Peer Review File

Manuscript Title: Young CSF restores oligodendrogenesis and memory in aged mice via Fgf17

Editorial Note: Parts of this Peer Review File have been redacted as indicated to maintain the confidentiality of unpublished data.

Reviewer Comments & Author Rebuttals

Reviewer Reports on the Initial Version:

Referees' comments:

Referee #1 (Remarks to the Author):

Iram et al. “FGF17 in cerebrospinal fluid boosts hippocampal oligodendrocyte growth and memory consolidation in aged mice”

This is as very interesting paper by Wyss-Coray Lab, reporting a novel aspect of brain aging: the authors show that cerebrospinal fluid, obtained from 2 months old “young” mice and infused into the ventricles of 18 months old “aged” mice preferentially stimulates the proliferation and differentiation of oligodendrocyte precursor cells (OPC). This effect is associated with the upregulation of several genes, including that of SRF (serum response factor), previously rather known as a neuronal transcription factor, and various established SRF target genes (but also upstream regulatory factors - that should be explained). A comparison of these data with published CSF proteome data bases lead the authors to the identification of FGF17 as a possible candidate that mediates the effect of young CSF in the original experiment. They go on and demonstrate that indeed FGF17 has the right properties (in vitro and in vivo) to stimulate OPC proliferation and differentiation, and to show improved outcome of behavioral experiments in aged mice as shown in the original experiments with “young” CSF infusions.
Taken together the paper comprises a set of remarkable findings that shed light on the role of oligodendrocytes on declining brain function at advanced age. Moreover it offers interesting candidates and pharmacological targets for future interventions.

I really like the study and even think that the second part by itself (concerning FGF17) would have made a nice story. That said, the “red line” of the paper as it stands (in continuation of a long line of research by the Wyss-Coray lab) has also a few remarkable gaps that should be closed.

1) It would be important to know how the perfusion process of “young” into “old” CSF has worked. What are the relative volume ratios and what is the predicted dilution factor? Did the authors perform dye or tracer injections that could yield that information?
2) From Fig. 1 the authors conclude “re-exposure of the aged brain to young factors induces OPC proliferation and differentiation“. While the first conclusion is sound the second requires more experimental proof. An increase in MBP-staining may be by a response of preexisting OL to CSF. Functionally, this would be a very different scenario from CSF-induced de-novo myelination of axons.

PDGFRalpha-CreER reporter induced by Tamoxifen should show whether the labeled OPCs differentiate and subsequently de-novo-myelinate previously naked axons. That is the standard in the field.
3) I think that aCSF, without any growth factors, is not a good control for real CSF. Why didn't the authors use "aged" CSF from other mice? Of course one could argue it doesn't matter because we found what we were looking for, but the aging aspect was an important part of the story.
4) That is why I am missing the information whether FGF17 is even contained at higher concentrations in young versus old CSF. It was made implicit, but not really shown. Also unclear is the FGF17 concentration in CSF (old or young) in comparison to what was used in in vitro experimebts (40ng/ml).
5) There is quite some literature on FGFR signaling in the oligodendrocyte lineage that should be cited/discussed.
6) Line 127: SRF target genes "both upstream and downstream". Please clarify
7) What are the effects (if any) on neuronal SRF? That may be important because neuronal SRF is required for myelination (I believe still the only known neuronal gene identified for the CNS).
8) How do the authors envision that the "transient" effects of SRF induction can relate to the chronic exposure of CSF to OPC in vivo. And why would aging oligodendrocytes compromise cognitive functions?
9) Strictly speaking, the authors have made two interesting observations on brain/CSF aging and FGF17/SRF signaling, both with causal explanations – however the connection that reduced FGF17 mediates the age-dependent decline of cognition remains (an important) correlation. Perhaps worth discussing.

10) The frequent references to "adaptive myelination" are also problematic. That is because the concept of Gibson et al (and others) rests on the specificity that some electrically more active components in the circuitry become myelinated in the contrast to other less active neurons. In their model, this specificity is essential to optimize circuit performance. In contrast "young" CSF would be a global factor in the brain and act rather unspecifically (possibly even counterproductive by increasing an OPC "noise"). That should be also discussed.

Minor:
11) On line 109 it must read "OPC" (not oligodendrocyte)
12) Page 7: Reference to Figure 4j,k is missing on
13) The title is somewhat misleading, as the bulk of the presented data is on OPC and not on mature OL.

Referee #2 (Remarks to the Author):

The manuscript by Iram et al,. seeks to test if young CSF improves brain function in aging mice. While the beneficial effects of CSF have previously been investigated in the context of brain development and adult neurogenesis, contributions to memory function have remained unknown. Here, the authors found that infusing young CSF directly into aged brains improved memory function in a foot shock memory paradigm. RNAseq analyses of hippocampal tissue uncovered oligodendrocyte associated genes as having the greatest changes in expression. Young CSF boosted OPC proliferation

and differentiation. SRF was identified as a key transcription factor driving these responses, and Fgf17 in CSF was sufficient to induce OPC proliferation and long-term memory consolidation in aged mice. This is an interesting study that has potentially broad impact on basic science and neurology and is likely relevant to the readership of Nature. The following points are important to address in a revision:

Major comments:

1. Naïve (un-operated) or sham controls for the foot shock experiments would be helpful to include in Figure 1.

2. Where does Fgf17 in young CSF originate? An extended figure comments on cortex and choroid plexus epithelail cells from human tissues potential sources, but each mentioned seem to have low expression. Is this confirmed, and what are the relevant sources in mice? Do Fgf17 KO mice show abnormalities in hippocampal myelination and learning and memory tasks?

3. Does Fgf17 account for full beneficial effects of young CSF infused into aging brain? The data, as presented, suggest this as a possible interpretation. Testing Fgf17-depleted CSF or CSF from Fgf17 KO mouse is an important experiment to address this point. Clarifying how the concentrations of infused Fgf17 (in aging) vs. endogenous CSF levels of Fgf17 (in young adulthood) compare would be informative.

4. Delivery of Fgf17 to affected cells in the hippocampus. In other CNS cell types, the cells binding factors originating in the CSF do so via primary cilia or by directly contacting the CSF. How are the OPC precursors positioned throughout the hippocampus and what is the mechanism / process by which CSF Fgf17 reaches them? It seems this process may need to be distinct from other parts of the brain, where proliferation in response to young CSF was not observed in the present study, or is that due to other factors (progenitor types, receptor expression, gradients of Fgf17).

5. Scholarship: The last sentence of the first paragraph states, “We hypothesized that intracerebroventricular (i.c.v.) administration of young CSF to aged mice will have rejuvenating effects on the brain (Fig. 1a).” One could argue that that this hypothesis has been previously tested and published in part by Silva-Vargas et al in Cell Stem Cell (not referenced in this paragraph, but included later in the manuscript). The concept of heterochronic CSF experiments to demonstrate age-specific CSF functions was also first demonstrated by Lehtinen et al., in Neuron (not referenced in this manuscript at all).

Minor comments:

1. Fgf17 is implicated in vascular development and growth. Is there evidence of vascular sprouting in CSF infused conditions that could contribute to observed effects in hippocampus?

2. How long do the beneficial effects of acute Fgf17 infusion last – can it be sustained longer term, or will OPC pool deplete?

Referee #3 (Remarks to the Author):

Iram and colleagues present intriguing findings that Fgf17 in young mouse CSF promotes oligodendrocyte proliferation and functional improvements to memory in aged mice. The CSF infusions from young mice into old mice is a highly novel experimental approach and they present evidence that CSF from young mice may improve age-related hippocampal phenotypes. These findings further emphasize the groundbreaking observations from previous studies that factors in young mice can reverse aging phenotypes in old mice. While Iram and colleagues offer intriguing new insights, the data presented fall short of fully supporting the conclusions. Additionally, the identification Fgf17 is not via a systematic analysis and is somewhat independent of the preceding findings making it seem a bit convoluted especially considering it is highlighted in the title of the manuscript. The authors should consider to more fully characterize the uniqueness and selectivity of the Fgf17 to the phenotype or alternatively focus more on SRF responses in the title of the manuscript. Specific comments are provided below.

Major Points

1. The memory recall improvements in Fig. 1b are significant but the two groups have different sample numbers, which isn't explained. To confirm this phenotype, it would be good to see additional behavioral assays associated with hippocampal learning and memory. Also, the study assumes that beneficial factor(s) are present in the YM-CSF and is responsible the hippocampal phenotypes. However, experiments to rule out that YM-CSF dilutes detrimental factor(s) in aged-CSF aren't included. This is particularly important considering the manuscript concludes by finding a good factor in YM-CSF.

2. The paper's foundation rests on the fact that oligodendrocytes are the cell-type most affected by infusion of young CSF, but this claim lacks supporting data. From the initial RNA sequencing experiment in Figure 1 c, the authors show a bar plot of the -log10 q-value of differentially expressed genes, comparing oligodendrocytes to mural cells, endothelial cells, and astrocytes. They claim that oligodendrocytes are more affected due to the higher average q-value of their cell-type specific genes. However, this conclusion is hard to rely on when many other major brain cell-types are lacking. It is unclear why the authors chose to only compare these four cell types, especially when there is evidence that vascular cells and mural cells are likely underrepresented in RNA sequencing. To make the case for oligodendrocytes being the most highly affected cell type, the authors should provide differential gene expression analysis for each cell type, including neurons, microglia, and oligodendrocyte precursors, including the number of cells captured per cell type and number of reads per cell. It is also unclear from the text whether this experiment was bulk or single-cell RNA sequencing.

3. The images in Figure 1e are very striking showing a high number of EdU/Pdgfra double positive cells in YH-CSF treated mice. However, the images in extended figure 2c depict a high number of Edu+ Pdgfra- cells in the hippocampus. Quantifying the number of Edu+, Pdgfra+ vs Edu+ Pdgfra- cells would provide further insight into the specificity of the YH-CSF to the oligodendrocyte lineages. Likewise, what are the identity of the Edu+ Pdgfra- cells and is there evidence of these types of cellular response in the transcriptomics data presented in Figure 1c.

4. The data suggests that in response to YM-CSF OPCs proliferate and differentiate into mature oligodendrocytes. Do these oligodendrocytes increase myelination in the hippocampus or do other mechanisms potentially account for improved memory consolidation? The example images are from two different bregma; the YM-CSF image is more posterior than the aCSF image. The dentate gyrus region of the aCSF example image appears brighter in the aCSF image than in the YM-CSF image. It is also particularly difficult to judge the increase or decrease of MBP "intensity" from a rendered image, without seeing the raw confocal images. More direct analysis of the functional outcome of increased Mbp staining such as TEM ultrastructural imaging would provide further insight into mechanisms by which OPCs lead to improved memory. Likewise, do these changes occur in conjunction with increased hippocampal neuronal electrophysiological and synaptic connectivity.

5. Figure 2d depicts DEGs upregulated in SLAMseq experiment and highlights several genes in red including Tagln, Ccn2, and Acta2. While these genes are cytoskeleton and actin genes they are also known markers of smooth muscle cells. It would be important to complement this analysis with IHC showing co-staining for these proteins with an OPC marker such as Pdgfra. This would rule out the possibility of this response coming from contaminating cell-types. Indeed, as shown in Figure 3a Srf is not selectively expressed in Pdgfra cells. Additionally, it is important to validate these in vitro findings in vivo in mice administered YH-CSF. Do OPCs in vivo show increased phalloidin and actin staining in response to YH-CSF? What are the upregulated SRF targets shown in Figure 3f? Do any overlap with those shown in 2d?

6. The SRF knock out experiments in Figure 2f and g suggest that SRF influences OPC proliferation in vitro. In parallel, the authors should delete Srf in OPCs in the mouse brain and assay its effect on proliferation to directly test the effect on OPC in vivo.

8. Figure 3f: why would target genes of Srf be both up and down regulated in each condition, if Srf expression is up, and the target genes are all upregulated in the volcano plot in Figure 2? Providing the names on the downregulated genes in the figure could provide further insight here.

9. Figure 4 introduces Fgf8 and 17 and demonstrates that Fgf17 can improve cognitive outcomes in aged mice. While this observation is interesting, the data falls well short of demonstrating that Fgf17 is responsible for all the previous phenotypes and cognitive improvements ascribed to YM-CSF. Does YM-CSF contain more Fgf17 than aged-CSF? If Fgf17 is immunodepleted from CSF is the OPC proliferation, increased MBP and cognitive improvements from YM-CSF lost? Does Fgf17 increase myelination in vivo? If so, does it affect neuronal activity? Determining whether the YM-CSF response is mediated in-part or wholly by Fgf17 will be an important addition to this paper.

Minor comments

1. In Figure 2, the authors argue that Srf drives the OPC proliferation caused by YM-CSF infusion. They support this with an experiment using primary OPC cultures from SRF floxed mice, and infecting with either Cre-GFP or truncated Cre-GFP. They argue that knocking out SRF in OPCs reduces proliferation (Figure 2g), but the representative images or data of the BRDU staining is not shown. It is impossible to understand the data without seeing representative examples. Again, sample sizes of three per condition are used, which is not appropriately high enough to rule out the possibility that the comparison is underpowered.

2. In Figure 3c, it would be helpful to explain why the aged and young mice do not cluster together on the dendrogram, especially since a main claim of the paper is that ageing specifically affects the oligodendrocyte cell population.

3. Figure 3e, it is unclear which region of the hippocampus is imaged, or whether this is the same region between the two slices. Inclusion of a nuclear stain would be helpful here. Also, in the representative image provided for the YM-CSF condition, there are seven arrows pointing to EDU+ Pdgfra+ cells, and perhaps three EDU- Pdgfra+ cells. This should put the percentage of EDU-positive OPCs at around 70%, but in the quantification, the percentage ranges from ~2 to ~18, making it unclear how the authors analyzed this data. Similarly, in Figure 3i, the authors should plot the % of BRDU+ Pdgfra+ cells, without normalization to the aCSF condition. In the example images of the highest CSF dose, there are approx. 16 BRDU+ nuclei in the aCSF condition, and 20 BRDU+ nuclei in the YH-CSF condition, making the increase about 1.25, assuming the cells are plated at equal density. Yet the quantification suggests an average change of greater than 2x, which is confusing.

4. In Figure 3k, OPCs are annotated according to different morphologies (ie “star”, “partial”, “arborized”) but what these annotations mean, or how they were judged, is not clear. Staining for more definitive markers such as MBP would be more conclusive.

Author Rebuttals to Initial Comments:

We thank the Reviewers for their constructive comments which, we believe, helped us to greatly improve the manuscript. Our responses are shown in blue.

Referees' comments:

Referee #1 (Remarks to the Author):

Iram et al. “FGF17 in cerebrospinal fluid boosts hippocampal oligodendrocyte growth and memory consolidation in aged mice”

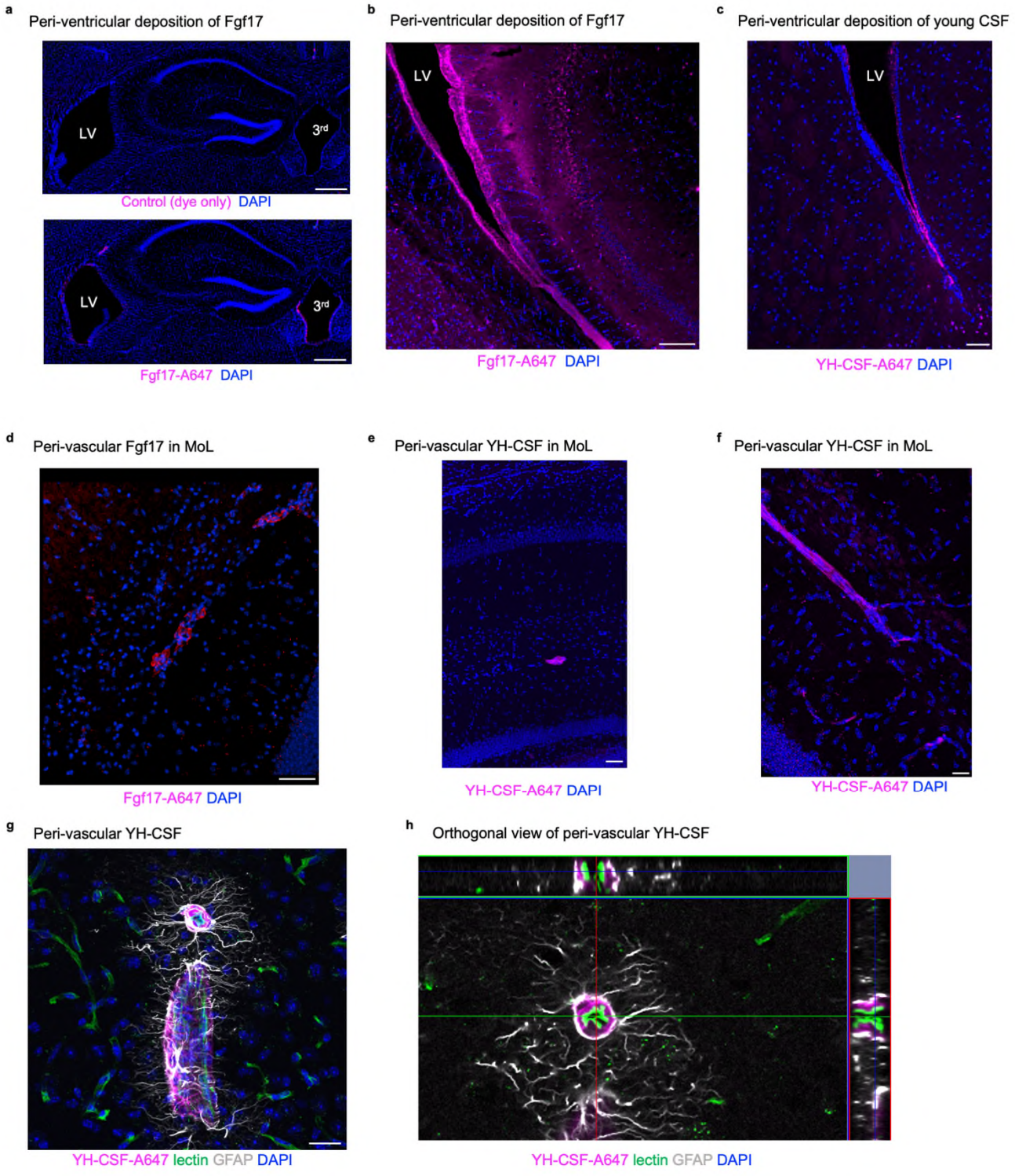
This is as very interesting paper by Wyss-Coray Lab, reporting a novel aspect of brain aging: the authors show that cerebrospinal fluid, obtained from 2 months old “young” mice and infused into the ventricles of 18 months old “aged” mice preferentially stimulates the proliferation and differentiation of oligodendrocyte precursor cells (OPC). This effect is associated with the upregulation of several genes, including that of SRF (serum response factor), previously rather known as a neuronal transcription factor, and various established SRF target genes (but also upstream regulatory factors - that should be explained). A comparison of these data with published CSF proteome data bases lead the authors to the identification of FGF17 as a possible candidate that mediates the effect of young CSF in the original experiment. They go on and demonstrate that indeed FGF17 has the right properties (in vitro and in vivo) to stimulate OPC proliferation and differentiation, and to show improved outcome of behavioral experiments in aged mice as shown in the original experiments with “young” CSF infusions.
Taken together the paper comprises a set of remarkable findings that shed light on the role of oligodendrocytes on declining brain function at advanced age. Moreover it offers interesting candidates and pharmacological targets for future interventions.

Thank you very much! We greatly appreciate these encouraging comments.

I really like the study and even think that the second part by itself (concerning FGF17) would have made a nice story. That said, the “red line” of the paper as it stands (in continuation of a long line of research by the Wyss-Coray lab) has also a few remarkable gaps that should be closed.

1) It would be important to know how the perfusion process of “young” into “old” CSF has worked. What are the relative volume ratios and what is the predicted dilution factor? Did the authors perform dye or tracer injections that could yield that information?

We thank the reviewer for the suggestion to explore the perfusion of young CSF into the parenchyma. We have calculated the approximate dilution of “endogenous” CSF as a result of young CSF infusion. Based on the literature, the mouse produces 0.32 ul of CSF per minute [1] or 460 ul/day and the minipumps we have been using release 12 ul of young CSF per day. This results in a dilution of endogenous “aged” CSF by roughly 2.6% (1:38). In addition, we performed injections of fluorescently labeled CSF and Fgf17, both of which showing strong perivascular staining in hippocampal blood vessels (mostly in the molecular layer) and thus indicating perfusion through the hippocampus (Reviewer Fig.1).

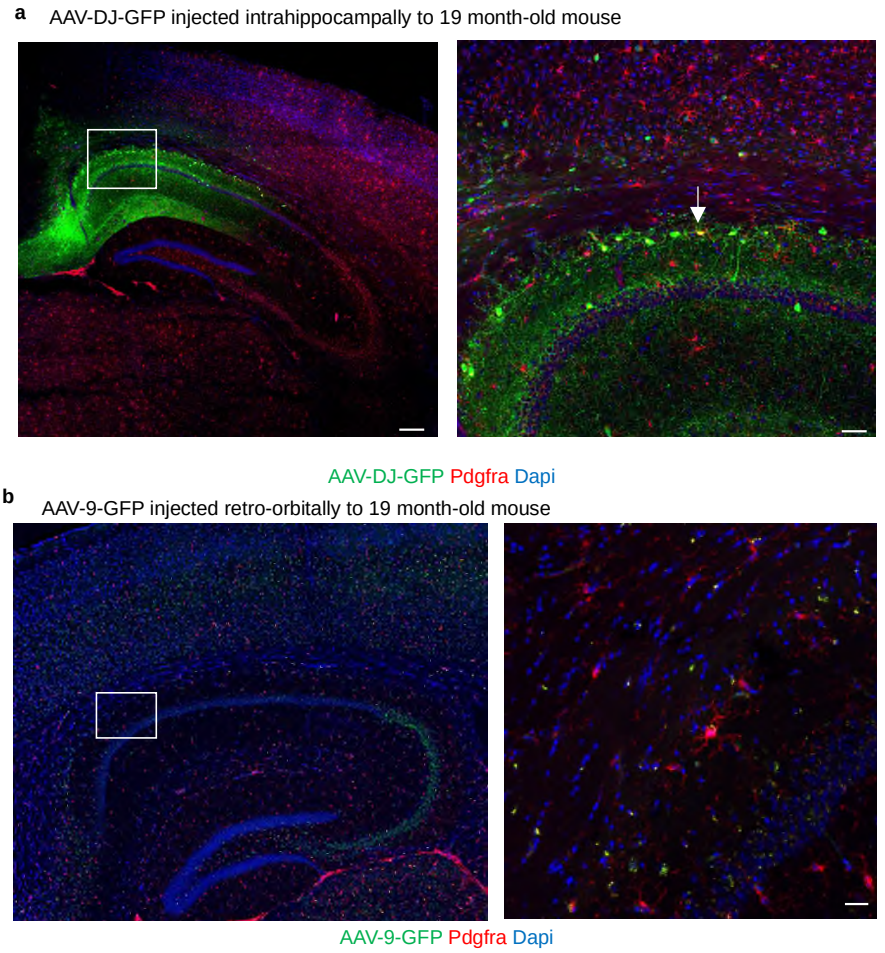


Reviewer Figure 1 –Perfusion of labeled YH-CSF and mouse Fgf17 to the brain parenchyma.
a, Deposition of labeled Fgf17 on ventricular walls 3 hours post ICV acute injection. Scale bar, 300μm.
b, Perfusion of labeled Fgf17 from lateral ventricle to the hippocampus. Scale bar, 100μm.
c, Deposition of labeled YH-CSF on lateral ventricle walls 2 hours post ICV acute injection. Scale bar, 100μm.
d, Labeled Fgf17 in perivascular spaces in the molecular layer of the hippocampus. Scale bar, 50μm.
e, Labeled YH-CSF in perivascular spaces in the molecular layer of the hippocampus. Scale bar, 50μm.
f, Labeled YH-CSF in perivascular spaces in the molecular layer of the hippocampus. Scale bar, 20μm.

g, Cross section showing YH-CSF in perivascular space, in between the vessel (green) and astrocyte endfeet (white). Scale bar, 20μm.
h, Orthogonal slice of YH-CSF (magenta) in perivascular space, in between the vessel (green) and astrocyte endfeet (white). Scale bar, 20μm.

2) From Fig. 1 the authors conclude "re-exposure of the aged brain to young factors induces OPC proliferation and differentiation". While the first conclusion is sound the second requires more experimental proof. An increase in MBP-staining may be by a response of preexisting OL to CSF. Functionally, this would be a very different scenario from CSF-induced de-novo myelination of axons. PDGFRalpha-CreER reporter induced by Tamoxifen should show whether the labeled OPCs differentiate and subsequently de-novo-myelinate previously naked axons. That is the standard in the field.

We appreciate that it will be important to determine whether young CSF and Fgf17 infusions drive ne-novo myelination or affect pre-existing oligodendrocytes. We agree with the reviewer that young CSF infusion to aged PDGFRalpha-CreER reporter mice would be an ideal experiment. However, our experimental paradigm would require us to age the mice to at least 18 months of age following acquisition and breeding of the mice. We attempted, instead, to deliver a fluorescent reporter to OPCs using lentivirus, AAV-DJ (AAV DJ-CMV-hrGFP, intra-hippocampal injection), AAV-PhP.eB (AAV PHP.eB-CMV hrGFP, intra-hippocampal injection) and AAV-9 (AAV 9/731-CMV-hrGFP, retro-orbitally) but failed to detect expression by OPCs with any of these vectors (Reviewer Fig. 2). We used a CMV promoter initially because it yields very good expression in OPCs infected with CRE *ex vivo* (Fig. 2h) but the lack of OPC infection *in vivo* discouraged us from pursuing further experiments with OPC specific promoters.
As alternative, we quantified the number of myelinated axons in the molecular layer of the hippocampus by TEM and found an increase in their numbers with no effect on the g-ratio (Fig. 1k and Extended figure 3). We believe that this lends additional support to the notion that young CSF promotes de-novo myelination rather than modifies current hippocampal myelin. That said, we cannot exclude the possibility that mature oligodendrocytes are affected and have expanded our discussion (lines 205-210): "Furthermore, mature oligodendrocytes are critical for maintenance of axonal health and for regulation of neuronal function [2]. We cannot exclude the possibility that CSF proteins directly affect mature oligodendrocyte function with beneficial outcomes on neuronal health and cognitive function. Myelin plasticity is emerging as an important mechanism in learning and memory [3] and the study of myelin dysfunction in aging related cognitive decline is gaining interest."



Reviewer figure 2- Attempts to infect OPCs *in vivo* in aged mice.
a, 19 months old mice were injected with AAV-DJ-GFP intra-hippocampally and infection was assessed 3 weeks later by co-localization of Pdgfrα (red) and GFP. Very few cells were double positive (arrowhead). Scale bar, 200 μm, insert, 50 μm
b, 19 months old mice were injected with AAV-9-GFP retro-orbitally and infection was assessed 3 weeks later by co-localization of Pdgfrα (red) and GFP. No GFP cells were detected. Scale bar, 200 μm, insert, 20 μm

3) I think that aCSF, without any growth factors, is not a good control for real CSF. Why didn't the authors use "aged" CSF from other mice? Of course one could argue it doesn't matter because we found what we were looking for, but the aging aspect was an important part of the story.

We concur with the reviewer's comment that aged CSF is a better control for young CSF. We initially used aCSF because the minimum volume to fill the smallest mini-osmotic pump is 120ul (pump reservoir + tube) which requires CSF collection from at least 15-20 mice for a single pump. Unfortunately, this would require at least 150 mice for one experiment, and we are unable to source that many aged mice. We have now included data from experiments using aged CSF in two alternative approaches:
a) Because human CSF is available in larger volumes, we were able to carry out a 6-day infusion with mini-osmotic pumps and compare the effects of young and aged healthy human CSF. Interestingly, young human CSF induced hippocampal OPC proliferation very similarly to young mouse CSF but aged human CSF did not promote proliferation (Fig. 1g-h).
b) Acute injection of young or aged CSF – this method requires only 3ul of CSF per mouse which is feasible with the limited amounts of aged mouse CSF. Hippocampal OPC nuclei were sorted 16 hours post injection of aCSF, young CSF and aged matched CSF (18

months) and we performed bulk RNAseq to compare gene signatures at the transcription level. When focusing on the initial list of oligodendrocyte genes that emerged from the hippocampus bulk RNAseq following a week of infusion, we found a similar increase in these genes as we saw in the chronic infusion paradigm as early as 16 hours post acute injection of young CSF but not following injection of aged CSF (Extended figure 1c).
Taken together, these two additional experiments provide a more direct comparison of the functional effects of young versus aged CSF in two species and support our claim that young CSF contains trophic factors for OPCs.

4) That is why I am missing the information whether FGF17 is even contained at higher concentrations in young versus old CSF. It was made implicit, but not really shown. Also unclear is the FGF17 concentration in CSF (old or young) in comparison to what was used in in vitro experimebts (40ng/ml).

We agree with the reviewer that measuring the levels of Fgf17 in mouse CSF is an important addition to this study. Here again, the low amounts of aged CSF are the main bottleneck for protein quantifications that are feasible. For example, a standard Fgf17 ELISA requires 100ul test solution per well in duplicates or triplicates which would require collection from dozens of aged mice (we tried diluting the CSF but it was under the detection limit). As an alternative, we measured young and aged CSF samples using the Somalogic platform but mouse Fgf17 was below the detection level. We also attempted, in a separate study, to compare the proteomes of young versus aged CSF by mass spectrometry but, again, out of 358 protein groups that were detectable in the abundance analysis between young and aged mouse CSF we failed to detect Fgf17 (Shuken et al. Nature Aging, In press).
As an alternative, to determine if Fgf17 might be regulated in an age dependent way, we decided to search for the cells producing it. We first consulted an existing human protein expression atlas (https://www.proteinatlas.org/ENSG00000158815-FGF17/tissue) and found Fgf17 is highly enriched in the brain (Extended data Fig. 11a). We next performed a meta-analysis of the Allen Brain Atlas mouse brain single cell smartseq dataset (https://portal.brain-map.org/atlases-and-data/rnaseq) which shows a subset of Fgf17 positive cells in the mouse cortex (Extended data Fig. 11b-d). Encouraged by these findings, we set out to compare Fgf17 mRNA and protein expression in young and old mouse brains in situ with RNAscope and immunofluorescence staining, respectively. Indeed, we found that in the young brain the highest expressing Fgf17 cells were cortical and hippocampal neurons. We quantified Fgf17 transcript and protein in young and aged mice and discovered that the expression of Fgf17 drops dramatically with age (Fig. 4f-g and extended data Fig. 11e-g).
In summary, while we couldn't measure Fgf17 protein directly in mouse CSF, our new analysis provides a more mechanistic explanation for the source of Fgf17 and its downregulation with age, as well as a potential therapeutic target for future studies.
With respect to the concentration of FGF17 used in our study we used the same range of concentrations routinely used for Fgf2 (bFgf) and other growth factors added to the OPC media [4].

5) There is quite some literature on FGFR signaling in the oligodendrocyte lineage that should be cited/discussed.

We thank the reviewer for this comment and have now included a short discussion paragraph on Fgfr signaling and more specifically Fgfr3 in oligodendrocytes (line 215-221): "Fgfr signaling is critical for oligodendrocyte development [5] with complex and diverse functions in disease processes such as demyelination and remyelination in Multiple Sclerosis [6]. Specifically, studies using Fgf3r-null mice show a delay in the terminal differentiation of pro-oligodendrocytes [7] and transient expression of Fgfr3 in subventricular zone progenitors drives oligodendrogenesis and promotes remyelination following a demyelinating injury [8]".

6) Line 127: SRF target genes “both upstream and downstream”. Please clarify

We apologize for the confusion and have removed this sentence.

7) What are the effects (if any) on neuronal SRF? That may be important because neuronal SRF is required for myelination (I believe still the only known neuronal gene identified for the CNS).

We thank the reviewer for this very important point. We have now included an analysis of Srf in the CA1 region by RNAscope in 2 experiments; (a) young and aged naïve mice, (b) aged mice acutely injected with aCSF or YM-CSF. We found that like OPCs, neuronal Srf is also downregulated with age but is not induced by young CSF infusion in aged mice. Moreover, while the effect on oligodendrocyte maturation in vivo could be mediated by SRF signaling in neurons in a non-cell autonomous manner, we believe that our in vitro studies in pure OPC cultures suggest that this is a direct effect of CSF proteins on OPCs.

8) How do the authors envision that the “transient” effects of SRF induction can relate to the chronic exposure of CSF to OPC in vivo. And why would aging oligodendrocytes compromise cognitive functions?

Many transcription factors and immediate early genes are expressed transiently but long enough to induce expression of downstream targets that carry on the functional effect. This was previously shown for SRF which is transiently activated by PDGF signaling[9,10] or BDNF signaling[11] and functionally impacting cytoskeletal rearrangements in neurons, muscle cells and other cell culture lines long after it is downregulated. Furthermore, in a positive control loop, SRF induces the expression of its own transcript, and this is what we used in our studies as a proxy for the activation of the pathway. However, depending on the stimulation, transcription binding co-factors and other regulatory elements, SRF might induce transcription of different sets of target genes. To our knowledge, this type of characterization has not previously been done in oligodendrocytes and is an interesting direction for future studies.

Regarding the reviewer’s second comment, myelination has recently emerged as an additional layer of network plasticity in learning and memory studies in rodents (see Reviewer table 1 for a list of recent studies causally linking oligodendrocytes to cognitive function). While most of these studies focus on the role of myelination in the young adult brain, we provide here one of the first studies establishing a link between OPCs and cognition in the old brain. Based on this growing body of literature and our own data we propose that with age, OPCs are deficient in responding adequately to proliferation and differentiation cues resulting in poor oligodendrogenesis and maintenance of myelination. To support that notion, we have now included a quantification of proliferating, EDU-Pdgfra+ OPCs in young and aged naïve mice which shows a stark decrease in hippocampal EDU-Pdgfra+ cells with age (Extended data figure 1h-i). Yet, the study of the role of oligodendrocytes in aging and neurodegeneration is in its infancy and there are many open questions regarding the mechanisms by which OPCs and mature oligodendrocytes regulate and modulate network function, and how these mechanisms change with aging and neurodegenerative disease.

9) Strictly speaking, the authors have made two interesting observations on brain/CSF aging and FGF17/SRF signaling, both with causal explanations – however the connection that reduced FGF17 mediates the age-dependent decline of cognition remains (an important) correlation. Perhaps worth discussing.

We agree that the connection between our two main observations was not as conclusive, and we provide now new data bringing them closer together. Specifically, we infused an anti-

Fgf17 blocking antibody ICV to young mice to test if the growth factor is necessary for normal memory function. We found that mice infused with anti-Fgf17, but not with control antibody, showed impaired performance in two hippocampal dependent cognitive tests (Y maze and contextual fear conditioning) and impaired neuronal plasticity (measured by lower c-fos levels in dentate gyrus granule cells) following behavioral tests (Fig. 4o-r).
In OPC cultures, the same concentration of anti-Fgf17 antibody also inhibits young CSF- and Fgf17-induced OPC proliferation. This indicates that the boost in proliferation is in part mediated by Fgf17 (Fig. 4s).
Taken together with the previous experiments showing that Fgf17 supplementation is sufficient to restore long term memory in aged mice, these additional necessity experiments provide a more extensive link between Fgf17 and cognitive function, in young and aged mice.

10) The frequent references to “adaptive myelination” are also problematic. That is because the concept of Gibson et al (and others) rests on the specificity that some electrically more active components in the circuitry become myelinated in the contrast to other less active neurons. In their model, this specificity is essential to optimize circuit performance. In contrast “young” CSF would be a global factor in the brain and act rather unspecifically (possibly even counterproductive by increasing an OPC "noise"). That should be also discussed.

We agree with the reviewer that activity dependent myelination occurs at very localized regions, and we tried to tone down the concept of “adaptive myelination”, remove the term in multiple locations, and discuss the concept in more detail. Intriguingly, recent studies show that multiple systemic manipulations (see Reviewer table 2 for details) affect the brain microenvironment to be globally more conducive to OPC proliferation and maturation with overall beneficial effects on regeneration following a demyelinating lesion and on cognitive function.

Minor:
11) On line 109 it must read “OPC” (not oligodendrocyte)
Corrected, thank you.
12) Page 7: Reference to Figure 4j,k is missing on
Corrected, thank you.
13) The title is somewhat misleading, as the bulk of the presented data is on OPC and not on mature OL.
We have changed the title to “Young CSF restores oligodendrogenesis and memory in aged mice via Fgf17”. We believe that the additional MBP and TEM analyses, along with the differentiation experiments in culture, support the claim that young CSF induces oligodendrogenesis which is commonly initiated by OPC proliferation.

Referee #2 (Remarks to the Author):

The manuscript by Iram et al,. seeks to test if young CSF improves brain function in aging mice. While the beneficial effects of CSF have previously been investigated in the context of brain development and adult neurogenesis, contributions to memory function have remained unknown. Here, the authors found that infusing young CSF directly into aged brains improved memory function in a foot shock memory paradigm. RNAseq analyses of hippocampal tissue uncovered oligodendrocyte associated genes as having the greatest changes in expression. Young CSF boosted OPC proliferation and differentiation. SRF was identified as a key transcription factor driving these responses, and Fgf17 in CSF was sufficient to induce OPC proliferation and long-term memory consolidation in aged mice. This is an interesting study that has potentially broad impact on basic science and neurology and is likely relevant to the readership of Nature. The following points are important to

address in a revision:

We greatly appreciate the reviewer's encouraging comments.

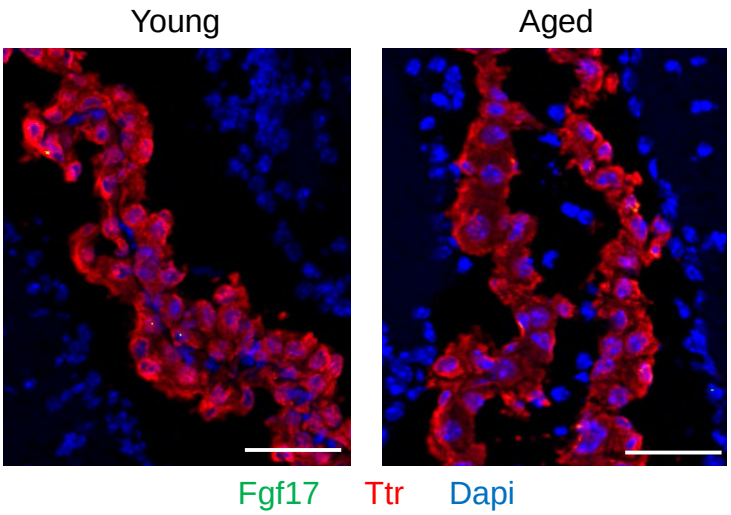
Major comments:

1. Naïve (un-operated) or sham controls for the foot shock experiments would be helpful to include in Figure 1.

We thank the reviewer for this comment. Unfortunately, in order to perform these behavioral studies we have to collect young CSF from 100-150 young mice depending on the cohort size. We therefore could not repeat the experiment to add the naïve control. Instead, and as detailed below, we included multiple additional controls that assess the cellular and molecular effects of young mouse or young human CSF on the aging hippocampus where multiple controls were included.

2. Where does Fgf17 in young CSF originate? An extended figure comments on cortex and choroid plexus epithelail cells from human tissues potential sources, but each mentioned seem to have low expression. Is this confirmed, and what are the relevant sources in mice? Do Fgf17 KO mice show abnormalities in hippocampal myelination and learning and memory tasks?

We agree with the reviewer that the source of Fgf17 is an important question. We first consulted an existing human protein expression atlas (https://www.proteinatlas.org/ENSG00000158815-FGF17/tissue) and found Fgf17 is highly enriched in the brain (Extended data Fig. 11a). We next performed a meta-analysis of the Allen Brain Atlas mouse brain single cell smartseq dataset (https://portal.brain-map.org/atlases-and-data/rnaseq) which shows a subset of Fgf17 positive cells in the mouse cortex (Extended data Fig. 11b-d). Encouraged by these findings, we set out to compare Fgf17 mRNA and protein expression in young and old mouse brains in situ with RNAscope and immunofluorescence staining, respectively. Indeed, we found that in the young brain the highest expressing Fgf17 cells were cortical and hippocampal neurons. We quantified Fgf17 transcript and protein in young and aged mice and discovered that the expression of Fgf17 drops dramatically with age (Fig. 4f-g and extended data Fig. 11e-g).
Based on the human datasets, we also searched for Fgf17 in mouse choroid plexus epithelial cells (co-localizing with a Ttr probe) in young and aged mice but could not find any cells positive for Fgf17 mRNA (Reviewer figure 3).



Reviewer figure 3- Undetected levels of Fgf17 in young and aged choroid plexus.
RNAscope analysis of Fgf17 in Ttr positive choroid plexus epithelial cells in young (2 months) and aged (22 months) mice. (n=3)
Scale bar, 50 μm.

Regarding the reviewer's last question, Fgf17 has critical roles during embryonic hind brain development [12]. Indeed, systemic Fgf17-KO mice have structural abnormalities in the cerebellum and dorsal frontal cortex and show an array of social abnormalities [12]. We mention these mice in our discussion but believe that the role of Fgf17 in the adult and aging nervous system should be separated from its roles in development.

3. Does Fgf17 account for full beneficial effects of young CSF infused into aging brain? The data, as presented, suggest this as a possible interpretation. Testing Fgf17-depleted CSF or CSF from Fgf17 KO mouse is an important experiment to address this point. Clarifying how the concentrations of infused Fgf17 (in aging) vs. endogenous CSF levels of Fgf17 (in young adulthood) compare would be informative.

These are important questions and while we believe Fgf17 is a key factor in mediating the beneficial effects of young CSF it is very likely that there are other factors involved. We emphasize this point in the revised discussion (line 187-189). To probe the importance of Fgf17 more directly we decided to block its activity in vivo and in vitro using a functional blocking antibody. This was done in part for technical reasons, because it is practically nearly impossible to obtain enough mouse CSF that would allow us to biochemically deplete Fgf17 and because Fgf17-KO mice have developmental abnormalities such that their CSF may be compromised in many other ways.
We thus infused an anti-Fgf17 blocking antibody ICV to young mice to test if the growth factor is necessary for normal memory function. We found that mice infused with anti-Fgf17 antibody, but not with control antibody, showed impaired performance in two hippocampal dependent cognitive tests (Y maze and contextual fear conditioning) and impaired neuronal plasticity (measured by lower c-fos levels in dentate gyrus granule cells) following behavioral tests (Fig. 4 o-r). In OPC cultures, the same concentration of anti-Fgf17 antibody also inhibits young CSF- and Fgf17-induced OPC proliferation. This indicates that the boost in proliferation is partially mediated by Fgf17 (Fig. 4s).
Taken together with the previous experiments showing that Fgf17 supplementation is sufficient to restore long term memory in aged mice, these additional experiments provide a more extensive link between Fgf17 and cognitive function, in young and aged mice.

Lastly, we agree with the reviewer that measuring the levels of Fgf17 in mouse CSF is an important addition to this study. Here again, the low amounts of aged CSF are the main bottleneck for the protein quantifications that could be done. For example- a standard ELISA requires 100ul per well in duplicates or triplicates which will require dozens of aged mice (diluted CSF was below detection levels). As an alternative, we measured young and aged CSF samples using the Somalogic platform but mouse Fgf17 was below the detection level.

We also attempted, in a separate study, to compare the proteomes of young versus aged CSF by mass spectrometry but, again, out of 358 protein groups that were included in the abundance analysis between young and aged mouse CSF we failed to detect Fgf17. In summary, while we couldn't measure Fgf17 protein directly in mouse CSF, our new analysis on the source of Fgf17 (described in point 2) provides a more mechanistic explanation for the source of Fgf17 and its downregulation with age, as well as a potential therapeutic target for future studies.

4. Delivery of Fgf17 to affected cells in the hippocampus. In other CNS cell types, the cells binding factors originating in the CSF do so via primary cilia or by directly contacting the CSF. How are the OPC precursors positioned throughout the hippocampus and what is the mechanism / process by which CSF Fgf17 reaches them? It seems this process may need to be distinct from other parts of the brain, where proliferation in response to young CSF was not observed in the present study, or is that due to other factors (progenitor types, receptor expression, gradients of Fgf17).

We thank the reviewer for this insightful comment. It is intriguing why the hippocampus is more prone to react to young CSF proteins and to Fgf17 infused at a relatively more anterior site into the lateral ventricles. In search for the receptor, we exposed SRF reporter cells to Fgf17 in the presence of inhibitory antibodies to the canonical Fgf receptors (Fgfr1/2/3) and found that inhibition Fgfr3 completely blocks reporter activation by Fgf17 (Extended data 12b). We then performed an RNAscope experiment with Pdgfra as an OPC marker and Fgfr3 to find patterns of expression within the hippocampus and around the ventricles. While this experiment was hard to interpret, because Fgfr3 is highly expressed by astrocytes, we found a subset of OPCs that expressed Fgfr3. These cells were found in the hippocampus and in hippocampal areas adjacent to the lateral and third ventricles (Extended data 12c). Furthermore, we performed injections of fluorescently labeled CSF and labeled Fgf17 both showing perfusion to the brain and strong perivascular stain in hippocampal blood vessels indicating perfusion through the hippocampus (Reviewer Fig. 1). These are of course only the beginning of unraveling how CSF proteins and Fgf17 might regulate the oligodendrocyte lineage and future studies will have to elucidate the uniqueness of hippocampal OPCs and the exact mechanisms of Fgf17 and OPC interaction.

5. Scholarship: The last sentence of the first paragraph states, "We hypothesized that intracerebroventricular (i.c.v.) administration of young CSF to aged mice will have rejuvenating effects on the brain (Fig. 1a)." One could argue that that this hypothesis has been previously tested and published in part by Silva-Vargas et al in Cell Stem Cell (not referenced in this paragraph, but included later in the manuscript). The concept of heterochronic CSF experiments to demonstrate age-specific CSF functions was also first demonstrated by Lehtinen et al., in Neuron (not referenced in this manuscript at all).

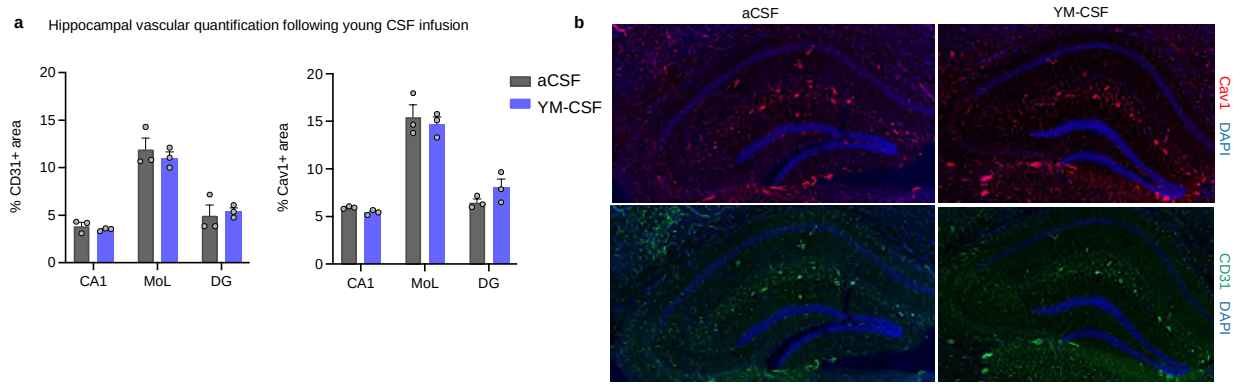
We apologize and have now included these studies in the discussion (line 192-194): "Heterochronic CSF experiments with cortical explants first demonstrated that developmental stage-specific CSF composition is critical for proper stem cell proliferation and brain development [13]".

Minor comments:

1. Fgf17 is implicated in vascular development and growth. Is there evidence of vascular sprouting in CSF infused conditions that could contribute to observed effects in hippocampus?

We quantified the hippocampal vasculature (by CD31 and Caveolin 1 immunostaining) in aged mice following a week of YM-CSF infusion and found no effect on the overall area

stained by these vascular markers (Reviewer figure 4).



Reviewer figure 4 – No effect of young CFS on hippocampal vascular area. Quantification of hippocampal blood vessels area by CD31 (gree) and Caveolin1 (red) immunostaining in aged mice infused with YM-CSF or aCSF for 1 week.

2. How long do the beneficial effects of acute Fgf17 infusion last – can it be sustained longer term, or will OPC pool deplete?

This is an important question and future experiments will have to explore how long the reported effects last. To clarify, Fgf17 was infused using mini-osmotic pumps in short and long-term paradigms but not injected acutely. In the long-term cohort, the last trial of the remote fear memory recall test was performed two weeks after termination of the 7-day infusion of Fgf17. We found that these mice still had better recall of the fear memory which indicates that the beneficial effect does not immediately disappear but is sustained many days past the infusion (Fig. 4n).

Referee #3 (Remarks to the Author):

Iram and colleagues present intriguing findings that Fgf17 in young mouse CSF promotes oligodendrocyte proliferation and functional improvements to memory in aged mice. The CSF infusions from young mice into old mice is a highly novel experimental approach and they present evidence that CSF from young mice may improve age-related hippocampal phenotypes. These findings further emphasize the groundbreaking observations from previous studies that factors in young mice can reverse aging phenotypes in old mice. While Iram and colleagues offer intriguing new insights, the data presented fall short of fully supporting the conclusions. Additionally, the identification Fgf17 is not via a systematic analysis and is somewhat independent of the preceding findings making it seem a bit convoluted especially considering it is highlighted in the title of the manuscript.

The authors should consider to more fully characterize the uniqueness and selectivity of the Fgf17 to the phenotype or alternatively focus more on SRF responses in the title of the manuscript. Specific comments are provided below.

We greatly appreciate the reviewer's enthusiasm and encouraging comments.
In the revised manuscript, we provide new data indicating both sufficiency and necessity of Fgf17 to mediate the observed beneficial effects of young CSF to a large extent. We also discuss the rationale for selecting Fgf17 and show data on the related protein, Fgf8, which had less pronounced effects than Fgf17.

Major Points

1. The memory recall improvements in Fig. 1b are significant but the two groups have different sample numbers, which isn't explained. To confirm this phenotype, it would be good to see additional behavioral assays associated with hippocampal learning and memory.

We apologize for the confusion. We start with similar sized groups but some of the aged mice did not survive the surgery and EDU injections, or in other cases the pumps had air bubbles following the preconditioning and were not implanted. We have now included these details in the methods section under Behavioral assays.
The amount of CSF we can extract from young mice is very limited and we use only CSF which passes strict purity criteria to exclude samples with blood traces[14]. This poses a major bottleneck throughout our study and unfortunately limits the experiments we can perform. We decided to follow behavioral paradigms described in previous publications that showed that de-novo myelination is necessary for long-term memory consolidation [15,16]. We chose the remote fear memory recall test where mice are exposed to foot shocks prior to pump implantation so young CSF, Fgf17 or artificial CSF infusion start only at the memory consolidation stage and 3 weeks before the long-term recall test. This type of long-term memory experiment doesn't permit to perform several behavioral tests on the same mice because they already receive the foot shock before the surgical procedure.
In addition, in the anti-Fgf17 inhibitory antibody infusion experiment which are now included in the study, we followed a paradigm previously used in our lab for anti-CD22 antibody infusions[17]. In this paradigm, the antibody is infused for 2-3 weeks and then the mice undergo two hippocampus depended behavioral assays – alternating Y maze and contextual fear conditioning (see figure 4o-p). These two experiments provide more evidence that Fgf17 blockade in young mice alters performance in cognitive tests (Fig. 4q-r). These experiments were performed on groups consisting of 10 young mice each, all of which survived the surgeries and were included in the analyses.

Also, the study assumes that beneficial factor(s) are present in the YM-CSF and is responsible the hippocampal phenotypes. However, experiments to rule out that YM-CSF dilutes detrimental factor(s) in aged-CSF aren't included. This is particularly important considering the manuscript concludes by finding a good factor in YM-CSF.

This is a good point and we have calculated the approximate dilution of "endogenous" CSF as a results of young CSF infusion. Based on the literature, the mouse produces 0.32 ul of CSF per minute [1] or 460 ul/day and the minipumps we have been using release 12 ul of young CSF per day. This results in a dilution of endogenous "aged" CSF by roughly 2.6% (1:38). We would argue that this is beyond detection with our biological and functional assays and not statistically measurable, and that it is thus highly unlikely that young CSF would result in a simple volumetric dilution of a detrimental factor in old mice. Furthermore, it should also be noted that since we infused aCSF as a control, it should similarly dilute detrimental factors if this were the mode of action. To compare the biological activities of young and aged CSF more directly we carried out two additional studies:
a) We repeated the 6-day mini-osmotic pump infusion using young and aged healthy human CSF as we had higher volumes of aged human CSF and could perform the experiment using human CSF instead of mouse. Interestingly, young human CSF induced hippocampal OPC proliferation very similarly to young mouse CSF but not aged human CSF (Fig. 1g-h).
b) Acute injection of young or aged CSF. At 16 hours following stereotaxic injection of 3 ul aCSF, young CSF or aged CSF (same 18 months of age as recipient) of young or aged mouse CSF into the lateral ventricle of aged mice, we isolated hippocampal OPC nuclei and performed bulk RNAseq to compare gene expression signatures. When focusing on the initial list of oligodendrocyte genes that emerged from the hippocampus bulk RNAseq following a week of infusion, we found a similar increase in these genes as early as 16 hours

post acute injection of young CSF but not following injection of aged CSF (Extended data Fig. 1c).

Taken together with the above calculations, these two additional experiments provide a more direct comparison between the functional effects of young versus aged CSF and strongly argue against a dilution effect.

2. The paper's foundation rests on the fact that oligodendrocytes are the cell-type most affected by infusion of young CSF, but this claim lacks supporting data. From the initial RNA sequencing experiment in Figure 1 c, the authors show a bar plot of the -log10 q-value of differentially expressed genes, comparing oligodendrocytes to mural cells, endothelial cells, and astrocytes. They claim that oligodendrocytes are more affected due to the higher average q-value of their cell-type specific genes. However, this conclusion is hard to rely on when many other major brain cell-types are lacking. It is unclear why the authors chose to only compare these four cell types, especially when there is evidence that vascular cells and mural cells are likely underrepresented in RNA sequencing. To make the case for oligodendrocytes being the most highly affected cell type, the authors should provide differential gene expression analysis for each cell type, including neurons, microglia, and oligodendrocyte precursors, including the number of cells captured per cell type and number of reads per cell. It is also unclear from the text whether this experiment was bulk or single-cell RNA sequencing.

We appreciate this point and apologize for not stating more clearly how we identified oligodendrocytes as the major responding cell type. The RNAseq experiment presented in figure 1 is a bulk RNAseq study which considers **all cell types** in the selected tissue; this is now clearly stated in the text and figure. In the analysis presented in Figure 1c, oligodendrocyte specific genes stood out in an unbiased gene set enrichment analysis (GSEA) whereas other cell type specific genes did not pass the significance threshold. In the revised manuscript we have now also included an independent analysis of the data using bioinformatic deconvolution algorithms based on hippocampal single cell RNAseq datasets produced in our lab[17]. This analysis, now added to extended data figure 1a-b, shows a relatively similar breakdown of cell type proportions between the two experimental groups (based on cell type specific genes). A downstream analysis of differential gene expression in each of these cell types identified an enrichment of differentially expressed genes in the OPC and mature oligodendrocyte groups. These two independent analyses pointed us to further study oligodendrocytes, but we do not exclude effects of young CSF proteins on other cell types. We have removed claims about unique / exclusive effects on oligodendrocytes and expanded our discussion accordingly (lines 187-189): "Expanding our examination of CSF proteins and SRF signaling in oligodendrocytes with aging to other cell types could illuminate complex regulatory interactions within parenchymal cells and with the environment during development, aging and aging-related diseases."

3. The images in Figure 1e are very striking showing a high number of EdU/Pdgfra double positive cells in YH-CSF treated mice. However, the images in extended figure 2c depict a high number of Edu+ Pdgfra- cells in the hippocampus. Quantifying the number of Edu+, Pdgfra+ vs Edu+ Pdgfra- cells would provide further insight into the specificity of the YH-CSF to the oligodendrocyte lineages. Likewise, what are the identity of the Edu+ Pdgfra- cells and is there evidence of these types of cellular response in the transcriptomics data presented in Figure 1c.

We thank the reviewer for the suggestion to explore the identity of the Edu+Pdgfra- cells. CSF and Fgf17 infusions induce proliferation of cells in an aged brain which has very few proliferating cells at the naïve state (see Extended Fig. 1f-g). Following the reviewer's request we have quantified the proportion of Edu+Pdgfra+ out of all EdU+ and found that roughly 25% of the proliferating cells in the hippocampus are OPCs. We co-stained with

other cell markers and find EdU+Iba1+ cells as well as EdU+Gfap+ as well (Extended Fig. 2g-j). We have removed claims about exclusive effects of CSF on oligodendrocytes and stressed this notion in the text (lines 62-63): “Notably, CSF infusions also triggered EdU incorporation in astrocytes and microglia (Extended data Fig. 2g-i)”. [REDACTED]

[REDACTED]

4. The data suggests that in response to YM-CSF OPCs proliferate and differentiate into mature oligodendrocytes. Do these oligodendrocytes increase myelination in the hippocampus or do other mechanisms potentially account for improved memory consolidation? The example images are from two different bregma; the YM-CSF image is more posterior than the aCSF image. The dentate gyrus region of the aCSF example image appears brighter in the aCSF image than in the YM-CSF image. It is also particularly difficult to judge the increase or decrease of MBP “intensity” from a rendered image, without seeing the raw confocal images. More direct analysis of the functional outcome of increased Mbp staining such as TEM ultrastructural imaging would provide further insight into mechanisms by which OPCs lead to improved memory. Likewise, do these changes occur in conjunction with increased hippocampal neuronal electrophysiological and synaptic connectivity.

Thank you for these insightful comments. We apologize for the confusing images and we have now included raw data and much higher-resolution MBP images (Fig. 1j). Per the reviewer’s suggestion, we quantified of the number of myelinated axons in the molecular layer of the hippocampus using TEM. This analysis shows an increase in the number of myelinated axons with no effect on the g-ratio (Fig. 1k and Extended data Fig. 3). We believe that this supports a hypothesis whereby young CSF promotes de-novo myelination rather than modifies existing

hippocampal myelin. However, we cannot exclude the possibility that there is also modification of existing myelin and now clearly state in the discussion (lines 205-210): "Furthermore, mature oligodendrocytes are critical for maintenance of axonal health and for regulation of neuronal function [2]. We cannot exclude the possibility that CSF proteins directly affect mature oligodendrocyte function with beneficial outcomes on neuronal health and cognitive function. Myelin plasticity is emerging as an important mechanism in learning and memory [3] and the study of myelin dysfunction in aging related cognitive decline is gaining interest.

5. Figure 2d depicts DEGs upregulated in SLAMseq experiment and highlights several genes in red including Tagln, Ccn2, and Acta2. While these genes are cytoskeleton and actin genes they are also known markers of smooth muscle cells. It would be important to complement this analysis with IHC showing co-staining for these proteins with an OPC marker such as Pdgfra.
This would rule out the possibility of this response coming from contaminating cell-types.

We have now included an NG2 and Acta2 staining of OPC treated with YM-CSF showing that after young CSF or aCSF treatment all OPCs are NG2 positive and ruling out potential contamination by smooth muscle cells (extended data Fig. 6).

Indeed, as shown in Figure 3a Srf is not selectively expressed in Pdgfra cells. Additionally, it is important to validate these in vitro findings in vivo in mice administered YH-CSF. Do OPCs in vivo show increased phalloidin and actin staining in response to YH-CSF? What are the upregulated SRF targets shown in Figure 3f? Do any overlap with those shown in 2d?

We thank the reviewer for this suggestion. We have imaged at least 30 Pdgfrα positive OPCs at high resolution (63x) and reconstructed the cell surface with IMARIS software. We then quantified phalloidin intensity within the OPC volume and indeed found that hippocampal OPC in mice infused with young mouse CSF had higher phalloidin staining intensity than those from mice infused with aCSF (Fig. 2f-g). This is in line with our finding in primary OPC cultures treated with young human CSF (Fig. 2d-e).

Regarding the last question, the SLAMseq experiment was done on postnatal rat OPCs while the boxplots show expression of SRF target genes in OPCs sorted from aged mouse hippocampi. We see similar trends at the pathway level but not at the gene level. We have now highlighted some of the genes plotted in figure 3f (see response to point 8 below).

6. The SRF knock out experiments in Figure 2f and g suggest that SRF influences OPC proliferation in vitro. In parallel, the authors should delete Srf in OPCs in the mouse brain and assay its effect on proliferation to directly test the effect on OPC in vivo.

We thank the reviewer for this suggestion. We would like to clarify that SRF-KO OPCs are still able to proliferate but they no longer respond to young human CSF (Fig.2j). We conclude from this experiment that the boost in proliferation induced by CSF proteins is mediated by SRF. This is now stated more clearly in the text.
That said, we do agree that an OPC specific inducible SRF-KO mouse would be very valuable to study the role of SRF in oligodendrocytes in the adult brain and we have started to breed floxed SRF mice with an inducible OPC specific CRE line (NG2-CRE) to carry out experiments along the lines the reviewer suggests. Unfortunately, such studies will take at least another 6 months and we believe they can form the basis of future in depth studies.

8. Figure 3f: why would target genes of Srf be both up and down regulated in each condition, if Srf expression is up, and the target genes are all upregulated in the volcano plot in Figure 2? Providing the names on the downregulated genes in the figure could provide further

insight here.

We thank the reviewer for this important question. We realized that the labeling of SRF targets in figure 2b and 2d included only the highlighted genes and is now more comprehensive by labeling the dots of all SRF target genes in red. It is now clearer to see that there are SRF target genes that are also downregulated in this experiment. Transcriptional control is complex and may involve several transcription factors to activate or repress a certain gene's expression. It is thus challenging to explain which of these regulatory mechanisms affected each gene. Moreover, the list of SRF predicted targets by TRANSFAC consists of more than 400 genes but only a subset of them is expressed by rat postnatal OPCs (Fig. 2) and aged mouse OPCs (Fig. 3) even after adjustment of the orthologous gene names by species. In both experiments we found a transcriptional signature indicating activation of the pathway, but we don't see many similarities at the gene level. We have now highlighted some of the genes up- and down-regulated in figure 3f. For example, Matr3, a nuclear matrix protein, is downregulated in OPCs with aging and upregulated both 1hr and 6hr post young CSF injection to aged mice.

9. Figure 4 introduces Fgf8 and 17 and demonstrates that Fgf17 can improve cognitive outcomes in aged mice. While this observation is interesting, the data falls well short of demonstrating that Fgf17 is responsible for all the previous phenotypes and cognitive improvements ascribed to YM-CSF. Does YM-CSF contain more Fgf17 than aged-CSF? If Fgf17 is immunodepleted from CSF is the OPC proliferation, increased MBP and cognitive improvements from YM-CSF lost? Does Fgf17 increase myelination in vivo? If so, does it affect neuronal activity? Determining whether the YM-CSF response is mediated in-part or wholly by Fgf17 will be an important addition to this paper.

These are important questions and while we believe Fgf17 is a key factor in mediating the beneficial effects of young CSF it is very likely that there are other factors involved. We emphasize this point in the revised discussion (line 187). To probe the importance of Fgf17 more directly we decided to block its activity in vivo and in vitro using a functional blocking antibody. This was done in part for technical reasons, because it is practically nearly impossible to obtain enough mouse CSF that would allow us to biochemically deplete Fgf17 and because Fgf17KO mice have developmental abnormalities such that their CSF may be compromised in many other ways. We thus infused an anti-Fgf17 blocking antibody ICV to young mice to test if the growth factor is necessary for normal memory function. We found that mice infused with Fgf17, but not with control antibody, showed impaired performance in two hippocampal dependent cognitive tests (Y maze and contextual fear conditioning) and impaired neuronal plasticity (measured by lower c-fos levels in dentate gyrus granule cells) following behavioral tests (Fig. 4o-r). In OPC cultures, the same concentration of anti-Fgf17 antibody also inhibits young CSF- and Fgf17-induced OPC proliferation. This indicates that the boost in proliferation is in part mediated by Fgf17 (Fig. 4s). Taken together with the previous experiments showing that Fgf17 supplementation is sufficient to restore long term memory in aged mice, these additional necessity experiments provide a more extensive link between Fgf17 and cognitive function, in young and aged mice.

We also attempted to measure the levels of Fgf17 in mouse CSF but encountered many technical and practical challenges. For example, a standard Fgf17 ELISA requires 100ul test solution per well in duplicates or triplicates which would require collection from dozens of aged mice (we tried diluting the CSF but it was under the detection limit). As an alternative, we measured young and aged CSF samples using the Somalogic platform but mouse Fgf17 was below the detection level. We also attempted, in a separate study, to compare the proteomes of young versus aged CSF by mass spectrometry but, again, out of 358 protein groups that were detectable in the abundance analysis between young and aged mouse CSF we failed to detect Fgf17 (Shuken et al. Nature Aging, In press). As an alternative, to determine if Fgf17 might be regulated in an age dependent way, we decided to search for

the cells producing it. We first consulted an existing human protein expression atlas (https://www.proteinatlas.org/ENSG00000158815-FGF17/tissue) and found Fgf17 is highly enriched in the brain (Extended data Fig. 11a). We next performed a meta-analysis of the Allen Brain Atlas mouse brain single cell smartseq dataset (https://portal.brain-map.org/atlases-and-data/rnaseq) which shows a subset of Fgf17 positive cells in the mouse cortex (Extended data Fig. 11b-d). Encouraged by these findings, we set out to compare Fgf17 mRNA and protein expression in young and old mouse brains in situ with RNAscope and immunofluorescence staining, respectively. Indeed, we found that in the young brain the highest expressing Fgf17 cells were cortical and hippocampal neurons. We quantified Fgf17 transcript and protein in young and aged mice and discovered that the expression of Fgf17 drops dramatically with age (Fig. 4f-g and extended data Fig. 11e-g).
In summary, while we couldn't measure Fgf17 protein directly in mouse CSF, our new analysis provides a more mechanistic explanation for the source of Fgf17 and its downregulation with age, as well as a potential therapeutic target for future studies.

Minor comments
1. In Figure 2, the authors argue that Srf drives the OPC proliferation caused by YM-CSF infusion. They support this with an experiment using primary OPC cultures from SRF floxed mice, and infecting with either Cre-GFP or truncated Cre-GFP. They argue that knocking out SRF in OPCs reduces proliferation (Figure 2g), but the representative images or data of the BRDU staining is not shown. It is impossible to understand the data without seeing representative examples. Again, sample sizes of three per condition are used, which is not appropriately high enough to rule out the possibility that the comparison is underpowered.

We apologize and have now included images of the infected OPCs in brightfield and the GFP, BRDU and DAPI images that were analyzed (Extended data Fig. 7e-i). Regarding the methodology we refer the reviewer to the methods paragraph –
Mouse OPC cultures
Mouse OPCs were purified from brains of SRF floxed mice (generated by David Ginty and kindly provided by Eric Small) by immunopanning as described above for rat OPCs [19].
On day 3 of the culture, SRF-f/f OPCs were split and plated in 384-well plates for proliferation experiments. When the cells were in suspension in proliferation media before plating, $1*10^{10}$ viral genomes of AAV DJ-CMV eGFP-deleted cre (GVVC-AAV-62) or AAV DJ-CMV eGFP-cre (GVVC-AAV-63) (both generated by the Stanford Gene Vector and Virus Core). The following day, media was fully replaced and 48hrs after infection 10% aCSF or YH-CSF were added to proliferation media with BRDU (20μM final concentration) for 16 hrs. Cells were fixed and cell proliferation was assessed as indicated above in the BRDU experiment.

2. In Figure 3c, it would be helpful to explain why the aged and young mice do not cluster together on the dendrogram, especially since a main claim of the paper is that ageing specifically affects the oligodendrocyte cell population.

The heatmap in figure 3c shows oligodendrocyte lineage marker genes to validate our gating strategy. We would not expect young and aged mice to differ by these genes and in fact believe it nicely shows that our gating strategy doesn't bias young or aged oligodendrocytes. We have removed the age identity from the heatmap to avoid any confusion.

3. Figure 3e, it is unclear which region of the hippocampus is imaged, or whether this is the same region between the two slices. Inclusion of a nuclear stain would be helpful here. Also, in the representative image provided for the YM-CSF condition,
there are seven arrows pointing to EDU+ Pdgfra+ cells, and perhaps three EDU- Pdgfra+ cells. This should put the percentage of EDU-positive OPCs at around 70%, but in the quantification, the percentage ranges from ~2 to ~18, making it unclear how the authors analyzed this data.

Similarly, in Figure 3i, the authors should plot the % of BRDU+ Pdgfra+ cells, without normalization to the aCSF condition. In the example images of the highest CSF dose, there are approx. 16 BRDU+ nuclei in the aCSF condition, and 20 BRDU+ nuclei in the YH-CSF condition, making the increase about 1.25, assuming the cells are plated at equal density. Yet the quantification suggests an average change of greater than 2x, which is confusing.

We commend the reviewer's keen eye. We have now chosen representative images that better reflect the group averages shown in the graphs. Regarding the nuclear stain in fig. 1f, unfortunately when we preform antigen retrieval for BRDU staining the nuclear staining is disrupted. This is not the case for EDU and all those images include DAPI staining.

4. In Figure 3k, OPCs are annotated according to different morphologies (ie "star", "partial", "arborized") but what these annotations mean, or how they were judged, is not clear. Staining for more definitive markers such as MBP would be more conclusive.

The morphological analysis is based on MBP staining and provides a way to score and compare the differentiation stage of various cells, as previously done by Zuchero et al, Developmental Cell, 2015. Extended data Fig. 4 shows the quantification of MBP intensity as well as enlarged representative images of MBP staining.

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Reviewer Reports on the First Revision:

Referees' comments:

Referee #1 (Remarks to the Author):

The authors have adequately addressed all my questions and have made a reasonable attempt to add experiments that were possible at the level of a revision. The discussion of FGFR signaling in the oligodendrocyte lineage has improved, but the 1996 reference is somewhat outdated for a research paper. Also the observation that FGF17 may block OPC differention (see ref 69) has been swept under the carpet and should be mentioned. Otherwise, the paper is very nice as it is.

Referee #2 (Remarks to the Author):

The authors have thoughtfully considered the comments from the previous review by clarifying the text and by performing additional experiments. As such, the study is significantly improved. I can appreciate the technical challenges involved, and also see that the present findings are of high importance that will open up many new lines of investigation. I agree with moving forward with this study at Nature. Congratulations!

Referee #3 (Remarks to the Author):

The authors present a set of findings supporting the idea that soluble factors from the cerebral spinal fluid of young mice can increase learning and memory in aged mice. This is an interesting and novel claim, and will likely be of interest to the readership of Nature. The paper makes three broad claims: that young CSF infusion to aged mice improves learning and memory; that young CSF infusion promotes OPC proliferation and differentiation, and myelination; and that these effects are mediated by the action of Fgf17.

The first claim is supported by an increase in learning and memory in aged mice infused with young CSF, compared to aged mice infused with artificial CSF. However, because there is no control of non-infused aged mice, it is difficult to tell whether young CSF infusion truly increases learning and memory, or just improves it relative to aged mice infused with aCSF. Testing a cohort of non-infused aged mice would resolve this confusion. Because of the difficulty in collecting young mouse CSF, the authors could compare sham-infused aged mice and aCSF-infused aged mice, to test whether freezing behavior is comparable.

The second claim, that young CSF infusion promotes oligodendrocyte maturation and myelination, is relatively well-supported. However, the third claim lacks direct evidence. The authors show that Fgf17 can recapitulate the effects of young CSF in promoting oligodendrocyte myelination and learning and memory, but stop short of actually demonstrating that Fgf17 is the causal factor in young CSF. The authors note the technical difficulty of collecting enough CSF to test whether Fgf17 levels, so this will likely form the basis of a future study.

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Author Rebuttals to First Revision:

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Fgfr citation from 1996 has been replaced with a reference from 2020.
Thanks you for bringing the Fortin at al paper to our attention, we are now referring to these contradictory results in line 166:
"Prior work in OPC culture suggested that Fgf17 slightly promotes proliferation although it may inhibit OPC differentiation in some contexts [32]."

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This is indeed a valid point and while we have not compared sham and artificial CSF (aCSF)-infused mice head-to-head, the fear conditioning paradigm is quite consistent and reliable in our hands and aCSF infusion typically results in roughly 20% freezing behavior. In an independent experiment not part of the manuscript we compared young with aged, non-infused (sham) mice and find that these aged mice freeze almost the exact amount of time. And while these groups were not tested and compared in the same experiment we find no significant difference between sham/non-infused mice and aCSF-infudsed mice.

In the figures below we plot the freezing rates of these sham/non-infused mice side by side with the plots of the infused mice from manuscript figures 1b (aCSF vs YM-CSF) and 4n (aCSF vs Fgf17).

Figure 1b + aged naïve mice

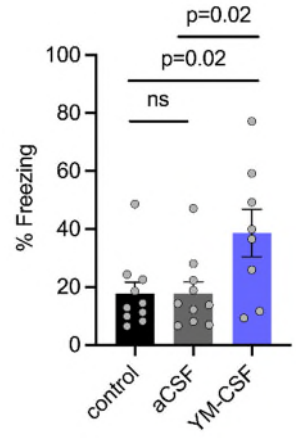
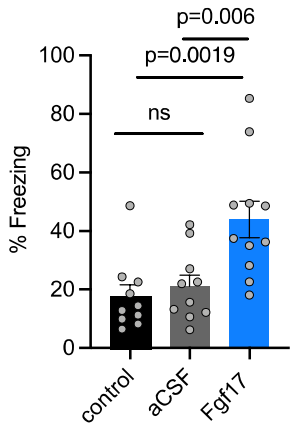


Figure 4n + aged naïve mice

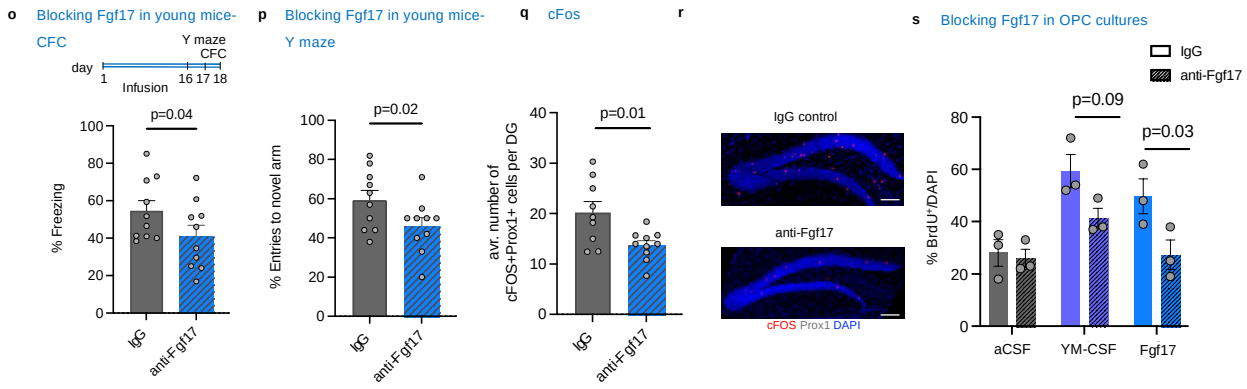


Together, these data suggest that aCSF does not artificially reduce freezing of mice compared with sham/non-infused mice and that aCSF infused mice are indeed an appropriate control for these complex experiments.

The second claim, that young CSF infusion promotes oligodendrocyte maturation and myelination, is relatively well-supported. However, the third claim lacks direct evidence. The authors show that Fgf17 can recapitulate the effects of young CSF in promoting oligodendrocyte myelination and learning and memory, but stop short of actually demonstrating that Fgf17 is the causal factor in young CSF. The authors note the technical difficulty of collecting enough CSF to test whether Fgf17 levels, so this will likely form the basis of a future study.

These are important questions and while we believe Fgf17 is a key factor in mediating the beneficial effects of young CSF we never say it is *the causal factor*, and it is very likely that there are other factors involved. We emphasize this point in the revised discussion (lines 187-189). To probe the importance of Fgf17 more directly we decided to block its activity in vivo and in vitro using a functional blocking antibody. This was done in part for technical reasons, because it is practically nearly impossible to obtain enough mouse CSF that would allow us to biochemically deplete Fgf17 and because Fgf17-KO mice have developmental abnormalities such that their CSF may be compromised in many other ways.

We thus infused an anti-Fgf17 blocking antibody ICV to young mice to test if the growth factor is necessary for normal memory function. We found that young mice infused with anti-Fgf17 antibody, but not with control antibody, showed impaired performance in two hippocampal dependent cognitive tests (Y maze and contextual fear conditioning) and impaired neuronal plasticity (measured by lower c-fos levels in dentate gyrus granule cells) following behavioral tests (Fig. 4 o-r). In oligodendrocyte precursor cell (OPC) cultures, the same concentration of anti-Fgf17 antibody also inhibits young CSF- and Fgf17-induced OPC proliferation. This indicates that the young CSF-induced increase in proliferation is partially mediated by Fgf17 (Fig. 4s).



Taken together with the previous experiments showing that Fgf17 supplementation is sufficient to restore long term memory in aged mice, these additional experiments provide a more extensive link between Fgf17 and cognitive function, in young and aged mice.

While we could not measure Fgf17 in mouse CSF due to technical limitations, we found that in the young brain the highest expressing Fgf17 cells were cortical and hippocampal neurons. We quantified Fgf17 transcript and protein in young and aged mice and discovered that the expression of Fgf17 drops dramatically with age (Fig. 4f-g and extended data Fig. 11e-g). This further supports the notion that Fgf17 deficiency in the aging brain may explain, in part, the cognitive deficits in aged mice.