

Synthetic tau seeds induce progressive tauopathy, gliosis and Alzheimer-like behaviour in PS19 mice

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Introduction

Study of prion-like propagation of tau pathology in transgenic murine models overexpressing pathogenic variants of human tau has been typically accomplished by intracranial infusion of pathogenic tau species isolated from Alzheimer's disease (AD) human post-mortem brains. However, this procedure raises safety and reproducibility concerns. Here, we tested the ability of synthetic tau fibrils (STF), a safer and more robust alternative, to induce an AD-like phenotype in PS19 mice overexpressing human tau with the P301S mutation.

Results

In the 14- to 17- weeks group, no differences were seen in the behavioural tests between the PS19_{STF} and the control group.

Behavioural tests 18- to 21- weeks

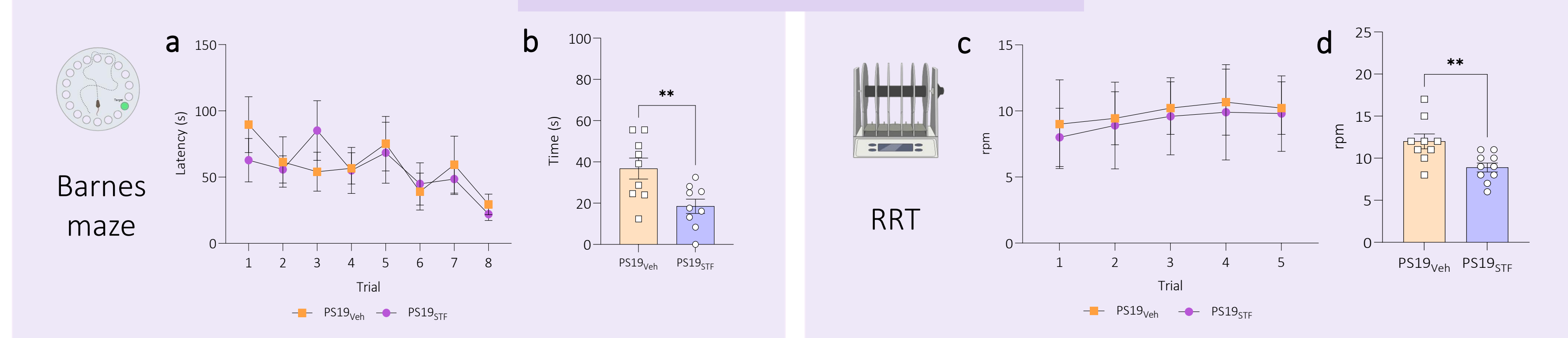


Fig1. Behavioural evaluation. For the **cognitive impairment evaluation**, target latency in Barnes maze test (**a**) throughout the **18- to 21-week group** trials is shown. Both animal groups learned the test correctly, as revealed by significant effect of the trial in the two-way repeated measures ANOVA ($F_{\text{trial Barnes}}(4.277, 72.09) = 2.459, p = 0.0493$). Time spent in target quadrant in the test is represented (**b**): notably, in the Barnes test, the PS19_{STF} group spend significantly less time in the target quadrant compared to the control group ($t=2.986, df=16, p = 0.0087$). For the **motor coordination evaluation**: latency to fall from RRT is shown. No differences in motor coordination were observed during the training sessions (**c**). However, t-test revealed that PS19_{STF} mice exhibited significantly worse motor performance compared to the control group (**d**) ($t=3.093, df=17, p = 0.0066$).

14- to 17- weeks

a

Alz50 and AT8 quantification

a

18- to 21-weeks

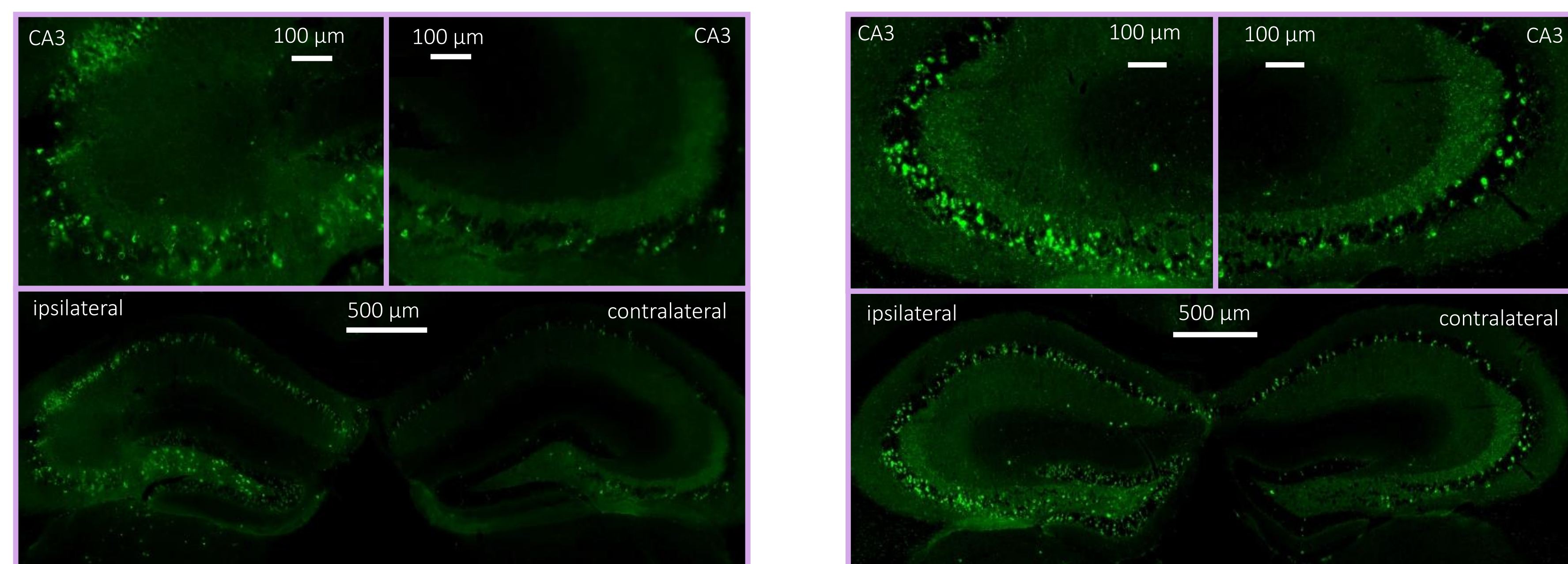


Fig. 2 Representative Alz50 staining and Alz50 and AT8 quantification in the 14- to 17-weeks group. Brain of a 17-week-old mouse that received 4 µg of STF (**a**) is shown: entire hippocampus (bottom), and magnified view of the CA3 (top) region. Quantification of Alz50- and AT8-positive structures in the CA3 mentioned hippocampal region is shown in the graphics (**b, c**). Both Alz50- and AT8-positive structures are present in the ipsilateral hippocampus of PS19_{STF} mice, and also present in the contralateral hemisphere, but with no differences with the control group. PS19_{veh} mice show nearly zero antibody-positive structures. Two-way ANOVA used for the statistical analysis, **, $p < 0,01$; ***, $p < 0,001$.

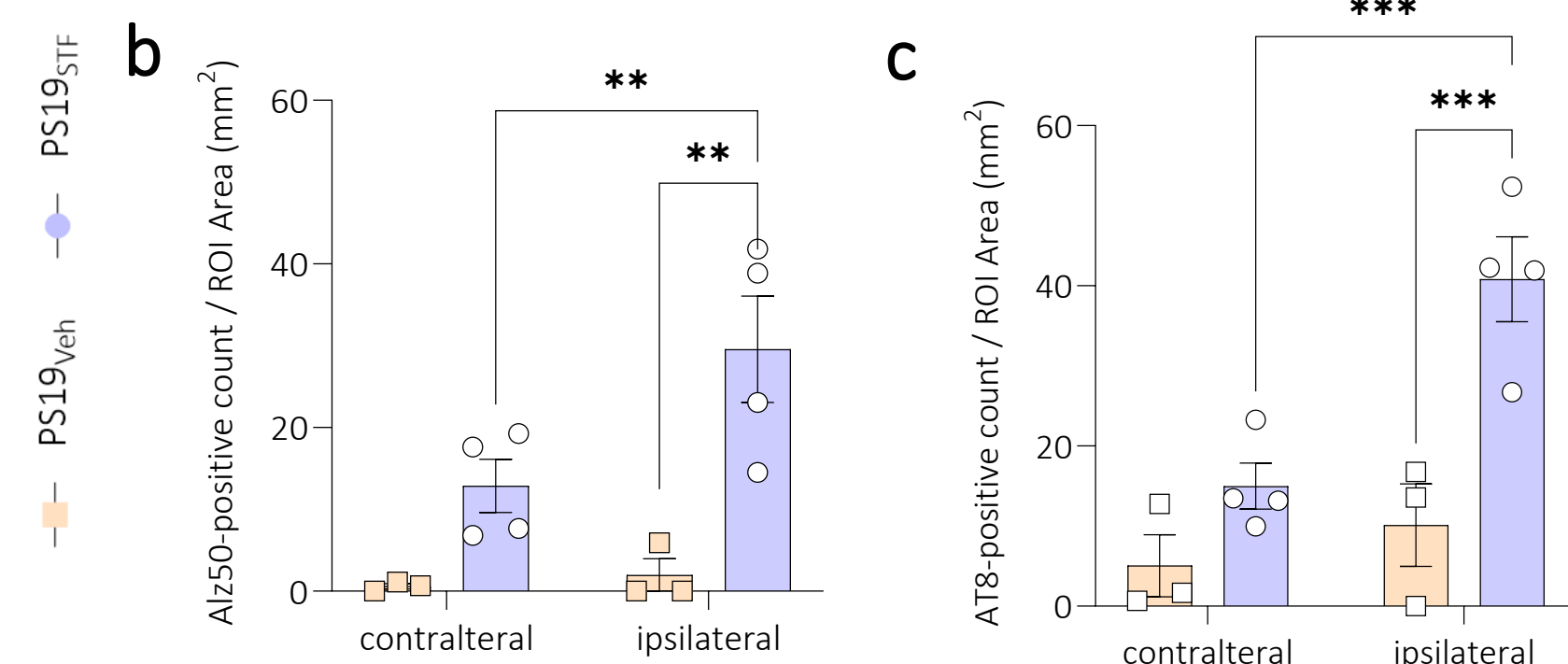
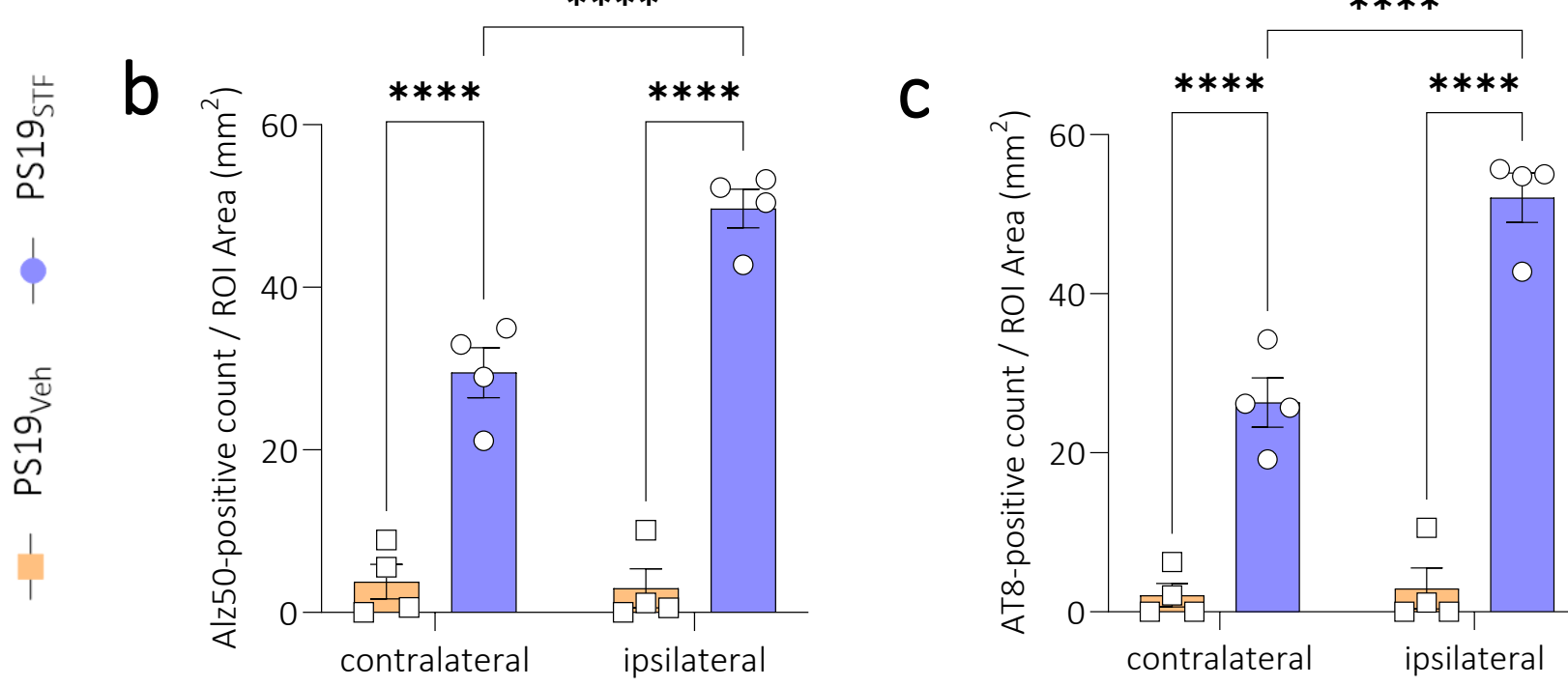


Fig. 3 Representative Alz50 staining and Alz50 and AT8 quantification in the 18- to 21-weeks group. A representative Alz50 staining of a 21-week-old mouse brain that received 4 µg of STF is shown (**a**), being the entire hippocampus (bottom), and a magnified views of the CA3 (top) shown. The quantification of Alz50- and AT8-positive structures (**b, c**) exhibit that both are present in the ipsilateral hippocampus of PS19_{STF} mice and also in the contralateral hemisphere, being significantly more numerous compared to the control group. PS19_{veh} mice show nearly zero antibody-positive structures. Two-way ANOVA used for the statistical analysis, ****, $p < 0,0001$.



Methods

Stereotaxic surgery and infusion: 2 µl of STF (n=21, PS19_{STF}, 2µg/µl) were unilaterally infused into the dentate gyrus of the right hippocampus of 10-week-old PS19 mice. Control mice (n=22, PS19_{veh}) received same amount of vehicle.

Behavioural evaluation (week 14 to 17 and week 18 to 21). The test used were: for **anxiety-like behaviour**: open field test, OFT; and elevated plus maze, EPM, for **cognitive performance**: Barnes maze; and for **motor coordination**: rotarod test, RRT.

Tissue processing: A transcardial perfusion and brain extraction was performed followed by a vibratome slicing of brains (20-µm-thick) and Immunofluorescence (IF) experiments with Alz50, AT8, Iba1 and GFAP antibodies.

a Iba1 quantification

14- to 17- weeks

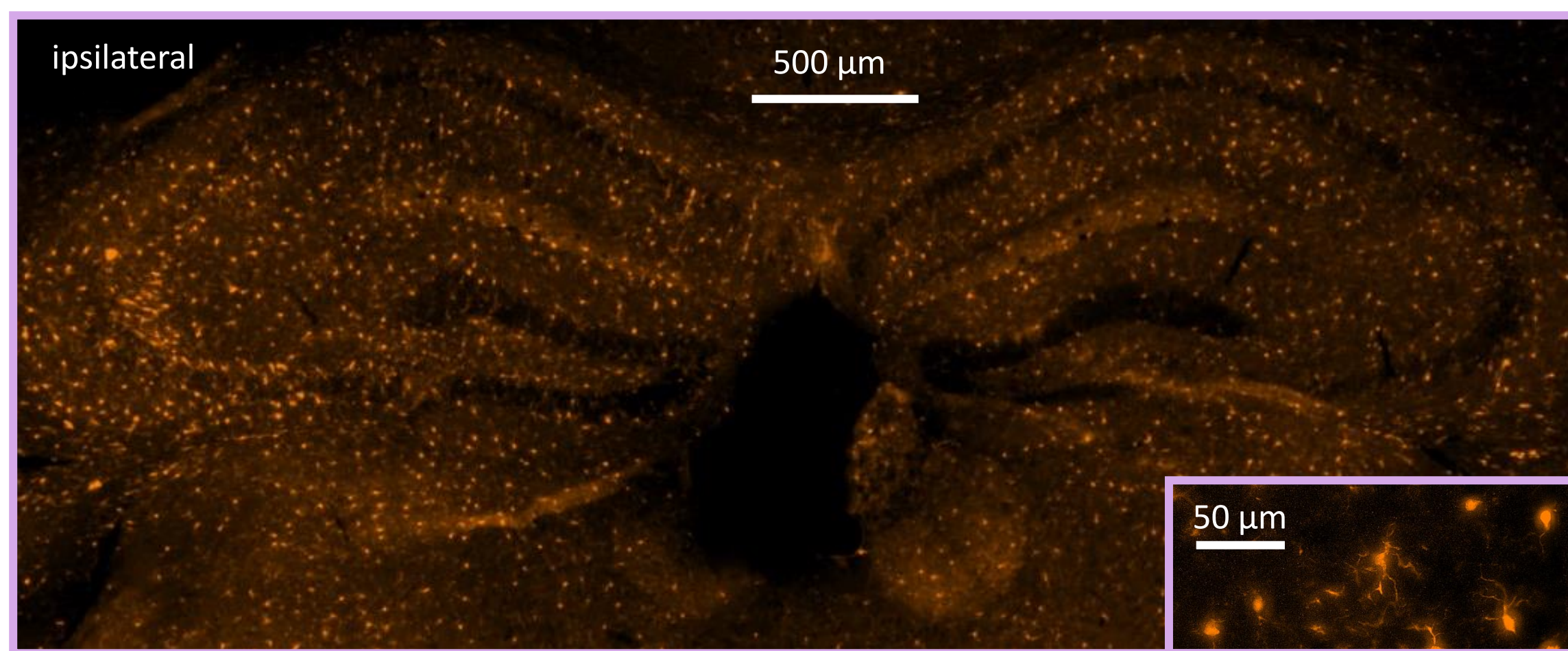
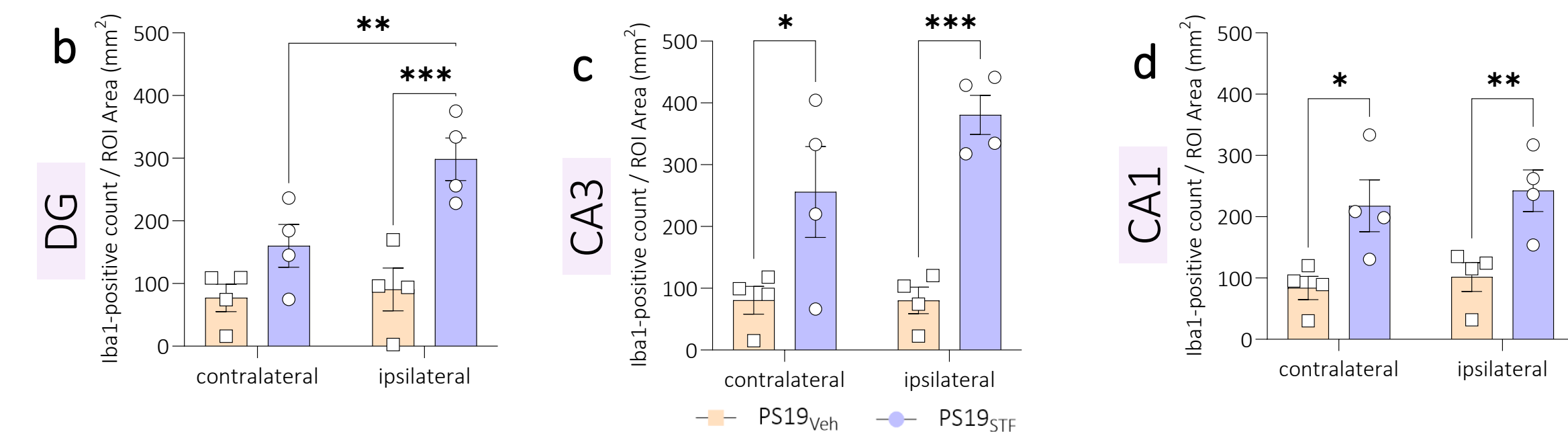


Fig. 4. Representative Iba1 staining and quantification in the 14- to 17-week group. The hippocampus of a 17-week-old mouse that received 4 µg of STF (**a**) is shown. Quantification of Iba1-positive structures in the dentate gyrus (DG), CA3, and CA1 hippocampal regions is shown in the graphs (**b-d**). Iba1-positive structures are observed in the ipsilateral hippocampus of PS19_{STF} mice across all regions. Furthermore, in the CA3 and CA1 regions, but no in the DG, Iba1-positive structures are also present in the contralateral hippocampus, in contrast to the control group. Two-way ANOVA used for the statistical analysis, *, $p < 0,05$; **, $p < 0,01$; ***, $p < 0,001$.



a GFAP quantification

14- to 17- weeks

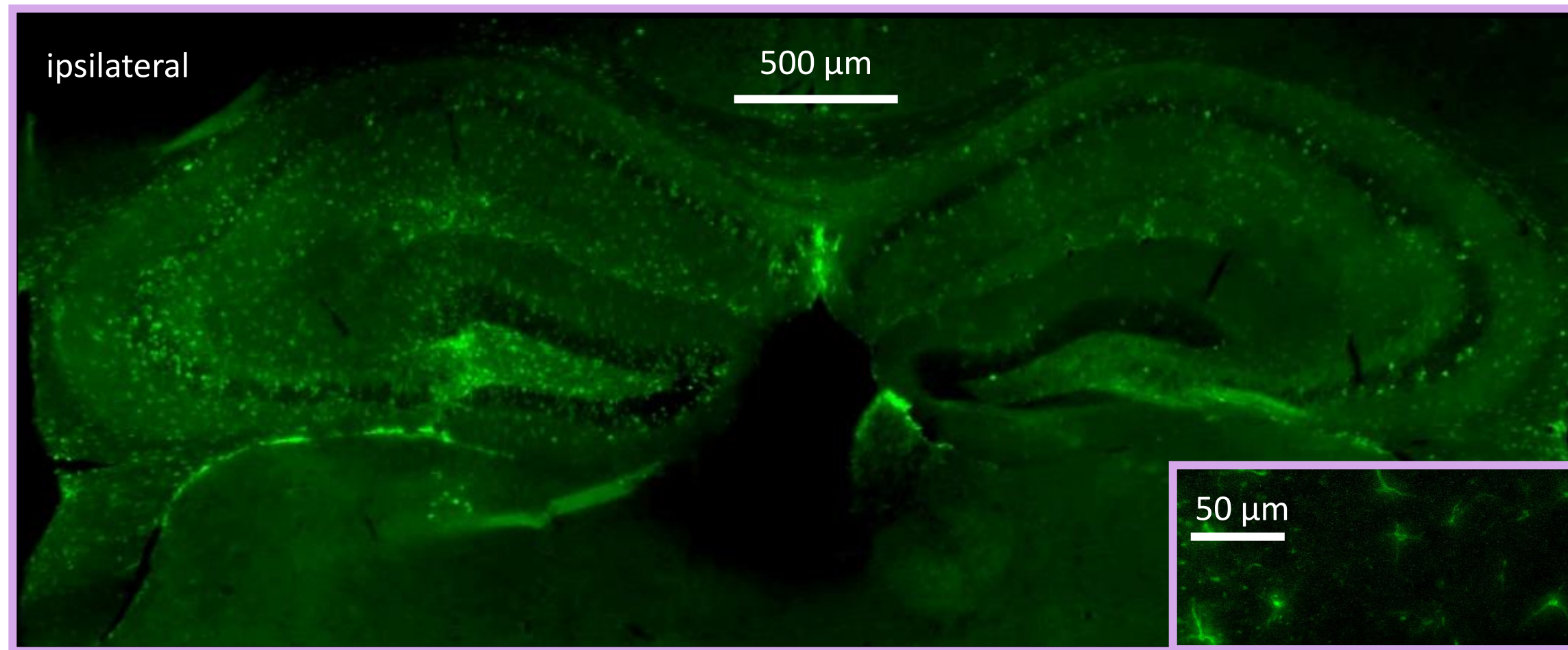
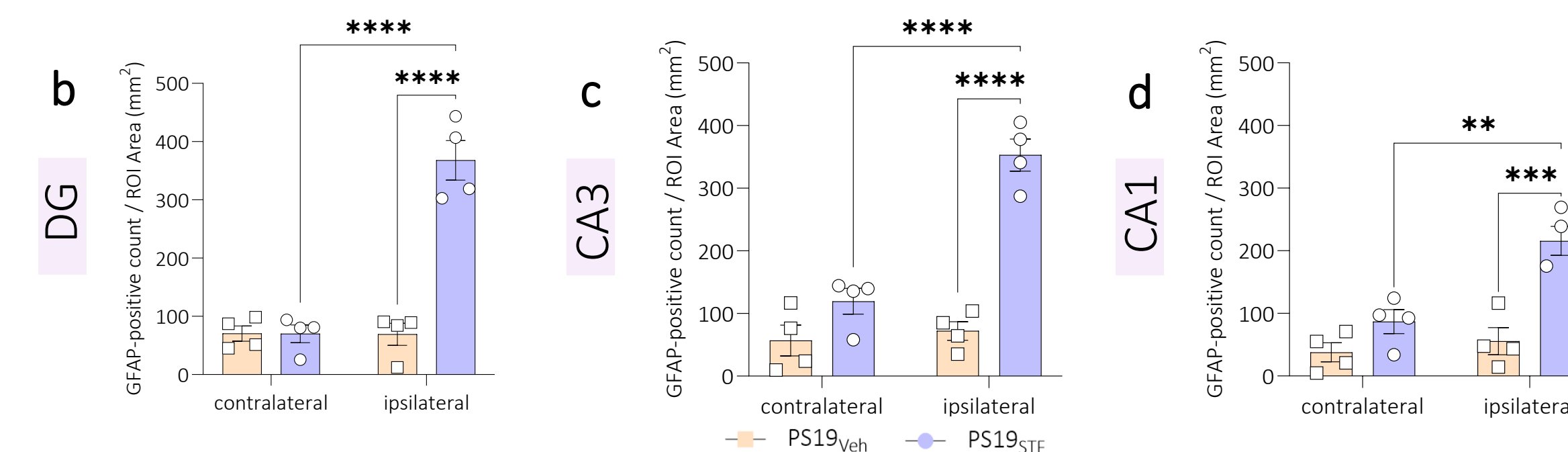


Fig. 5. Representative GFAP staining and quantification in the 14- to 17-week group. The hippocampus of a 17-week-old mouse that received 4 µg of STF (**a**) is shown. Quantification of GFAP-positive structures in the DG, CA3, and CA1 hippocampal regions is presented in the graphs (**b-d**). GFAP-positive structures are observed only in the ipsilateral hippocampus of PS19_{STF} mice across all regions compared to the control group. Two-way ANOVA used for the statistical analysis, **, $p < 0,01$; ***, $p < 0,001$; ****, $p < 0,0001$.



Conclusions

These results validate STF as a suitable tool for studying tau propagation and associated gliosis, as well as the AD-like behavioural phenotype in PS19 mice. Moreover, the use of defined STF concentrations may reduce within-group variability and improve experimenter safety.