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Dysregulated neural firing has been proposed as an early-stage feature of Alzheimer’s disease (AD) and is thought to negatively impact elements of stable neuronal network function. However, the underlying cellular and synaptic processes involved in amyloid-related micro-circuit dysregulation remain unclear. Given that synapses exhibit a highly heterogenous molecular composition, which is thought to support specialized functionality, it is possible that early-stage synaptic pathophysiology does not similarly impact all synapses in the local amyloid micro-environment. Instead, it may be driven by a selective vulnerability of molecularly and functionally related, or independent, cells or synapses. Here, we use the App^{NL-G-F} mouse model to investigate synaptic pathophysiology in early-stage amyloidosis and test the degree to which it occurs globally or in specific subsets of cells and synapses.

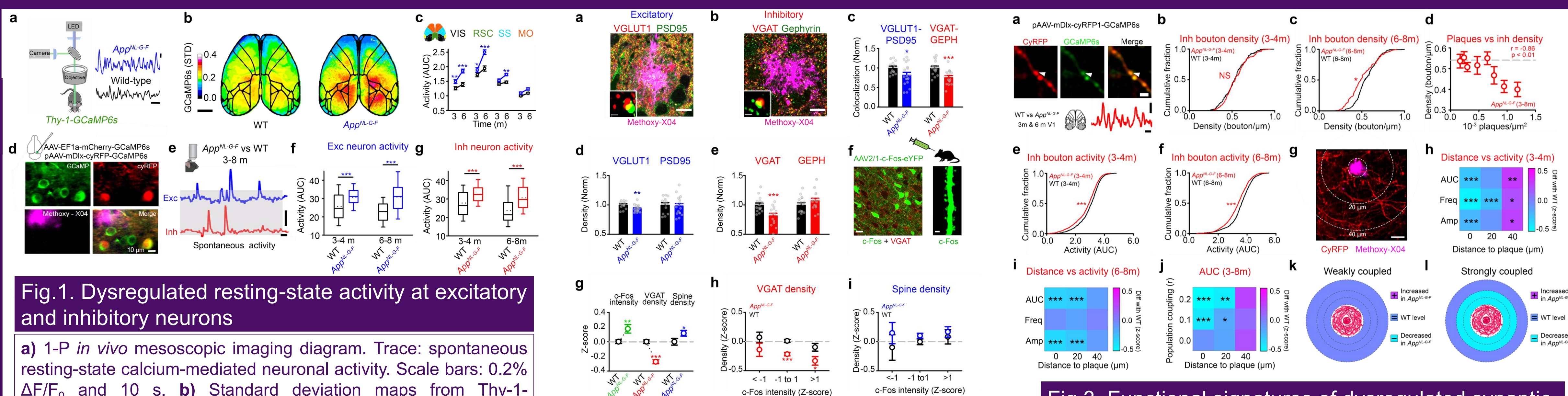


Fig.1. Dysregulated resting-state activity at excitatory and inhibitory neurons

a) 1-P *in vivo* mesoscopic imaging diagram. Trace: spontaneous resting-state calcium-mediated neuronal activity. Scale bars: 0.2% ΔF/F₀ and 10 s. **b)** Standard deviation maps from Thy-1-GCaMP6s (WT, left) and App^{NL-G-F} x Thy-1-GCaMP6s (right) mice. **c)** Average activity measured from broad parcellated regions (VIS: visual/parietal, RSC: retrosplenial, SS: somatosensory, and MO: motor cortices) in WT (black) and App^{NL-G-F} (blue) mice. **d)** Example region of *in vivo* 2-P imaging showing expression of co-injected viral constructs to label excitatory (green only) and inhibitory (red and green) neurons, and amyloid plaques via methoxy-X04 (magenta). Scale bar: 10 μm. **e)** Example of ΔF/F₀ calcium traces from excitatory (blue) and inhibitory (red) neurons during resting-state activity. Scale bars: 100% ΔF/F₀, 20 s. **f,g)** Average AUC of ΔF/F₀ calcium traces measured in excitatory (f, blue) and inhibitory (g, red) neurons in App^{NL-G-F} mice and age-matched WT (black) animals. Error bars: mean ± SEM (c), median ± IQR (f-g), *p<0.05, **p<0.01, ***p<0.001.

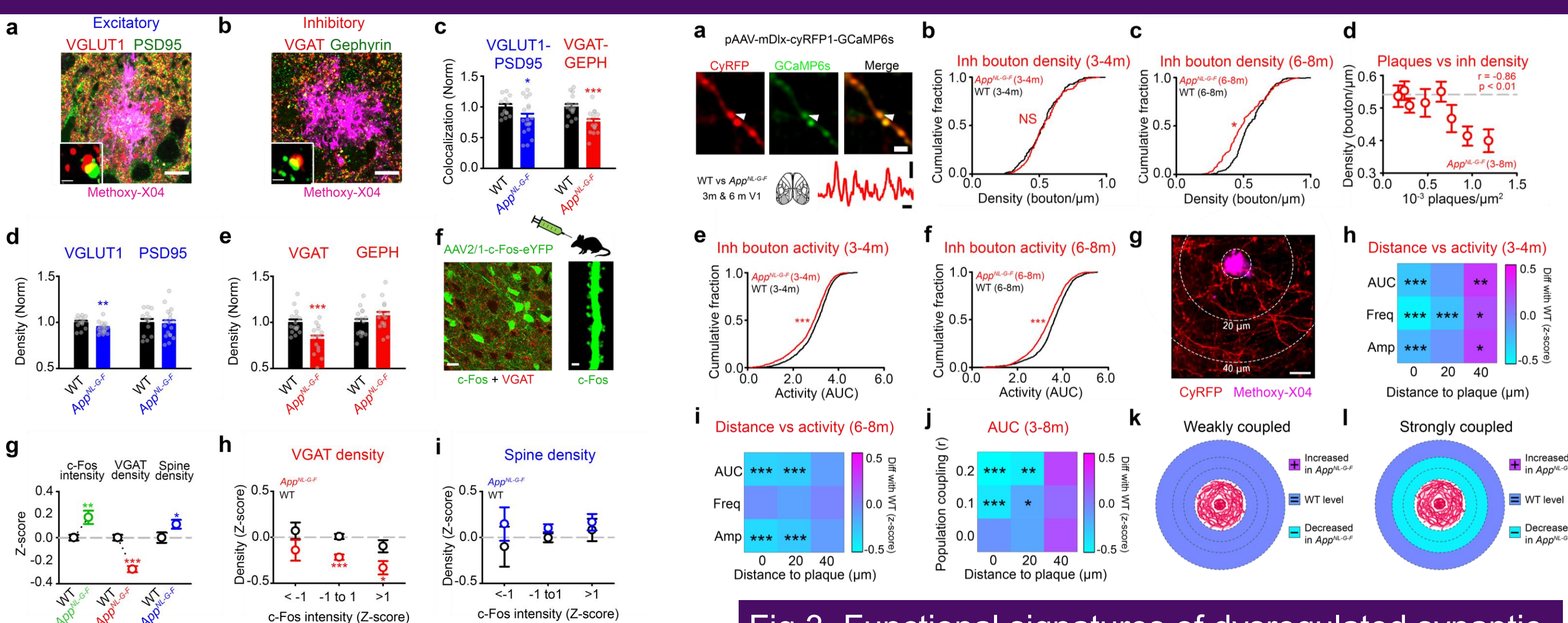


Fig.2. Cortical presynaptic GABAergic vulnerability

a,b) Immunofluorescence (IF) labelling of presynaptic (red) and postsynaptic (green) glutamatergic (GLUT, a) and GABAergic (b) markers in the vicinity of amyloid plaques (magenta, methoxy-X04) in App^{NL-G-F} mice. Scale bar: 10 μm. Insets, colocalization between the pre- and post-synaptic markers (yellow). Scale: 1 μm. **c-e)** Normalized (to WT) colocalization (c) or density of GLUT (d) and GABAergic (e) pre- (left) and post- (right) synaptic proteins. **f)** Cartoon depicting viral-mediated c-Fos-based activity tagging in WT and App^{NL-G-F} mice. Image showing IF labelling of VGAT (left) and c-Fos-eYFP positive dendritic spines (right). Scale bars: 20 μm (left), 2 μm (right). **g)** Plot of c-Fos intensity (green), VGAT (red) and dendritic spines (blue) density from 3-8 m App^{NL-G-F} mice normalized to age-matched WT values (black). **h,i)** Normalised excitatory c-Fos expression versus VGAT (h) and spine (i) density. Error bars: mean ± SEM, *p<0.05, **p<0.01, ***p<0.001.

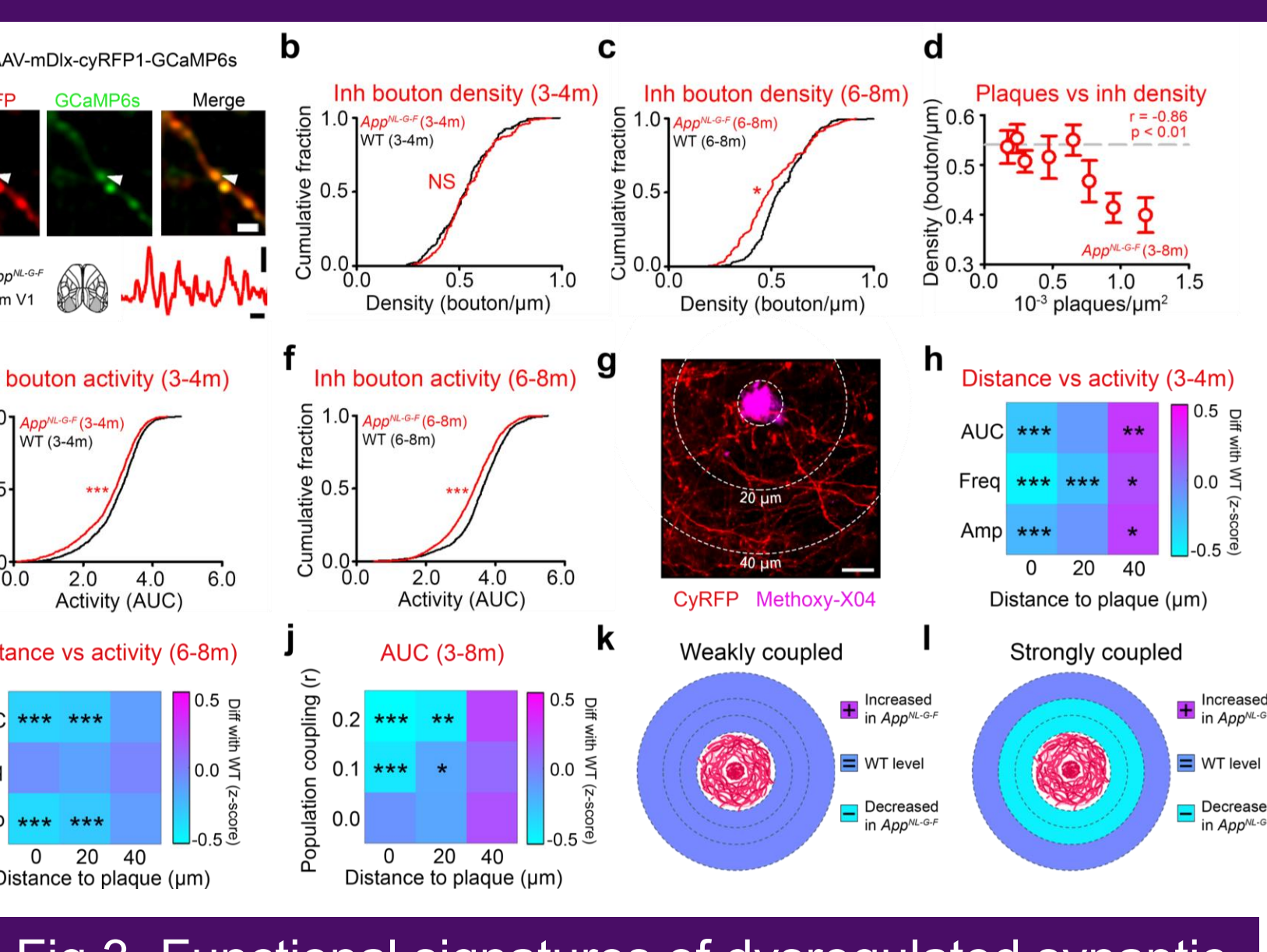


Fig.3. Functional signatures of dysregulated synaptic activity

a) *In vivo* 2-P imaging of superficial GABAergic axon expressing cyRFP (red, structure) and GCaMP6s (green, function); imaged VIS cortex of WT and App^{NL-G-F} mice, with example trace. Scale bars: 2 μm, 50 ΔF/F and 2 s. **b,c)** Density of GABAergic boutons in 3-4m (b) and 6-8m (c) App^{NL-G-F} (red) and WT (black) mice. **d)** Methoxy-X04 labelled plaque and bouton density per region. **e,f)** Calcium activity (AUC) at GABAergic boutons during resting-state at 3-4m (e) and 6-8m (f). **g)** Superficial GABAergic axons expressing cyRFP (red) and a methoxy-X04-labelled plaque (magenta). Dashed circles show plaque edge and 20 μm increments. Scale bar: 10 μm. **h,i)** Difference (App^{NL-G-F}-WT) in the z-scored (to WT) average AUC, frequency (Freq), and amplitude (Amp) values of GABAergic boutons located at increasing distances from methoxy-X04 labelled plaques in 3-4m (h) and 6-8m (i) App^{NL-G-F} mice. **j)** Difference (App^{NL-G-F}-WT) in the z-scored (to WT) AUC values of GABAergic boutons with increasing population-coupling values (y-axis) and distance from methoxy-X04 labelled plaques (x-axis). **k,l)** Schematics of periplaque regions showing change in activity (AUC) of GABAergic boutons. Error bars: mean ± SEM, *p<0.05, **p<0.01, ***p<0.001.

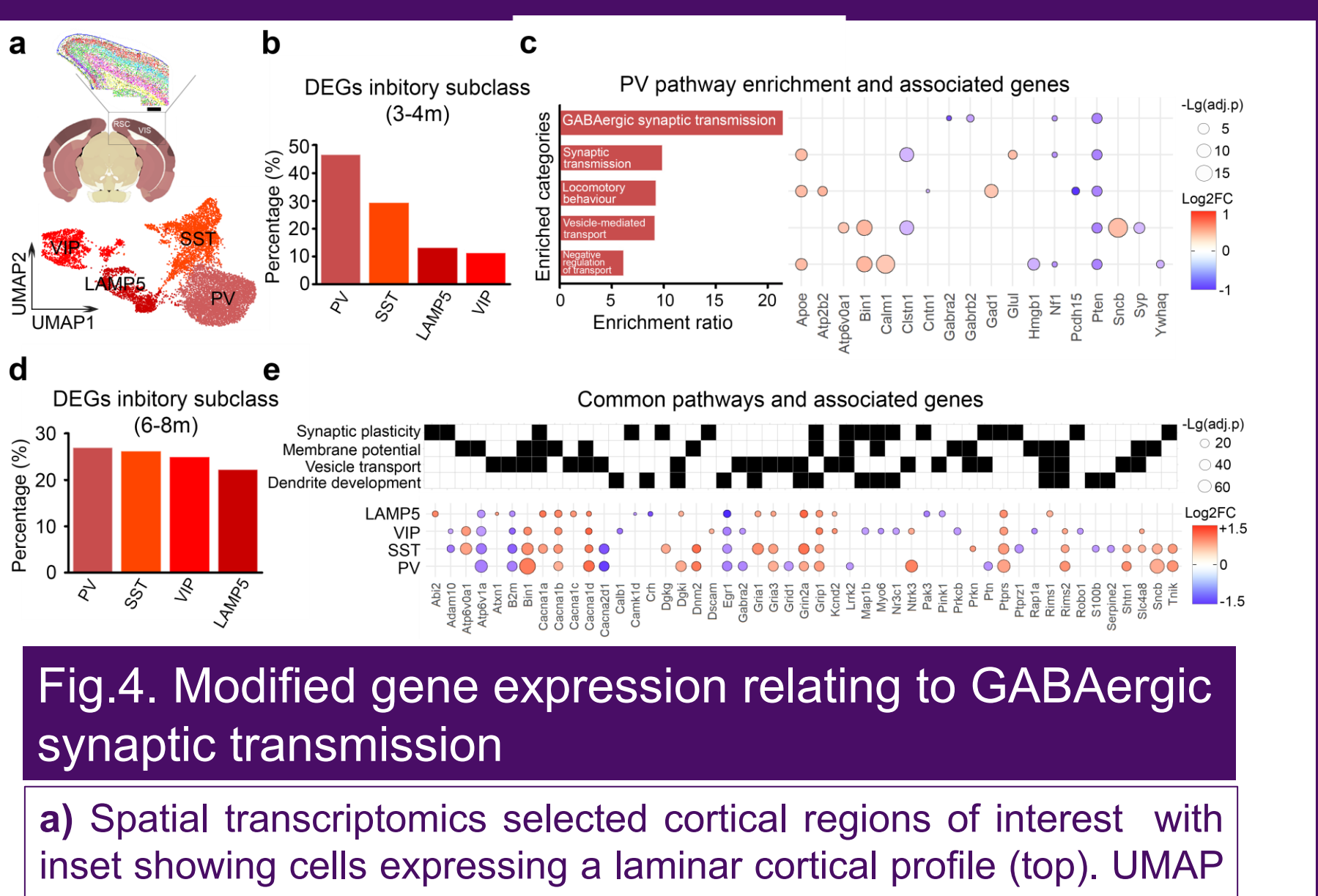


Fig.4. Modified gene expression relating to GABAergic synaptic transmission

a) Spatial transcriptomics selected cortical regions of interest with inset showing cells expressing a laminar cortical profile (top). UMAP plot of identified GABAergic subclasses (PV, SST, LAMP5 and VIP; bottom). Scale bar 300 μm. **b)** Percentage of DEGs across GABAergic subclasses when comparing App^{NL-G-F} and WT mice at 3-4m (Chi-squared test, p < 0.001). **c)** PV interneurons biological processes and associated genes following pathway enrichment at 3-4m. **d)** Percentage of DEGs across GABAergic subclasses for App^{NL-G-F} vs WT at 6-8m (Chi-squared test, p = 0.130). **e)** Subset GABAergic DEGs (dot plot) and top associated biological processes (grid plot) at 6-8m. DEG threshold: abs(log2FC) > 0.25 and adj. p < 0.05. Biological processes: ORA; BH-FDR < 0.05. Dot plots: -Lg (FDR) for size; Log2FC for colour bar. Grid plot: black shows association of genes (x-axis) with biological processes (y-axis).

Conclusion
We propose that early-stage AD-related synaptic pathophysiology is focused at population coupled synapses, with molecular measures implicating abnormal synaptic processing as an early-stage feature in parvalbumin-positive interneurons.
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