Stem Cell Reports
Letter
ISSCR
OPEN ACCESS

Questioning the evidence for a Janus-faced nature of adult neurogenesis in Alzheimer's disease

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https://doi.org/10.1016/j.stemcr.2021.05.006

A substantial number of studies support the notion that hippocampal neurogenesis is deficient in mouse models of Familial Alzheimer's disease (FAD) as well as in Alzheimer's disease (AD) patients (Disouky and Lazarov, 2021). In agreement with that, interventions that upregulate neurogenesis have led to the attenuation of pathology and rescue of memory impairments (see for example Choi et al., 2018). Likewise, previous studies have showed that ablating neurogenesis in FAD mice exacerbated learning and memory (Choi et al., 2018; Hollands et al., 2017). In contrast, a recent study by Zhang et al. suggests that ablating neurogenesis improves synaptic and cognitive function in FAD mice (Zhang et al., 2021). Upon closer examination of the experimental approach, we raise questions concerning the study design and the authors' interpretation of the results, and thus, reveal critical differences between this study and the existing body of literature (see for example [Choi et al., 2018; Hollands et al., 2017]). To deplete neurogenesis in APPswe/PS1ΔE9, the authors bred this FAD line with mice expressing glial fibrillary acidic protein-driven thymidine kinase (GFAP-TK). These mice were then treated with valganciclovir to indiscriminately deplete all proliferating GFAP+ cells. It is essential to note that neural stem cells (NSCs) comprise only a small portion of all GFAP+ cells with the majority of GFAP expression found in astrocytes (Kriegstein and Alvarez-Buylla, 2009). In a second approach, the authors used systemic treatment with methylazoxymethanol acetate (MAM) for the depletion of neurogenesis. Similarly to the first approach, the choice of ablating neurogenesis by this treatment suffers from lack of specificity. This approach ablates dividing cells and may include the abolishment of astrocytes, microglia, neural progenitor cells, and others. In addition, MAM could alter the composition of the gut microbiota, decrease microbes, and minimize neuroinflammation. Thus, it is plausible that the apparent improvement in MAM-treated FAD mice was due to the reduction of systemic inflammation rather than hippocampal neurogenesis. Moreover, the experimental timeline and design requires a close examination.

The increasingly recognized heterogeneity of the astrocyte populations in the central nervous system (CNS) is reviewed elsewhere (Escartin et al., 2021). This heterogeneity calls for discrimination and specificity, particularly when assessing the implications of the ablation of GFAP+ cells in the CNS. AD etiology adds multiple layers of complexity to this assessment (González-Reyes et al., 2017). A wealth of information describes the altered phenotype of astrocytes in AD (for review, see Garwood et al., 2017). We provide several examples of current literature: The phenotype of astrocytes, rather than their numbers, changes in AD patients. In addition, the astrocytic L-serine pathway, generating the co-agonist of NMDAR required for synaptic plasticity, is defective in AD patients and mice. Another study observed that the inhibitory gliotransmitter γ-amino-butyric acid (GABA) and monoamine oxidase-B (Maob) are significantly -increased in reactive astrocytes in postmortem AD brains and in the dentate gyrus of FAD mice. Inhibiting GABA release from reactive astrocytes restored impaired spike probability, synaptic plasticity, and learning and memory in these mice. Isolation of astrocytes from APPswe/PS1ΔE9 mice revealed proinflammatory phenotypes, displaying reduced expression of genes involved in neuronal communication and support. These datasets were remarkably comparable in FAD mouse models and human AD brains. Single-nuclei RNA-seq analysis of astrocyte gene networks associated with AD pathology in 5XFAD and human AD revealed weakened metabolic coordination with neurons. Thus, in summary, eliminating these astrocytes may account for the improvement in the GFAP;TK;FAD mouse model observed by Zhang et al.

As mentioned above, the phenotype of astrocytes is not uniform, neither in the healthy brain nor in the AD one (Garwood et al., 2017). Examination of astroglial phenotypes in the 3XTg-AD mouse model revealed that some difference may be attributed to their proximity to amyloid plaques, which may account for the progressive disruption of neural networks and neurotransmitter imbalance. Another study describes hyperactivity and abnormal calcium waves in astrocytes in FAD mice. Numerous reports provide evidence for the mechanism by which astrocytic phenotype in AD drives pathogenesis. For example, astrocytes expressing the APOε4 isoform display impaired Aβ

uptake, cholesterol accumulation, and autophagy. Interestingly, converting APOε4 into APOε3 in these cells was sufficient to attenuate AD pathology. Another insight into the mechanism by which reactive astrocytes may accelerate pathology in AD comes from a study suggesting that the expression of α1-antichymotrypsin in astrocytes increases plaque burden and compromises presynaptic markers in the dentate gyrus in mice expressing human APP. Another study suggests that activated microglia induce a reactive neurotoxic phenotype of astrocytes. Lastly, a recent study reported that reactive astrocytes were implicated in microhemorrhage and were potentially a main culprit in causing cerebral amyloid angiopathy in AD models.

In support of that, studies examining therapies for AD suggest that targeting inflammatory pathways in astrocytes in AD significantly attenuates pathology. For example, interfering with the calcineurin/NFAT (nuclear factor of activated T cells) signaling pathway specifically in astrocytes ameliorates pathology and improves synaptic and cognitive function in FAD mice. Likewise, inhibition of the transcription factor signal transducer and activator of transcription 3 (Stat3), a canonical inducer of astrogliosis activated in AD patients and mouse models, reduced plaque pathology, upregulated amyloid clearance pathways, and enhanced synaptic and cognitive activity. Inhibiting the P2Y1 purinoreceptor (P2Y1R) pathway in astrocytes, a contributor to neuronal-glial network dysfunction, in APPswe/PS1ΔE9 mice, reduced astrocyte hyperactivity and preserved spatial learning and memory.

Taken together, the examples above are merely a small portion of mounting evidence implicating astrocytes in neuroinflammation in AD and supporting the notion that astrocytes play a major role in neurotoxicity, exacerbation of neuroinflammation, and acceleration of amyloid and tau pathology. Thus, reduced astrocyte disease phenotype has been an overarching goal for the amelioration of AD pathology and cognitive impairment. It is conceivable that Zhang and colleagues' findings could be explained by the reduction of neuroinflammation in their mouse model. The authors argued against this possibility and claimed that proliferative astrocytes and microglia are barely observed in the hippocampus of APP/PS1 and hAPP-J20 mice at 2–4 months of age. However, several studies suggested otherwise and reported significant astrocytic and microglial activation and neuroinflammation in APP/PS1 mice as young as 3 and 4 months of age . RNA-seq data from APP/PS1 animals of different ages have shown an increase in microglial and astroglial accumulation starting from 2 months of age, in comparison to wild-type animals. Zhang et al. did not quantify or characterize GFAP+ cells in their experimental group. Instead, they cited others' work. Overall, a thorough quantification of astrocytes and characterization of their phenotype in their model is lacking.

In essence, by abolishing astrocytes and/or microglia, either genetically or pharmacologically at 4 months of age, Zhang et al. eliminated major drivers of pathology, which would have escalated as a function of time, in these mice. In contrast, in healthy physiological conditions, astrocytes were recently shown to play an important role in remote memory formation (Kol et al., 2020). Thus, the effect of ablating GFAP+ cells in FAD mice may have vastly different implications than ablating these cells in wild-type mice. In another line of reasoning Zhang and colleagues suggest that they treated APP/PS1 with valganciclovir to control for drug side effects. However, this control does not address the effect of eliminating proliferating GFAP+ cells.

Another noteworthy experimental detail in the Zhang study is related to the timeline of treatment. The authors treated the APPswe/PS1ΔE9 mice with valganciclovir at 2.5 months of age, for 1 month. Then, mice were put on a normal diet. Behavioral testing was performed 4.5 months later, and amyloid quantification was performed 5.5 months post valganciclovir. One would expect that following this lengthy period, levels of neurogenesis would recover, at least in part. Indeed, the authors reported what seems like a significant increase in the number of DCX+ cells between 4 and 9 months of age in valganciclovir-treated GFAP;TK;APPswe/PS1ΔE9 mice. Furthermore, synaptic functions were restored after 6 weeks from the removal of valganciclovir. Thus, any behavioral effects cannot be attributed to the depletion of neurogenesis. Clearly, a very important assessment lacking in this study is the number of new neurons at every age point. Significant recovery in DCX+ cells in the 4–9 months age groups evidently indicates possible recovery in the number of new mature neurons too, which might be a causal factor in cognitive improvement. Along these lines, the attenuation of Calbindin shown (Zhang et al.'s Figure S7A) may not be the result of depletion in neurogenesis but of its recovery. In both approaches, namely, the TK:GFAP and the MAM treatment, quantification of levels of neurogenesis at the time of behavior was not provided. Of note, previous studies suggest that the dosage of MAM used in this study (7 mg/kg) was insufficiently potent to block neurogenesis (Dupret et al., 2005). Finally, it was not clear whether the authors used males, females, or both, an important factor that affects the onset of pathology and its progression in AD. In summary, we propose that these results cannot be exclusively interpreted as the effect of ablating hippocampal neurogenesis on FAD pathology and cognition.

Understanding the role(s) of adult hippocampal neurogenesis in AD and related dementia is important as it offers an insight into dentate gyrus and hippocampal function as they are related to memory failure in AD. However, we believe that it is pertinent to consider the experimental

models and the implications of their usage in order to avoid confusion in the field.

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