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Location, Location, Location: Transcriptional Control of Astrocyte Heterogeneity

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Abstract

Huang *et al*. have found that deletion of astrocyte lineage-specifying transcription factor NFIA from mature astrocytes alters astrocyte morphology, molecular identity, and synaptic-support capacity in a region-specific manner. We discuss the implications of these findings in light of emerging roles for astrocytes in immune cell crosstalk.

Astrocytes in the central nervous system (CNS) play increasingly appreciated roles in supporting brain development and function. Astrocytes are highly ramified cells uniquely adapted to maintain the intense metabolic demands of neurons, performing functions such as recycling neurotransmitters, serving as an energy supply, and facilitating synaptic communication. However, in another light, they can be considered a brain-specialized type of stromal cell. They share many conserved features with fibroblasts in other organs, such as production of extra-cellular matrix, close association with vasculature, and responsiveness to mechanical stress. Like fibroblasts, astrocytes form a close partnership with tissue-resident macrophages, known as microglia in the brain [1], and can become reactive in response to injury and inflammation. This reactive response is often associated with fibrosis in peripheral tissues and termed reactive astrogliosis when it occurs in the CNS. Stromal cells are integral to immune responses and they support tissue resident immune cell survival and function both at rest and during inflammation. As such, defining the genetic and epigenetic mechanisms that regulate the heterogeneity and function of stromal cells, including astrocytes in the brain, is relevant to understanding both their tissue-specific functions and their interactions with the local and systemic immune system.

In a recent study by Huang *et al*. [2], the authors define a novel role for an astrocyte lineage-defining transcription factor in regulating astrocyte heterogeneity and CNS function in mice. During CNS development, distinct, temporally separated sets of transcription factors control

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the initiation of neural cell-type differentiation. The transcription factor nuclear factor 1-A (NFIA) was one of the first transcription factors shown to be required for specification of the murine glial lineage, including oligodendrocytes and astrocytes [3]. While astrocytes, like many cell types, have ongoing expression of lineage-defining transcription factors, how this ongoing transcription factor expression determined astrocyte identity and function remained unknown. In the present study, the Deneen group provides persuasive evidence that the functional role of NFIA in astrocytes does not end in development, further defining NIFIA-dependent mechanisms by which regional astrocyte heterogeneity is maintained [2].

Huang and coworkers conditionally deleted NFIA from mature astrocytes in mice using a recently developed astrocyte-specific Cre line, *Aldh1l1*-Cre/ERT2 [4]. After administering tamoxifen at 5 weeks of age to induce astrocyte deletion at a time-point when astrogenesis is largely complete, the researchers waited 4 months to evaluate the effect of late postnatal NFIA deletion on astrocytes [2]. This perturbation led to stunted astrocyte morphology with decreased branching. However, these results were observed predominantly in the hippocampus (a brain region that mediates learning and memory) and, to a lesser extent, in the cerebral cortex. Astrocytes in two other regions, the brain stem and the olfactory bulb, remained unaffected. This region-restricted phenotype was also observed in functional assays. For example, NFIA conditional knockout (cKO, *Aldh1l1*-Cre/ERT2$^{+/-}$, *NFIA*$^{fl/fl}$) animals showed deficits in hippocampus-dependent learning and memory behavioral tasks, but performed similarly to wild type (WT) mice in assays that probed olfactory bulb function. These findings indicated that astrocytic NFIA was necessary for complex behavioral tasks, but only when those tasks were dependent on a region already seen to exhibit astrocytic morphologic dependence on NFIA.

Consistent with the fact that loss of NFIA made hippocampal astrocytes smaller than in WT mice, their physical association with neurons was also impaired, as measured by a Förster resonance energy transfer-based assay. Potentially as a result of their decreased proximity to neurons, hippocampal astrocytes lacking NFIA also presented impaired sensing of glutamate and GABA, the two major neurotransmitters by which neurons communicate. While basal properties of hippocampal neural circuits were unaltered by this perturbation, synaptic plasticity (the ability of connections between neurons to change in response to their environment) was affected. In one classic readout of synaptic plasticity, long-term potentiation, the authors found that high-frequency stimulation in the hippocampus led to synaptic strengthening in WT animals, as expected. In contrast, in NFIA cKO animals, the same induction paradigm had only a transient increase in synaptic strength, indicating impaired plasticity. These data suggest that NFIA activity is important for the maintenance of hippocampal astrocyte synapse-supporting functions, particularly in states of plasticity [2].

To define how NFIA led to these region-specific effects on astrocyte function, the authors examined its impact on gene expression in the hippocampus, compared with the olfactory bulb, where NFIA did not seem to play a functional role. RNA-sequencing of WT versus NFIA cKO astrocytes revealed threefold more differentially expressed genes in hippocampal astrocytes versus olfactory bulb astrocytes. In ChIP-seq experiments, the researchers observed a strongly increased frequency of promoter binding by hippocampal NFIA; while

NFIA bound nearly 2000 promoters in the hippocampus, it bound only 75 in the olfactory bulb. Concurrently, they performed immunoprecipitation followed by mass spectrometry and found that another glial-lineage transcription factor, NFIB, sequestered NFIA in the olfactory bulb, but not in the hippocampus, thus preventing NFIA from modulating transcription. These data provide an elegant description of how the role of a 'developmental' transcription factor does not end with development, exemplifying how combinatorial usage can generate diversity in a stromal cell population (Figure 1). They further demonstrate that astrocytes are functionally heterogeneous based on brain region, raising the question of how other astrocyte functions, including immune modulation, may also vary in a region-specific manner.

How might such mechanisms be relevant to understanding the roles of astrocytes in the regulation of immune function? Recently published mouse single cell-RNA-sequencing datasets highlight between-region [5] and within-region [6] astrocyte heterogeneity, including the astrocytic expression of cytokines and immune response genes (e.g., *Il33*, *Il18*, *Tlr3*). Functional implications of these expression patterns are beginning to emerge. One clear example is that astrocytes can regulate myeloid cell function. For example, IL-33 and TGF-β can regulate the physiologic function of microglia in the mouse developing brain, including in synaptic pruning [1]. Moreover, in experimental autoimmune encephalitis, a mouse model of multiple sclerosis (MS), reactive astrocytes activate the unfolded protein response and recruit inflammatory monocytes to the brain [7]. Recruitment of peripheral macrophages is a pathognomonic feature of numerous neuroinflammatory and neuro-degenerative conditions, including MS, Alzheimer's disease, and others. As such, defining novel subsets of astrocytes in these disorders is a topic of increasing interest [8]. Conversely, peripheral immune cells can modulate astrocyte reactivity. For example, in mouse models of ischemic stroke, amphiregulin derived from regulatory T cells can prevent reactive astrogliosis and promote functional recovery [9]. Thus, defining the astrocyte-immune 'interactome' may yield insight into the often-underappreciated crosstalk between astrocytes and immune cells and reveal mechanisms for selective vulnerability in immune-mediated CNS pathologies.

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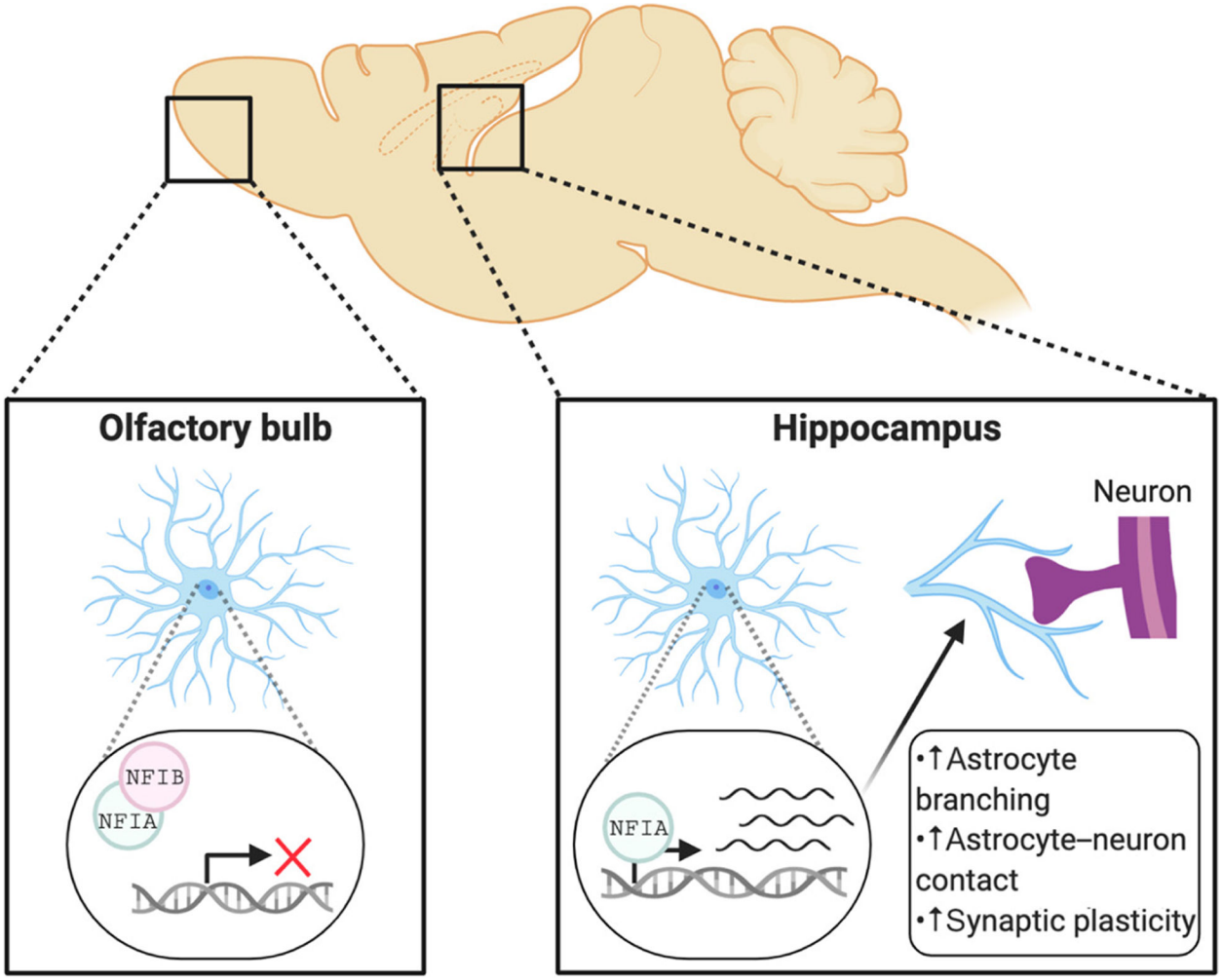

Figure 1. Regional Heterogeneity in the Activity of Transcription Factor Nuclear Factor 1-A (NFIA) in Mature Mouse Astrocytes.

Huang *et al.* [2] found that conditional deletion of transcription factor NFIA from mature astrocytes in mice severely affected astrocyte morphology, transcriptional state, and neural circuit-associated functions, but only in the hippocampus. Astrocytes in other regions, including the olfactory bulb, were relatively spared by the perturbation. In the olfactory bulb, transcription factor NFIB bound and sequestered NFIA away from the genome, mitigating the impact of NFIA loss in this region. This figure was created using BioRender.com (https://biorender.com/).