

Unexpected Microglial “De-activation” Associated With Altered Synaptic Transmission in the Early Stages of an Animal Model of Multiple Sclerosis

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ABSTRACT: Multiple sclerosis, and its animal model—experimental autoimmune encephalomyelitis (EAE), is a demyelinating disease causing motor and sensory dysfunction, as well as behavioral comorbidities. In exploring possible functional changes underlying behavioral comorbidities in EAE, we observed increased excitatory drive onto the major cells of the basolateral amygdala. This was associated with increased numbers of dendritic spines. An unexpected finding was that microglial cells at this time were in a “deactivated” state, and further studies suggested that the microglial deactivation was responsible for the increased excitatory drive. This is the first report of microglial deactivation in an inflammatory disease and raises many questions as to the underlying mechanisms and functional relevance.

KEYWORDS: Microglia, EAE, synaptic pruning, amygdala, inflammation, comorbidity

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Multiple sclerosis (MS) is an autoimmune inflammatory demyelinating disease of the central nervous system, characterized by the presence of sclerotic plaques and peripheral immune cell infiltration. Myelin-specific T cells destroy the myelin sheath, leading to demyelination and consequent motor and sensory impairment.¹ In addition to these symptoms, MS is also characterized by cognitive and emotional changes—about 30% to 50% of the population presents with depressive symptoms and 40% to 60% show cognitive impairments, which is 3 times the prevalence in the general population.²

Experimental autoimmune encephalomyelitis (EAE) is an animal model of MS (in our case, induced in the C57BL/6 mouse) that has been widely used to study the disease. The model recapitulates principal features of the disease, including motor symptoms, demyelination, and inflammation. It is induced by injecting a myelin oligodendrocyte glycoprotein peptide (MOG₃₅₋₅₅) along with pertussis toxin and complete Freund’s antigen (CFA). In most studies, CFA without MOG is the control, which initially activates the innate immune system. This enables investigators to tease out how the activation of the acquired immune system by the MOG affects brain function, over and above that affected by innate immune system activation by CFA.

The classic behavioral symptom of EAE is ascending flaccid paralysis starting ~d10–11 post induction. Most of the studies in the EAE model have been focused on the spinal cord because the motor symptoms are correlated with spinal cord inflammation.³ Less studied are the emotional and cognitive changes that accompany MS. One of the challenges of studying the cognitive/emotional changes in a rodent model is that most of these behavioral assessments are based on motor

functioning and these tests cannot be done on mice that are paralyzed. Haji et al⁴ and Pollak et al⁵ have addressed the behavioral comorbidity in EAE by studying the mice at an early stage when the motor symptoms are not yet apparent. Work from our group and others has shown that EAE mice show emotional changes indicative of increased anxiety and altered cognitive function in the early stages of EAE.⁶ To understand the cause of the changes in emotional behavior, we explored cellular correlates in the amygdala, which is a brain region involved in these emotional changes.⁷

In the early, presymptomatic stages of EAE, we did not find any peripheral infiltrating cells in the brain, or any demyelinating lesion. However, when we studied synaptic properties in the basolateral amygdala, we found that excitatory synaptic transmission was enhanced without any change in the inhibitory synaptic properties. With Dr Adrienne Benediktsson (Mount Royal University), we performed anatomical investigations of the pyramidal neurons which revealed that dendritic spine numbers were increased. As these are the receptive sites of excitatory synapses, this could explain the enhanced excitatory drive. There was also a change in AMPA/NMDA ratio, again suggestive of more active synapses.

Change in synaptic function has been linked to microglia. These are the immune surveillance cells of the brain that are activated during inflammatory processes, and this activation has been linked to pruning of synapses. However, the dichotomy of increased spine number in a presumed inflammatory environment characteristic of EAE was unexpected and prompted us to further study the microglial phenotype. Microglial activation is often characterized by morphological changes—the cells are ramified in the resting state and display an amoeboid structure



during the inflamed state. We performed a detailed analysis on confocal images of microglia (data not included in this article) and did a 3-dimensional (3D) reconstruction of microglia in the amygdala and found that there were no significant changes in the microglial process length, area, volume, or branching depth. Next, in collaboration with Dr Marie-Eve Trembley (Laval University), we investigated the microglial ultrastructure at the electron microscope (EM) level. This analysis indicated that microglia displayed more ramified morphology, reduced Iba-1 (a microglial marker) staining, and reduced extracellular digestion. These data suggested that microglia were not activated in the conventionally described manner that is associated with inflammation. Consistent with this, we observed reduced CD68 staining, which is a lysosomal marker associated with “activated” microglia.

The complement system, specifically C1q and CD11b/C3, is known to be involved in neuronal-microglial interaction. For example, C1q deposition is associated with synaptic loss during early stages of Alzheimer disease⁸ and inhibition of C1q, C3, or CR3 reduces early synaptic loss. Complementary to our morphological and synaptic observations, we found decreased C3 transcript in the amygdala in early stage of EAE. The complement transcripts were unchanged in the microglia, indicating the source of complement was not the microglia, consistent with previous findings that its source is the neuron,⁹ raising the possibility that neuronal signaling alters microglial phenotype in EAE.

To investigate whether this altered microglial phenotype was responsible for the electrophysiological changes, we injected minocycline (which reduces microglial activation) intracerebroventricularly and found that glutamatergic transmission was upregulated. A similar finding was noted when we depleted microglia by Mac-1 Saporin infusion directly into the amygdala. Our findings led us to suggest that a “deactivated” state of microglia was responsible for the reduced synaptic pruning and increased glutamatergic signaling we had previously observed in early EAE.

To our knowledge, this is the first study reporting a “deactivated” state of microglia during an inflammatory insult. Although our results are compelling, it is somewhat surprising based on our previous understanding of microglial involvement in inflammatory disease. This study raises several questions:

1. *Is microglial morphology a true indicator of its immune system mediated activation?* In our study, we found changes only at the EM level and not at the light microscopic level. Thus, the striking morphological changes that are often visible at the light microscopic levels¹⁰ may not be essential for microglia to influence neuronal functions.
2. *How do these changes in early EAE transition to a different functional state during the highly inflammatory milieu characteristic of fully developed EAE?* An earlier study in a different brain area¹¹ reported decreased numbers of spines at the peak of the disease. There is good evidence that

microglia at this time display the amoeboid shape and cellular expression characteristic of activated microglia, and it will be interesting to determine how and if the microglia play a role in these later synaptic changes. Transcriptomic and proteomic analysis of microglial cells at various stages of the disease could shed light on the molecular changes within the cell.

3. *What is the signaling mechanism?* Our investigations were done at the presymptomatic stage of EAE, where there is no cellular infiltration into the amygdala from the periphery. Nor was there evidence of increased levels of pro-inflammatory cytokines. It is intriguing that microglia function can be altered in the absence of infiltrating T cells. However, recent evidence points to a role for meningeal T cells and their derived cytokines in brain function.¹² T cells activated by the autoimmune challenge could send specific signals, possibly at the blood brain interface, which could alter microglial homeostasis. Future experiments studying these interactions in the EAE model could shed light on the signaling mechanism.
4. *What is the functional significance of such changes?* During many neurodegenerative diseases, such as Alzheimer disease and schizophrenia, it is thought that microglia activation leads to synaptic loss.^{13,14} In contrast, in the early stages of EAE, excitatory synapses and spine number were increased. Is this a form of protection from neurodegeneration in the early stages of inflammation that is subsequently overwhelmed at later stages of the disease? Last, but not the least, how do such changes affect the behavior? Does the microglial “deactivation” have anything to do with the increased anxiety and altered cognitive changes we have observed in early EAE?

In summary, this study raises many questions that warrant further investigation. Answers to these questions could increase our understanding of inflammation-related behavioral changes.

Author Contributions

SA and QP wrote and edited the manuscript.

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