Journal Club

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CX3CR1 Does Not Universally Mediate Microglia-Neuron Crosstalk during Synaptic Plasticity

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Review of Schecter et al.

Tissue macrophages play essential roles during organogenesis and maintenance of tissue homeostasis by adopting specialized, tissue-specific phenotypes. These phenotypes are dictated by their distinct origins (i.e., their lineage), as well as the microenvironmental cues they encounter in their resident tissue. Both of these influences, lineage and environment, converge at the genomic level to confer tissue-specific functionality (Gosselin et al., 2014). For example, microglia, the resident macrophage population of the brain, rely on the environmental cue TGF-β to express the complement proteins used to opsonize and eliminate inactive synapses during development (Schafer et al., 2012). The heterogeneity observed across macrophages of different tissues suggests that individual tissues may also be composed of heterogeneous populations of macrophages depending on the temporal and spatial availability of different environmental cues; indeed, multiple recent studies have found that microglia exhibit brain region-dependent heterogeneity in their transcriptomic makeup despite sharing a core gene profile (Grabert et al., 2016; De Biase et al., 2017). Thus, microglia in different brain regions, or across different developmental phases, may rely on different signaling mechanisms to perform various functions. In a paper published recently in *The Journal of Neuroscience*, Schecter et al. (2017) provide compelling evidence in support of this notion by demonstrating that the fractalkine receptor (CX3CR1), which has an established role in mediating synaptic refinement and transmission in the developing hippocampus (Paolicelli et al., 2011), has no such effect during experience-dependent synaptic plasticity in primary visual cortex (V1).

The visual system has been widely used to study the role of microglia in sculpting neural circuits during development. Synaptic plasticity in V1 can be studied by manipulating a mouse's visual experience. For example, repeated exposure to a visual stimulus strengthens neuronal responses in V1 in a process called stimulus-selective response potentiation. In contrast, depriving one eye of visual input by suturing the eyelid closed shifts the responsiveness of V1 neurons from the deprived eye to the contralateral eye; this is called an ocular dominance shift.

To investigate a role for microglial CX3CR1 in V1 plasticity, Schecter et al. (2017) used transgenic mice in which the *Cx3cr1* gene was replaced with GFP (Jung et al., 2000). These mice can be used to study the effect of *Cx3cr1* gene dosage on microglial function by comparing wild-type C57BL/6 mice to mice heterozygous ($Cx3cr1^{gfp/+}$) or homozygous ($Cx3cr1^{gfp/gfp}$ or $Cx3cr1^{KO}$) for the GFP-containing allele. After confirming that $Cx3cr1^{KO}$ mice exhibit normal segregation of contralateral and ipsilateral retinal inputs in the LGN, Schecter et al. (2017) used electrophysiological measures to test whether fractalkine signaling is necessary for plasticity in the visual system. Interestingly, V1 layer IV neurons in $Cx3cr1^{KO}$ mice responded normally to stimulus-selective response potentiation, showing an increasing magnitude of response with repeated exposure to a visual stimulus. Moreover, monocular deprivation of $Cx3cr1^{KO}$ mice failed to perturb the shift of responsiveness of V1 layer IV neurons from the deprived eye to the contralateral eye receiving normal visual input.

A contemporaneous study (Lowery et al., 2017) largely supports the findings of Schecter et al. (2017). Extending their analyses to include heterozygous $Cx3cr1^{gfp/+}$ mice in addition to WT and $Cx3cr1^{KO}$ mice, Lowery et al. (2017) observed normal segregation of eye-specific retinal inputs in the LGN across all *Cx3cr1* genotypes, as well as intact ocular dominance plasticity in V1. Furthermore, these authors found that the density and behavior of V1 (layer II) microglia, including process motility, microglia-dendritic spine interactions, and hyper-ramification following

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monocular deprivation, was unperturbed across all *Cx3cr1* genotypes (Lowery et al., 2017).

That separate groups using divergent approaches were able to reach the same conclusion, namely, that microglial CX3CR1 is dispensable for synaptic plasticity in V1, suggests that the findings of the studies are not artifactual. However, there are multiple reports that point toward a role for fractalkine in synaptic plasticity and microglia development (Paolicelli et al., 2011; Pagani et al., 2015). Paolicelli et al. (2011) used acute hippocampal slices explanted from *Cx3cr1*^{KO} mice to investigate whether loss of fractalkine signaling perturbed synaptic plasticity in Schaffer collateral inputs to the CA1 region of the hippocampus. Loss of CX3CR1 signaling enhanced LTD in acute hippocampal slices derived from postnatal day (P) 13 mice, but not P40 mice, indicating that fractalkine signaling participates in the early forming of brain circuits in the hippocampus (Paolicelli et al., 2011). Paolicelli et al. (2011) also observed a transient decrease in microglial density in the hippocampus of *Cx3cr1*^{KO} mice, which they reasoned was responsible for the developmental delay in the processes they studied. The same group further observed abnormal microglia development, characterized by less complex process arbors and diminished response to ATP-induced process rearrangement, in the hippocampus of *Cx3cr1*^{KO} mice (Pagani et al., 2015). One important caveat is that these hippocampal experiments were conducted in acute brain slice preparations bereft of a vascular supply and normal afferent and efferent connections rather than in the intact brain. In contrast to work performed in the hippocampus, both Schecter et al. (2017) and Lowery et al. (2017) found no difference in microglial density or morphology in V1 of *Cx3cr1*^{KO} mice, although Lowery et al. (2017) replicated previous work (Hoshiko et al., 2012) showing a transient delay in the entry of microglia into thalamocortical axon clusters in somatosensory cortex of *Cx3cr1*^{KO} mice.

How can these disparate findings be reconciled? One possibility is that *Cx3cl1* is differentially expressed across different brain regions. Indeed, two reports indicate that *Cx3cl1* is only faintly expressed in layer IV of the mouse cerebral cortex (Tarozzo et al., 2003; Kim et al., 2011), the region of cortex investigated by Schecter et al. (2017). Despite this, Lowery et al. (2017) also observed no effect of *Cx3cr1* KO on synaptic plasticity in layer II of V1, a layer in which fractalkine is highly expressed (Tarozzo et al., 2003; Kim et al., 2011). Thus, the availability of *Cx3cl1* differs across brain regions and may partially explain the lack of an effect in *Cx3cr1*^{KO} mice observed by Schecter et al. (2017).

Another possibility is that microglia exhibit spatial and/or temporal heterogeneity in the mechanisms they use to sculpt circuits, as suggested by Lowery et al. (2017). Indeed, the development of next-generation sequencing technologies has led to the identification of significant microglial heterogeneity across brain regions (Grabert et al., 2016). Strikingly, there are gross differences in microglial morphology, dynamics, and transcriptomic makeup even within substructures of the basal ganglia (De Biase et al., 2017). This microglial heterogeneity also exists during brain development, the time period during which microglia actively develop their CNS-specific functional specialization. As brain development progresses in a stepwise fashion, so does the phenotype of microglia to facilitate each phase of brain development (Matcovitch-Natan et al., 2016). Moreover, the temporal evolution of the microglial phenotype likely depends on the changing availability of local environmental cues that instruct macrophage specialization during organogenesis (Schafer et al., 2012). Such findings are consistent with the results that demonstrate temporally demarcated effects of loss of fractalkine on microglia density and synaptic plasticity in the somatosensory cortex and hippocampus (Hoshiko et al., 2012). Because microglia express *Cx3cr1* throughout much of their development (Bennett et al., 2016), the expression of *Cx3cl1* by neurons could be temporally restricted.

Finally, the observation that experience-dependent plasticity occurs in V1 in *Cx3cr1*^{KO} mice does not completely exclude a role for microglial CX3CR1 in this process. For example, fractalkine signaling could serve a redundant function in V1, but not the hippocampus. This is consistent with the hypothesis that the lack of effect observed by Schecter et al. (2017) stems from spatial and/or temporal heterogeneity in *Cx3cl1* expression or heterogeneity in microglial synapse elimination machinery. Thus, more work is necessary to (1) further define *Cx3cl1* expression dynamics during development as well as during monocular deprivation and (2) understand alternative mechanisms through which microglia refine synaptic connections.

In conclusion, Schecter et al. (2017) observed no effect of *Cx3cr1* knock-out on visual system plasticity using an electrophysiological approach. This finding has been independently replicated by others (Lowery et al., 2017) but stands in contrast to previous work performed in the hippocampus. Such apparently conflicting findings warrant further investigation into the diversity of synaptic refinement mechanisms that microglia use to sculpt brain circuits. In an organ as complex and regionally diverse as the brain, single-cell resolution may be necessary to illuminate the full extent of heterogeneity within cell populations such as microglia during both homeostasis and disease.

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