spatially and temporally limited to the primary site of damage, but also takes place in remote brain regions that are anatomically and functionally connected to the site of injury (Viscomi et al., 2008; Folkersma et al., 2011).

In such remote regions, microglia respond differentially than the primary site, adopting a specific state, depending on the specific interactions with the environment derived from the insult.

In response to extensive and prolonged damage at the primary injury site, microglia can switch from a "physiological" ramified to hypertrophic, hyper-ramified shape or adopt an amoeboid morphology. Recent morphological data of remote microglia show the presence of a hyper-ramified phenotype, with an increasing number of branches at early time points after injury before assuming the classically morphology of amoeboid cells (Bisicchia et al., 2018). This "bushy" shape of microglia has been associated with a state of alertness or priming of microglia. Priming is a state of mostly pro-inflammatory microglia, driven by changes in the microenvironment and considered to trigger an exaggerated inflammatory response to a further dangerous stimulus. The presence of hyper-ramified microglia at remote regions provides evidence of a possible contribution of microglia to remote degeneration and suggests that remote neuronal dysfunction and degeneration might be due to the potential exaggerated immune response of early priming microglia (Bisicchia et al., 2018). Although the demonstration of "bushy" microglia is indicative of a state of alertness, however, further studies are needed to explained what triggers microglia hyper-ramification in remote areas at early time points after damage, even before neuronal death. Considering that these cells respond more rapidly than neurons to the signals that they encounter, the distinctive morphological changes of remote microglia might result from the specific environment developed in such areas as a consequence of damage. Remote damage initiated by the primary insult, in fact, is driven by a number of events including apoptotic cell death, excitotoxicity, oxidative stress, and mitochondrial dysfunction, all of which could affect and sustain remote microglia responses (Zhang et al., 2012; Viscomi and Molinari, 2014). Factors selectively released in these regions by the different cellular populations as well as molecules that are linked to the loss of neuronal viability might influence the microglia phenotype. The altered crosstalk between activated microglia and damaged neurons establishes a vicious loop that, one hand, accelerates remote degeneration and, on the other, keeps alive microglia responses. However, since the remote damage is a multifactorial phenomenon in which many components become active both in specific as well as in the same time frames, the characterization of the factors/mechanisms associated with the remote damage and in particular wit remote microglial responses is just beginning and requires further studies. For these reasons, researches on experimental models of remote damage induced by a focal brain injury are essential and mandatory to reach, not only a more expansive understanding of the mechanisms and players involving in remote damage, but to identify new effective treatments for this deceitful aspect of CNS damage.

Remote microglial responses are long-lasting events that may continue for a long time after CNS damage has occurred (**Figure 1**). Experimental findings as well as neuroimaging studies in humans demonstrate that microglial activation can persist for weeks, months, years, even after the inflammatory response in the primary injury site diminished (Viscomi et al., 2008; Folkersma et al., 2011). The functional meaning of this remote long-lasting activation is not well-known. However, although early microglia response may be beneficial in response to injury, chronic or inadequate microglia activation can contribute to neuronal dysfunction, and disease progression. Persistent remote microglia responses alter neuron-microglia crosstalk and orchestrate neuronal destructive processes. At this regard recent findings demonstrate that remote microglia by losing their ability to extend processes toward the suffering axotomized neurons affected by damage, lose its "innate" function of removing cellular and molecular debris caused by injury (Kluge et al., 2018). Considering that the loss of microglia process extension in remote injury site continues to increase over time it has been suggested that it might be contributing to the progression of brain injury in the acute, subacute and chronic stages. Interestingly, this structural/morphological impairment is not a global property of microglia that undergo activation after injury, but it seems to be quite a prerogative of remote microglia.

Although the sustained and long-lasting microglial responses in remote areas is a clear sign of the fact that the scenario in distal areas is not only quite different, but can evolve independently of the primary site of injury, future studies are crucial to elucidate what region- and environment-specific mechanisms exactly drive microglia specific responses. Improving our understanding of signals that initiate and drive remote microglia responses is essential to disentangle the complex and long-lasting mechanism of remote degeneration induced by focal brain injury. Furthermore, a more comprehensive understanding of the differences between primary and remote microglia will help us to increase our knowledge of the windows of opportunity for therapeutic interventions following brain injury, which may contribute to a translational bridge from bench to bedside. At this regard, it seems increasingly clear that the development of therapies effective for counteracting brain injuries cannot ignore the key role of remote damage in influencing functional recovery after CNS injury as well as the influence of microglia on both propagation of secondary injury far from the site of injury (Bisicchia et al., 2019).

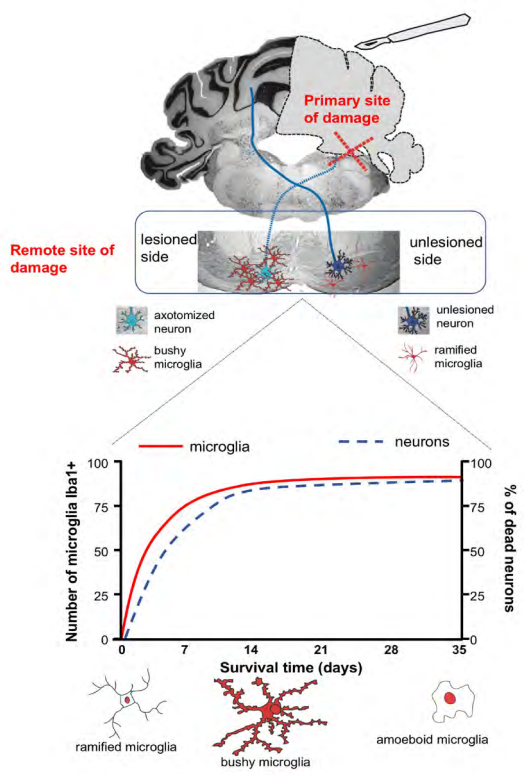

Figure 1 Schematic of the long-lasting activation and of the dynamic changes of microglia in remote regions induced by focal brain injury.
Schematic showing the long-lasting activation and the morphological changes of microglia induced by a focal brain injury —cerebellar lesion, here indicated as "primary site of damage" —in a remote brain region that is anatomically and functionally connected to the primary site of damage, namely pontine nuclei (PN, "remote site of damage"). Due to the anatomical and functional connections between the "primary site of damage" and the "remote site of damage", almost all the neurons of PN of the contralateral side to the lesion (lesioned side) receive death signals from the primary site of damage that, progressively, lead to their degeneration, a phenomenon not observable in the ipsilateral PN (unlesioned side). The events triggered by the primary damage affects also microglia, that undergo to proliferation and to substantial morphological changes (graph and panel below). Interestingly, in the remote site of damage, the neuronal death and the microglia responses continue for months after the primary damage has occurred (graph) and both events progress in parallel. In fact, in the PN of the lesioned side, starting from few days after damage (x-axis), the number of microglia Iba-1$^+$ cells (y-axis on the left of the graph) increases progressively and lasts for several weeks after damage (red line in the graph). This event is accompanied by a progressive neuronal death of axotomized neurons [y-axis on the right of the graph; adapted from Viscomi et al. (2008)]. In this study the Authors follow the two events at different time points after damage and demonstrate that microglia activation was observed as early as 7 days after the primary insult, tended to peak about 3 weeks after the lesion, and remained high for more than one month, following the wave of neuronal death. The increasing number of microglia after damage is accompanied by substantial morphological changes (panel below the graph). After the primary lesion, remote microglia respond promptly to damage switching from a "physiological" ramified to hypertrophic, hyperramified bushy phenotype, with a large number of branches and enlarged cell body noticeable for throughout the period in which neurons undergo death, suggest that microglia might contribute to remote degeneration by exacerbating the severity of remote neuronal loss. How this occurs and the players of this intricate mechanism need further investigations.

In conclusion, it is undeniable that the microglia responses to injury are complex and multifaceted and that its dynamism can be considered a distinctive epiphenomenon of the altered cell-to-cell communication induced by damage. Although it is widely accepted that microglia contribute directly or indirectly to brain pathology, further studies are needed to elucidate the triggering events leading to microglia responses, the complex interplay among signaling molecules, as well as how microglia intervene in the spread of injury in regions far from the primary site of injury. Identifying the complex cell-specific signals and the interplay among signaling molecules, as well as region-specific signals may facilitate the design of appropriate and timing treatments for achieving outcomes in CNS injuries.

I appreciate Blue Pencil Science for the professional editorial work.

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***Received:** May 29, 2019*
***Accepted:** July 6, 2019*

***doi:** 10.4103/1673-5374.264448*

***Plagiarism check:** Checked twice by iThenticate.*
***Peer review:** Externally peer reviewed.*

***Open peer reviewers:** Mehmet Ilyas Cosacak, German Center for Neurodegenerative Diseases, Germany; Saman Sargolzaei, University of California at Los Angeles, USA.*
***Additional file:** Open peer review reports 1 and 2.*

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P-Reviewers: Cosacak MI, Sargolzaei S; C-Editors: Zhao M, Li JY; T-Editor: Jia Y