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

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Adult hippocampal neurogenesis (AHN) is impaired in mouse models of Alzheimer's disease (AD) and AD patients (Krezymon et al., 2013; Moreno-Jiménez et al., 2019; Sun et al., 2009; Toda et al., 2019). An obvious assumption, therefore, is that enhancing AHN will ameliorate, while inhibiting AHN will exacerbate, the pathology and memory deficits in AD. Recently, we examined the effects of inhibiting AHN on synaptic and cognitive functions in AD mice by using two AD models with two approaches to inhibit AHN. Our data suggest that inhibiting AHN improved synaptic and cognitive functions in AD mice (Zhang et al., 2021). In a previous study, Hollands et al. reported that depletion of adult neurogenesis exacerbates cognitive deficits in AD by compromising hippocampal inhibition (Hollands et al., 2017). In this issue of *Stem Cell Reports*, they've written a letter raising some points regarding the interpretation of some results in our paper (Phan et al., 2021). We thank the authors of this letter for taking time to look through our data. After carefully reading the letter with great interest, we want to discuss the points raised in the letter.

Phan et al. mentioned that the approaches (GFAP-TK plus GCV treatment and MAM) we used to delete adult neural stem cells (aNSCs) were not specific. We understood this point at the beginning of our study. Actually, to our knowledge, approaches that specifically delete/inhibit AHN are unavailable so far. That is exactly why we applied different methods to delete the aNSCs in our study. In their own paper, they showed that their approach (Nestin-δ-HSV-TK with valganciclovir) not only inhibited neurogenesis but also affected the number of glia in the hippocampus of APP/PS1 mice (Hollands et al., 2017). Reactive astrocytes express Nestin (Clarke et al., 1994). However, in their paper, they did not assess the effect of their approach on the number and phenotypes of astrocytes in APP/PS1 mice. They started to delete adult neurogenesis in mice right after weaning. However, we believe that mice at this age (right after weaning) are too young to be called adult.

Phan et al. provided a great number of references to demonstrate that astrocytes are important in neuroinflammation in AD and that targeting astrocytes/inflammation affects AD pathology and memory (2–5 paragraphs in their letter). We are very much aware of this connection and totally agree with that. Phan et al. then implied that the improved cognition in AD mice reported in our study was due to abolishing astrocytes and/or microglia. While this possibility could not be completely excluded, we believe it is unlikely based on the following considerations/evidence: both GFAP-TK/GCV and MAM treatment may kill the proliferating glial cells, and ganciclovir (GCV) treatment alone was also reported to inhibit the activation of microglia; however, in our studies, TK^+ mice were treated with GCV at the age of 2–4 months when proliferative astrocytes and microglia are barely observed in the hippocampus for both APP/PS1 and hAPP-J20 mice; both TK^- and TK^+ mice of APP/PS1 and hAPP-J20 were treated with GCV; hAPP-J20 mice were treated with MAM at the age of 4.5–5 months when gliosis is minimal; and furthermore, our extensive analysis showed that the two approaches we used did not affect both the number and morphology of astrocytes and microglia in the hippocampus of WT and AD mice at different time points (Figure S2 and Figure S4 in our paper). Phan et al. stated in their letter that several studies reported significant astrocytic and microglial activation, and neuroinflammation in APP/PS1 animals as young as 3 and 4 months old. However, no references supporting this claim were provided.

Phan et al. wrote in their letter that, "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." Actually, as mentioned above, we did analyze the number and morphology of microglia ($Iba1^+$) and astrocytes ($GFAP^+$ or $ALDH1L1^+$) in the hippocampus of both APP/PS1 and hAPP-J20 mice with different treatments and at different time points. Our results showed that GFAP-TK/GCV or MAM did not affect the number and morphology of microglia and astrocytes in the hippocampus of APP/PS1 or hAPP-J20 mice (Figure S2 and Figure S4 in our paper).

Therefore, we disagree with the claim by Phan et al. in their letter that, "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." While we do not want to exclude any other possibilities, we still believe it is unlikely that reduced gliosis accounts for the attenuated deficits of synaptic and cognitive functions of AD models in our study.

Indeed, it is a concern that there was a lengthy period of time following the GCV treatment and before the behavioral tests in APP/PS1 mice. Many things could be happening during that period of time, which made the explanation of the behavioral data in APP/PS1 mice complicated. However, we believe that the claim by Phan et al. that, "Thus, any behavioral effects cannot be attributed to the depletion of neurogenesis" is inaccurate. Depletion of AHN may not be the only factor accounting for the improved memory in APP/PS1 mice; there is definitely no reason to completely exclude the effects of AHN ablation on improved memory in APP/PS1 mice. Cho et al. reported previously that ablating adult neurogenesis in Nestin-TK mice treated with GCV for 4 weeks led to long-term (more than 40 weeks) suppression of spontaneous recurrent seizures (SRS) (Cho et al., 2015), suggesting that the effects of deleting AHN could last for a long period of time.

On the other hand, it is very unlikely that recovery of newborn neurons is a causal factor in cognitive improvements of APP/PS1 mice with AHN deletion. Even if there were a recovery of new neurons following the GCV treatment (we are not sure about that in our case because we do not want to compare the number of DCX^+ cells directly between 4- and 9-month-old mice; the staining was done at different times), it did not reach the level of DCX^+ cells in APP/PS1 mice without deletion of AHN. Then, if the recovery of neurogenesis is the causal factor, how is it possible that the memory was better in APP/PS1 mice with AHN deletion than APP/PS1 mice without AHN deletion?

This concern is actually another reason why we tried to evaluate the effects of inhibiting AHN on pathology and memory of AD mice in different models, and with different approaches. As shown in Figure 7I in our paper (Zhang et al., 2021), deleting AHN with GFAP-TK/GCV improved memory in hAPP-J20 mice as well, and there was only a short period of time following the GCV treatment before behavioral tests. This effect was also in line with the results of MAM treatment in hAPP-J20 mice as shown in Figure 3 in our paper.

Phan et al. mentioned that, "Clearly, a very important assessment lacking in this study is the number of new neurons at every age point." As shown in Figure 2 and Figure 3 in our paper, we quantified the number of DCX^+ cells in the hippocampus of APP/PS1 mice at 4, 6, and 9 months old and in hAPP-J20 mice at around 6 months old. These time points include the times for behavioral tests and electrophysiological recordings.

As Phan et al. pointed out, some references indicated that the dosage of MAM (7 mg/kg) was insufficiently potent to block neurogenesis. Hsiao et al., however, showed that this dosage sufficiently reduced AHN (Hsiao et al., 2014), which is consistent with our data as shown in Figure 2 in our paper.

Phan et al. wrote in their letter that, "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." While they made a good point regarding the sex of animals and the AD pathology, we did mention, "mice of both sexes were used for experiments" in our supplemental experimental procedures.

In summary, our data suggest that deleting AHN improves synaptic and cognitive functions in AD mice. Clearly, however, more studies are needed to further appreciate the effects and the underlying mechanisms of modulating AHN (either enhancing or inhibiting) on AD pathology. As we mentioned in our paper, we believe that the effects of deleting abnormal new neurons and enhancing healthy neurogenesis on AD are not mutually exclusive.

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