Linking synaptopathy and gray matter damage in multiple sclerosis

A Musella, G Mandolesi, F Mori, A Gentile and D Centonze

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Introduction
Diffuse microglial and astroglial reactions indicate that the innate immune system is chronically activated in the gray matter of multiple sclerosis (MS) and of experimental autoimmune encephalomyelitis (EAE). How neuronal survival is influenced by reactive microglia and astroglia, whose activation should be protective or restorative in neurological diseases, is unclear. Based on recent observations in EAE and in MS studies, here we propose a mechanism of neuronal damage triggered by uncontrolled synaptic activation and excitotoxicity secondary to glial release of proinflammatory molecules able to regulate the sensitivity of glutamate receptors in neurons.

Synaptic scaling
Those neurons whose activity is persistently silenced, as in response to brain diseases damaging surrounding excitatory neurons, increase their sensitivity to glutamate in an attempt to restore normal excitability. This adaptive, homeostatic response is known as synaptic up-scaling and is secondary to increased surface expression of glutamate AMPA receptors (AMPARs). Up-scaling is a self-limiting phenomenon, since excitability normalization prevents further synaptic sensitization due to synaptic down-scaling, the opposite form of adaptation induced by neuronal hyperstimulation and leading to reduced synaptic sensitivity.

Compared with long-term potentiation (LTP), another form of synaptic plasticity increasing neuronal sensitivity, synaptic up-scaling has a number of remarkable differences. Up-scaling is a negative feedback response as it is induced by synaptic hypostimulation. LTP, in contrast, is a positive feedback mechanism, since synaptic responsiveness is increased in response to hyperstimulation. In addition, up-scaling involves all synaptic sites in a neuron, while LTP is input specific, as only stimulated synapses undergo adaptive changes. Furthermore, LTP induction requires the activation of glutamate N-methyl-D-aspartate (NMDA) receptors, while up-scaling is an AMPAR-dependent phenomenon.

Up-scaling in EAE and in MS
Evidence exists that up-scaling are its dependency on the inhibition of Arc/Arg3.1, a synaptic protein controlling AMPAR expression and, importantly in the context of our working hypothesis, on inflammatory molecules. Glial tumor necrosis factor-α (TNF) was in fact soon recognized as a key player in synaptic up-scaling induction, and later other inflammatory molecules were added to the list of molecules orchestrating up-scaling. The involvement of inflammatory molecules in synaptic scaling is consistent with the observation that tissue damage in the central nervous system (CNS) is associated with transient microglial and astroglial reaction.

Other critical features of synaptic up-scaling are its dependency on the inhibition of Arc/Arg3.1, a synaptic protein controlling AMPAR expression and, importantly in the context of our working hypothesis, on inflammatory molecules. Glial tumor necrosis factor-α (TNF) was in fact soon recognized as a key player in synaptic up-scaling induction, and later other inflammatory molecules were added to the list of molecules orchestrating up-scaling. The involvement of inflammatory molecules in synaptic scaling is consistent with the observation that tissue damage in the central nervous system (CNS) is associated with transient microglial and astroglial reaction.

Up-scaling in EAE and in MS
Evidence exists that up-scaling is induced in the course of EAE and MS, likely contributing to excitotoxic neurodegeneration. In EAE, in fact, significantly and persistently higher levels of TNF in the gray matter have been reported, coupled with the other molecular and synaptic changes typical of up-scaling. Expression of AMPARs at synaptic membranes increases in EAE, and AMPAR-mediated glutamatergic currents are up-regulated in parallel with widespread downregulation of Arc/Arg3.1. Consistent with current literature showing that up-scaling is regulated by inflammatory mediators, application of a Th1 cytokine mix in neuronal cultures replicates the downregulation of Arc/Arg3.1 occurring in EAE brains. Similar to activity deprivation-induced up-scaling, EAE-induced alteration of glutamate synapses is also prevented by...
pharmacological inhibition of TNF, as shown in vitro or after intracerebroventricular administration of etanercept, a TNF blocker. Thus, all these data converge in indicating that synaptic hyperexcitability in EAE follows TNF-induced up-scaling.

During MS relapses, diffuse cortical hyperexcitability has been reported by means of paired-pulse transcranial magnetic stimulation but not following single pulses of stimulation, according to the conclusion that such hyperexcitability is synaptically mediated. In addition, cerebrospinal fluid (CSF) taken from MS patients but not from control individuals has the property of exacerbating glutamatergic transmission recorded in mouse brain slices in a TNF-dependent manner. Pre-treatment with etanercept, in fact, blocked the ability of MS-derived CSF to induce synaptic scaling in vitro. These data indicate that during MS, TNF is released by inflammatory cells in the circulating CSF at concentrations high enough to induce diffuse synaptic up-scaling in neurons exposed to this cytokine (Figure 1).

Figure 1. Schematic representation of the synaptic up-scaling in MS and EAE. MS: multiple sclerosis; EAE: experimental autoimmune encephalomyelitis; TNF: tumor necrosis factor; IL: interleukin; sEPSC: spontaneous excitatory postsynaptic currents; AMPAR: AMPA receptor; GLAST: glutamate/aspartate transporter. In the basal condition, neurons have a stable level of activity as a result of glutamatergic synaptic inputs mediated by AMPARs (a). In response to brain damage, reactive microglia and astroglia release inflammatory mediators (IL-1β and TNF), leading to enhanced AMPAR localization at the postsynaptic surface, and to reduced GLAST expression at the astrocyte membrane. These events result in the homeostatic process of synaptic up-scaling, causing an increase of glutamatergic transmission (sEPSC) (b). Transient activation of microglia and astroglia leads to a self-limiting up-scaling phenomenon, restoring basal condition. Conversely, persistent inflammation triggers uncontrolled synaptic activation and excitotoxicity (c). The chronic inflammatory nature of MS disease turns up-scaling into synaptic damage and dendritic retraction, contributing to gray matter atrophy in the brain.
Chronic inflammation turns up-scaling into synaptic damage and dendritic retraction

Up-scaling is a self-limiting phenomenon, representing an adaptive response to synaptic silencing and, accordingly, acute brain damage is generally associated with transient activation of microglia, a major source of TNF. In MS and EAE, in contrast, TNF signaling persists virtually indefinitely, owing to the chronic nature of the diseases and, most important, to their inflammatory nature. As a result, chronic up-scaling exposes synapses to excitatory transmission and finally to excitotoxic dendritic degeneration. Consistently, in EAE mice the apoptotic cascade was demonstrated to be selectively activated in the synaptic compartment of neurons, leading to dendritic retraction, synaptic loss, and neuronal disconnectivity far before overt neuronal loss can be detected. Notably, the apoptotic cascade has been recognized to exert actions on the structural integrity of synapses, and to modulate synaptic plasticity, even in cells that are not necessarily destined to die.

Treatment of EAE mice with an AMPAR antagonist attenuates dendritic spine loss and reduces the clinical manifestation of the disease, confirming that excessive up-scaling of AMPAR-mediated synaptic excitation plays a role in the neuronal damage associated with central inflammation. Being that dendritic spines are highly represented in neurons, we propose that their retraction secondary to up-scaling-driven synaptopathy could well explain diffuse gray matter atrophy in EAE and in MS brains.

Up-scaling and dendritic loss are correlates of chronic gray matter microglial activation

Neuropathological and positron-emission tomography (PET) studies have demonstrated a correlation between chronic and diffuse gray matter microglial activation and the neurodegeneration associated with irreversible disability in MS. The mechanistic link between microglial activation and neuronal degeneration is, however, unknown, and we hypothesize that up-scaling and the resulting excitotoxic process could be involved in microglia-driven neurodegeneration. Microglial activation in EAE brains is associated with TNF release, and activated microglia applied onto brain slices prepared from healthy mice induces up-scaling of AMPAR-mediated synaptic transmission by releasing TNF. Furthermore, activated microglia applied on slices from EAE mice failed to further increase glutamate transmission, indicating that EAE-induced up-scaling and microglia-induced up-scaling share common mechanisms. Addition of microglial cells to neuronal cultures also causes a significant reduction of the dendritic spine number, further supporting the hypothesis that both up-scaling and dendritic spine loss are regulated by activated microglia in EAE and MS brains.

Astroglial cells contribute to synaptic degeneration in EAE

Astroglial cells are also activated and proliferating during MS and EAE and represent, along with microglial cells, crucial elements of the innate immune system chronically activated in these disorders. Astrocytes play significant functions in shaping synaptic transmission, and form with the presynaptic nerve terminal and the postsynaptic dendritic spine the so-called “tripartite synapse” (Figure 1).

Removal of glutamate from the synaptic cleft is a prominent action of astrocytes, limiting excitatory signaling. During neuroinflammatory conditions, release of proinflammatory mediators such as interleukin (IL)-1β causes the activation of astrocytes, which significantly downregulate their membrane expression of the glutamate transporter GLAST, involved in glutamate uptake. As a result, AMPAR-mediated currents are increased in duration during EAE, and excitotoxic synaptopathy is favored, thereby mimicking the effects of TNF-induced synaptic up-scaling. Since astroglial cells also release TNF at synapses, the downregulation of GLAST seen in EAE and in MS brains appears as an additional mechanism of synaptic adaptation to neuroinflammatory insults, contributing to dendritic and synaptic degeneration, and ultimately explaining the role of chronic glial activation in the development of gray matter atrophy and chronic disability in MS patients.

Conclusion and perspectives

The adaptation of synaptic transmission to the inflammatory milieu generated within the CNS by the pathological process of MS has emerged in EAE investigations, and has been confirmed in neuropathologic, genetic, and TMS studies in MS patients. Although neuronal loss in MS is not as extensive as in primarily neurodegenerative diseases, our hypothesis provides a plausible mechanistic link between chronic astroglial and microglial activations and diffuse gray matter damage occurring in MS brains. Inflammation-induced hyperfunctioning of glutamate transmission, which can be useful to restore excitability of neurons that have lost part of their synaptic inputs after focal or multifocal brain damage, is exaggerated in MS and EAE, and triggers excitotoxic dendritic spine loss and neuron-to-neuron disconnectivity even before...
irreversible neuronal death. However, MS is primarily an inflammatory demyelinating disease, causing focal lesions in the white and gray matter, such as in subpial cortical layers. It can be postulated that focal demyelination influences synaptic pathology, but this hypothesis deserves ad hoc studies. In addition, neurodegeneration is a prominent pathological hallmark especially in the progressive phase of MS, which is characterized by limited gray matter inflammation. Factors other than TNF are therefore very likely to be equally important to trigger tissue damage.

The recognition that not only myelin but also synapses are early and privileged sites of damage in MS and involved in tissue loss could have profound therapeutic implications, as dendritic spines are highly dynamic structures, and dendritic spines can be for example rescued in EAE mice by physical exercise, which causes in parallel the correction of the synaptic alterations and the attenuation of motor deficits of these mice. Regular physical exercise, therefore, should be seriously explored as an effective disease-modifying therapeutic strategy, to counteract cortical atrophy and irreversible disability in MS. Pharmacological or brain stimulation procedures able to induce synaptic adaptations, dendritic spine formation, and network restoration in the diseased brain could have similar therapeutic potential.

Conflicts of interest
Dr Diego Centonze has acted as an advisory board member of Merck-Serono, Teva, Genzyme, Bayer Schering, Biogen Idec, Novartis, Almirall, and GW Pharmaceuticals; and has received funding for traveling and honoraria for speaking or consultation fees from Merck Serono, Teva, Genzyme, Novartis, Bayer Schering, Sanofi-Aventis, Biogen Idec, and Almirall. He is the principal investigator in clinical trials for Novartis, Merck Serono, Teva, Bayer Schering, Sanofi-Aventis, Biogen Idec, and Roche. The other authors have nothing to declare.

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