LOSS OF LAMP5 INTERNEURONS DRIVES NEURONAL NETWORK DYSFUNCTION IN ALZHEIMER'S DISEASE

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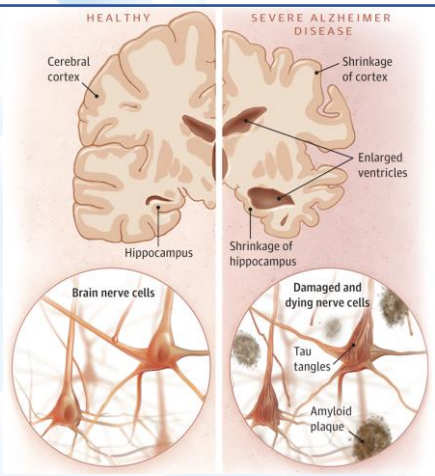
ABSTRACT

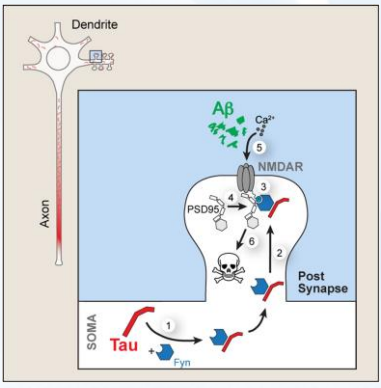
In Alzheimer's disease (AD), where amyloid-β (Aβ) and tau deposits in the brain, hyperexcitation of neuronal networks is an underlying disease mechanism, but its cause remains unclear. Here, we used the Collaborative Cross (CC) forward genetics mouse platform to identify modifier genes of neuronal hyperexcitation. We found LAMP5 as a novel regulator of hyperexcitation in mice, critical for the survival of distinct interneuron populations. Interestingly, synaptic LAMP5 was lost in AD brains and LAMP5 interneurons degenerated in different AD mouse models. Genetic reduction of LAMP5 augmented functional deficits and neuronal network hypersynchronisity in both Aβ- and tau-driven AD mouse models. To this end, our work defines the first specific function of LAMP5 interneurons in neuronal network hyperexcitation in AD and dementia with tau pathology.

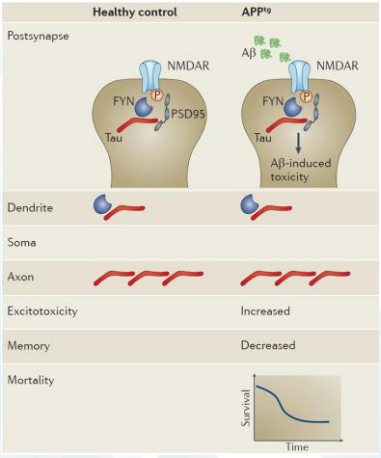
DRC Dementia Research Centre

BACKGROUND



Alzheimer's disease (AD) is the most common neurological disease and an increasing global health problem. The disease is characterised by extracellular Aβ plaques and intracellular tau tangles.





Others and us have previously reported tau-dependent neuronal hyperexcitation as a pathomechanism contributing to functional deficits in AD mouse models. Neuronal network dysfunction with spontaneous non-convulsive seizures have been reported in both AD patients and mouse models. However, the cellular events that mediate neuronal network dysfunction remain unclear.

Here, we used forward genetics in mice to identify modifier genes of neuronal hyperexcitation and reveal novel pathways that contribute to neuronal network dysfunction in AD. The combination of both Aβ and tau-dependent transgenic mouse models of AD with validation in human donor brain tissue led us to identify the loss of distinct interneuronal sub-populations likely contributing to neuronal network failure in AD pathogenesis.

References: Wiley, J. (2021) Alzheimer's & Dementia 17, 327-406; Ittner A, Ittner LM (2018) Neuron 99: 13-27; Ittner LM, et al (2010) Cell 142: 387-397; Ittner L *et al.*, (2011) *Nature Reviews Neuroscience*, 12: 67-72.

RESULTS

LAMP5 limits neuronal hyperexcitation in vivo

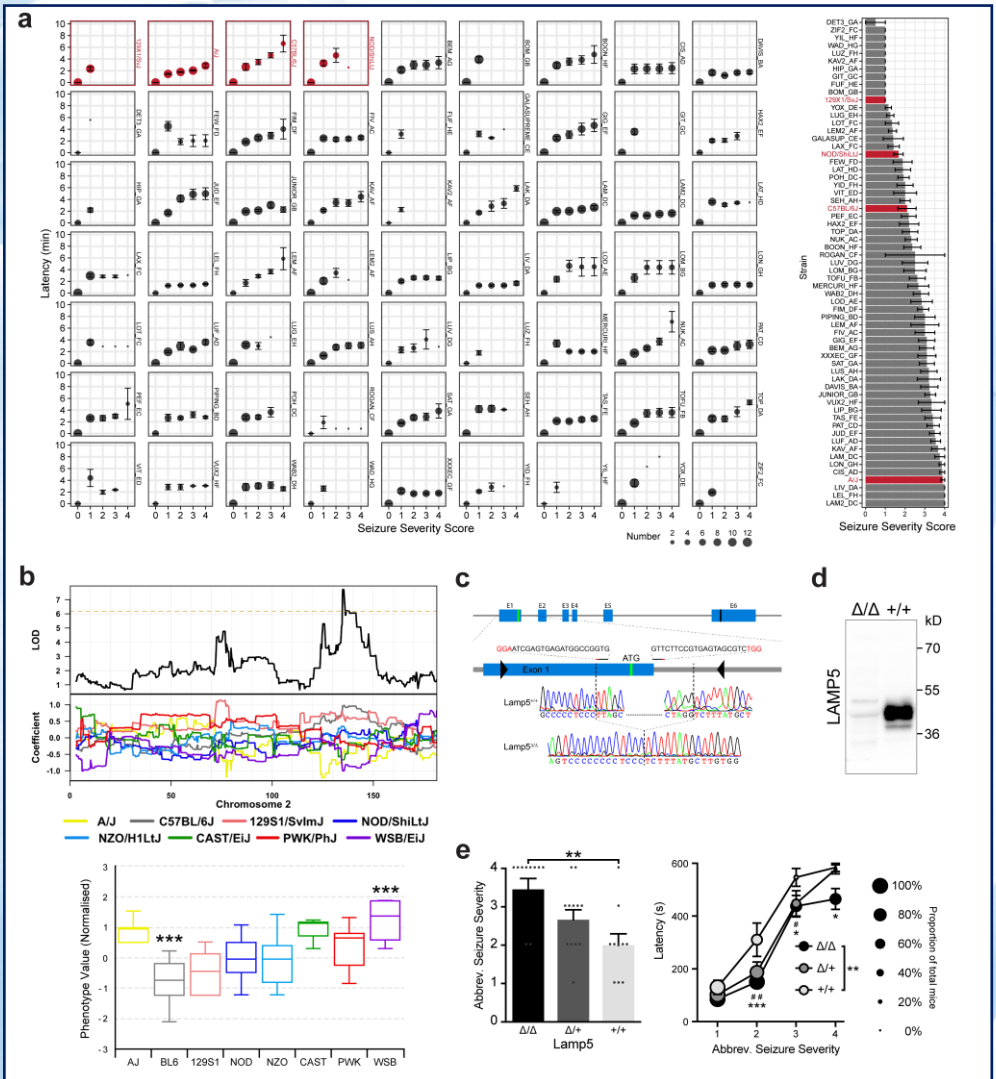


Fig. 1 (**a**) Latency to develop more severe seizures (*left*) and ranking of mean seizure severity score (*right*) reached within 10 minutes after intraperitoneal administration of 50mg/kg pentylenetetrazole (PTZ) in 59 collaborative cross (CC; black) and 4 inbred strains (red). Symbol size indicate number of mice that reached the indicated seizure severity score. (**b**) QTL mapping using mean seizure severity identified a seizure susceptibility locus on the murine chromosome 2 (*top/middle*) including contribution of the 8 CC background strains to this locus. (**c**) Gene targeting approach to generate Lamp5$^{\Delta/\Delta}$ mice by deleting 250 bp of the exon1/intron1 junction including the translational start codon (ATG) of the murine *Lamp5* locus, as confirmed by genomic sequencing. (**d**) Western blotting confirms absence of LAMP5 in Lamp5$^{\Delta/\Delta}$ mice. (**e**) Mean seizure severity (*left*) and latency to more severe seizures (*right*) in response to PTZ in Lamp5$^{+/+}$, Lamp5$^{\Delta/+}$, Lamp5$^{\Delta/\Delta}$ mice (n=10-12; *, p<0.05; **, p<0.01; ***, p<0.001; one-way ANOVA). Latency symbol sizes indicate proportion of mice reaching each seizure score.

RESULTS

Loss of LAMP5 interneurons in AD brains and models

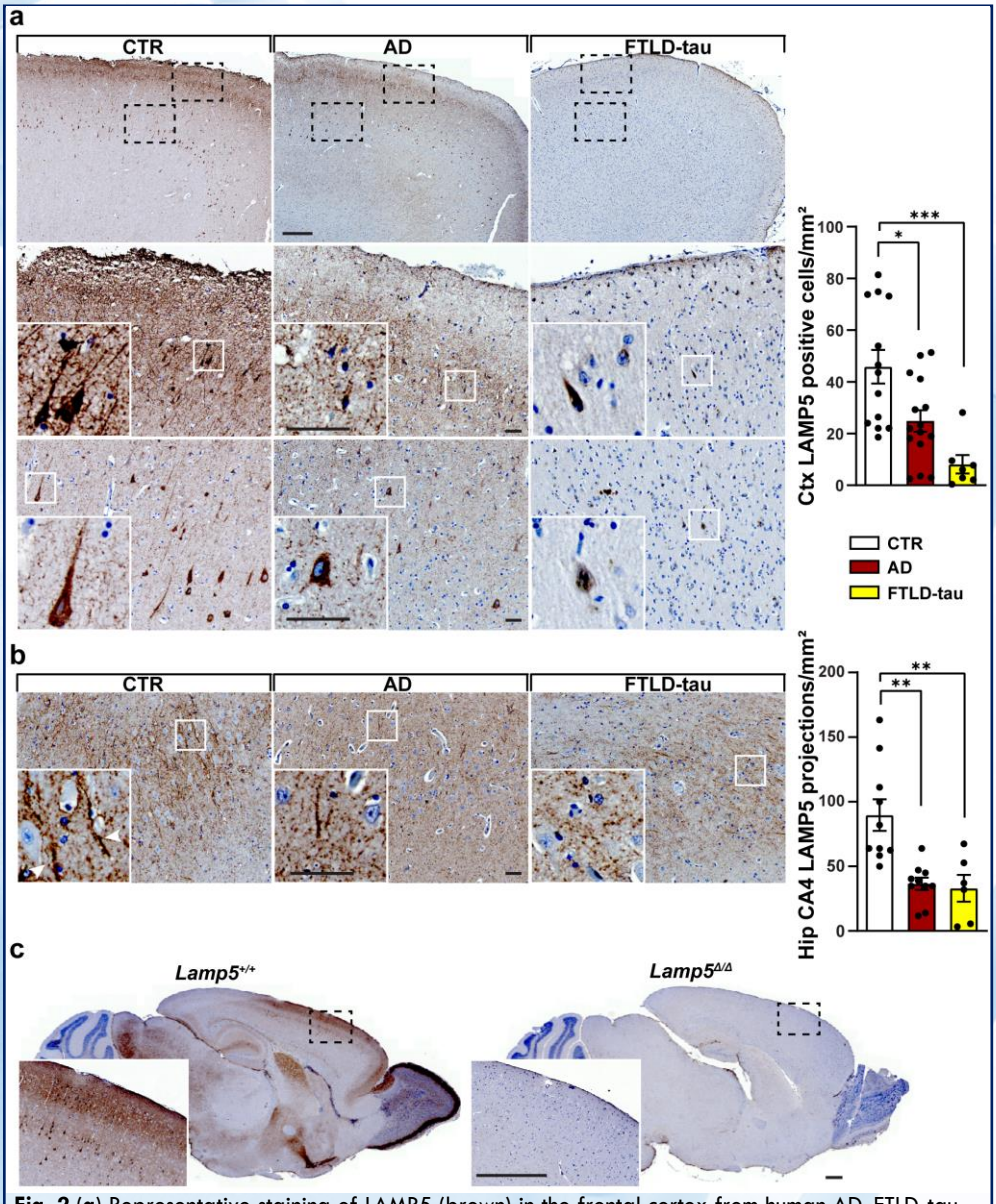


Fig. 2 (**a**) Representative staining of LAMP5 (brown) in the frontal cortex from human AD, FTLD-tau and control (CTR) brains. Overview (top row) and higher magnification (broken boxes) of layer I/II (middle row) and layer IV (bottom row) are shown. Insets show individually stained neurons. Quantification of numbers of LAMP5+ neurons (*, $p<0.05$; ***, $p<0.001$; Student *t* test). Scale bar, top row 500 μm, middle and bottom row 50 μm. (**b**) Representative staining of LAMP5 in the hippocampus (CA4) from human AD, FTLD-tau and CTR brains. Quantification of LAMP5+ projections (**, $p<0.01$; Student *t* test). Scale bar, 50 μm. (**c**) Sagittal sections of wild-type (*Lamp5*$^{+/+}$) and LAMP5 deficient (*Lamp5*$^{\Delta/\Delta}$) mouse brains stained for LAMP5 (brown) confirms antibody specificity. Higher magnification show frontal cortex (broken box). Scale bar, 500 μm.

RESULTS

Loss of LAMP5 interneurons in Aβ and tau-expressing mouse models of AD and FTLD-tau

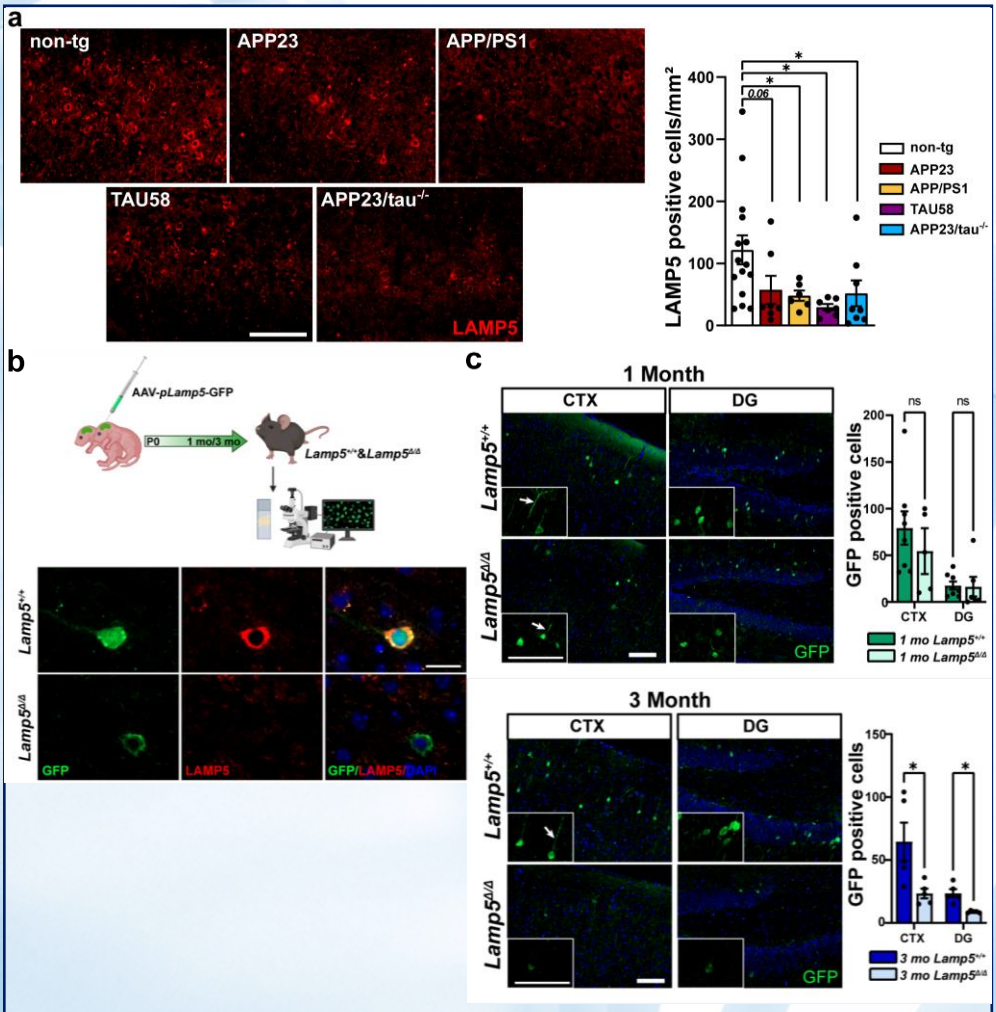


Fig. 3 (**a**) Representative staining of LAMP5 in 12-month-old of control (non-tg), APP23, APP/PS1, TAU58 and APP23/tau$^{-/-}$ brains. Quantification of numbers of LAMP5+ interneurons in the cortex of non-tg, APP23, APP/PS1, TAU58 and APP23/tau$^{-/-}$ mice (*, $p<0.05$, Student *t* test). Scale bar, 100 μm. (**b**) Newborn wild-type *Lamp5*$^{+/+}$ and *Lamp5*$^{\Delta/\Delta}$ littermates were injected with adeno-associated virus (AAV) for expression of green fluorescence protein (GFP) under control of the murine *Lamp5* promoter. At 1 month of age, GFP and LAMP5 expression were stained. Scale bar, 20 μm.(**c**) Representative imaging of AAV-mediated eGFP reporter activity controlled by the murine Lamp5 promoter in wild-type (Lamp5$^{+/+}$) and Lamp5$^{\Delta/\Delta}$ mice at 1 and 3 months of age. Insets show higher magnification of eGFP reporter-positive neurons, including lack of dendritic arborization in 3-month-old Lamp5$^{\Delta/\Delta}$ brains. Quantification of cells with Lamp5 promoter-driven eGFP reporter expression in the cortex (CTX) and dentate gyrus (DG) of Lamp5$^{+/+}$ and Lamp5$^{\Delta/\Delta}$ mice (*, $p<0.05$; Student *t* test). Scale bar, 100 μm.

RESULTS

LAMP5 reduction accelerates deficits of APP23 mice

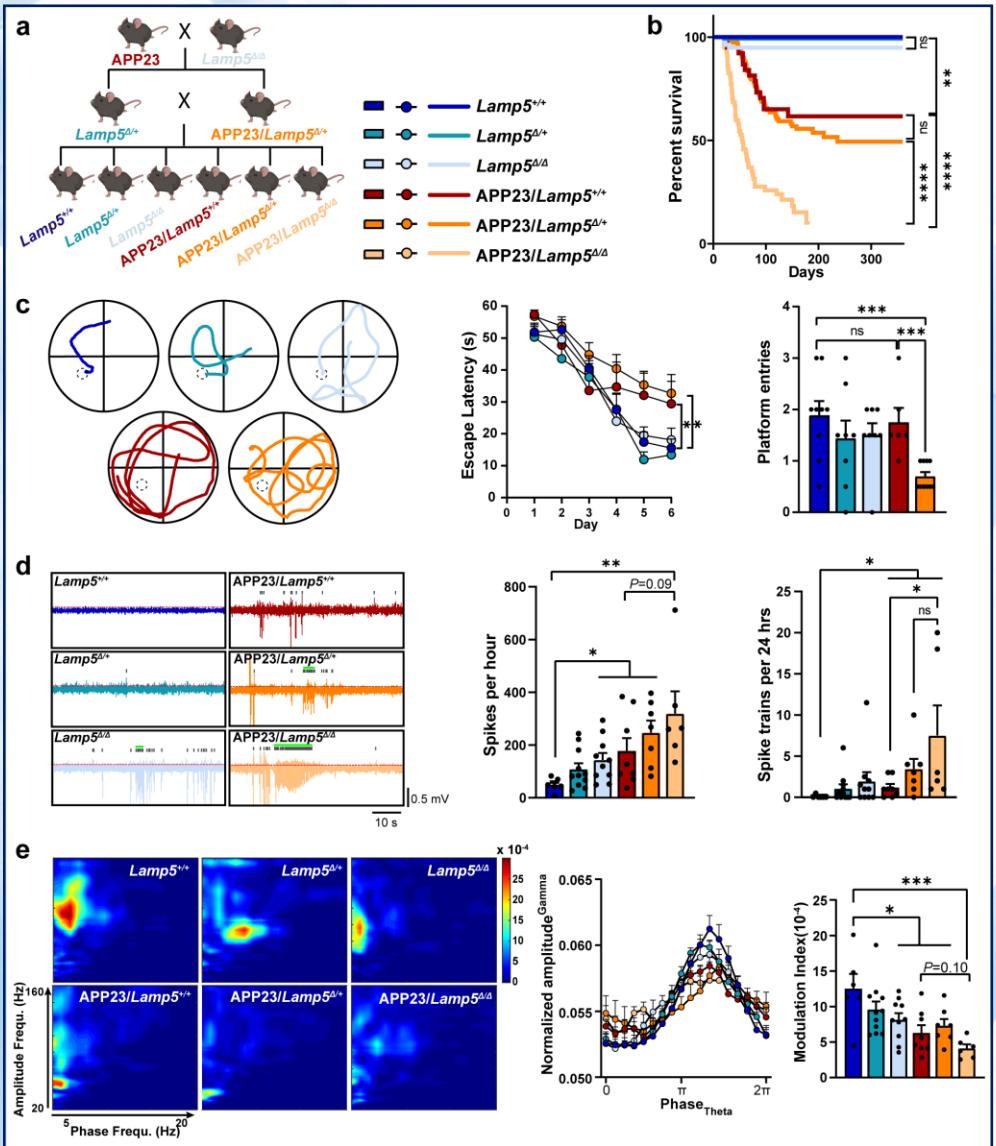


Fig. 4 (**a**) Crossbreeding scheme of APP23 and Lamp5$^{\Delta/\Delta}$ strains to obtain experimental colony. Color-coding of indicated genotypes apply to all figure panels. (**b**) Survival rate. (**c**) Memory testing at 12 months of age in the Morris water maze: example shown of day 6 swim traces per genotype (*left*), mean latency to find escape platform on individual days of the acquisition trials (*middle*) and platform zone entries during probe trials without escape platform on day 7 (*right*). (**d**) Hippocampal electroencephalography (EEG) recording in freely moving mice at 4.5 months of age: example of EEG traces showing episodes of spontaneous hyperexcitatory discharges (spikes: grey bars; spike trains: green bars).(**e**) Interictal cross-frequency coupling (CFC) of theta phase and gamma amplitude was disrupted in *Lamp5*$^{\Delta/\Delta}$ and APP23 crossings (*left*). Phase amplitude blots of interictal EEG recording with reduced CFC in APP23/*Lamp5*$^{\Delta/+}$ and more so in APP23/*Lamp5*$^{\Delta/\Delta}$ mice (*middle*). Modulation index of CFC (*right*) (*, p<0.05; ***, p<0.001; ns, not significant)

RESULTS

LAMP5 reduction augments impairments in TAU58 mice

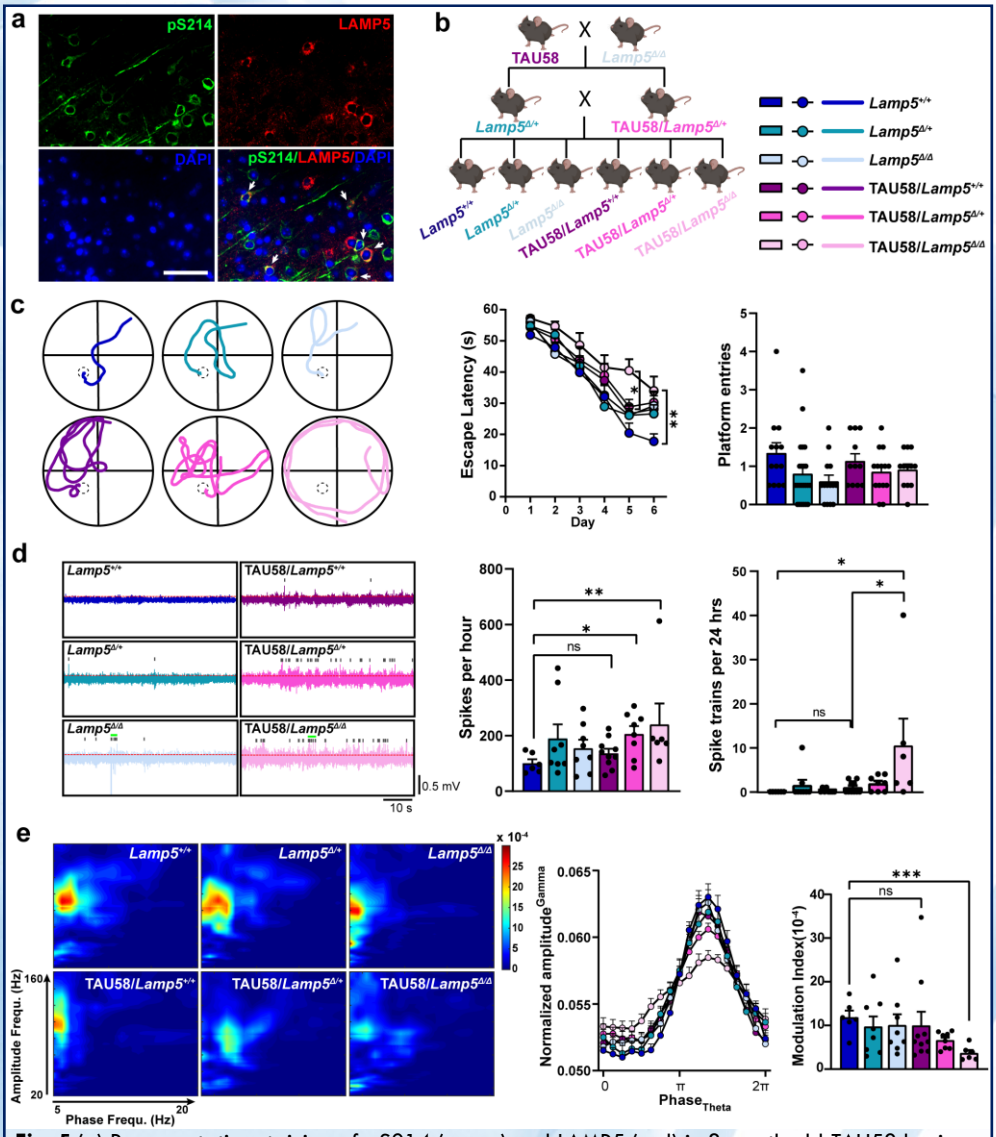


Fig. 5 (**a**) Representative staining of pS214 (green) and LAMP5 (red) in 3-month-old TAU58 brains. Scale bar, 50 µm. (**b**) Crossbreeding scheme of TAU58 and Lamp5$^{\Delta/\Delta}$ strains. (**c**) Memory testing at 6 months of age in the Morris water maze: example shown of day 6 swim traces per genotype (*left*), mean latency to find escape platform on individual days of the acquisition trials (*middle*) and platform zone entries during probe trials without escape platform on day 7 (*right*). (**d**) Hippocampal electroencephalography (EEG) recording in freely moving mice at 6.5 months of age: example shown of EEG traces showing episodes of spontaneous hyperexcitatory discharges (spikes: grey bars; spike trains: green bars) in *Lamp5*$^{\Delta/\Delta}$ and TAU58 crossings, and increase amplitudes in TAU58/*Lamp5*$^{\Delta/+}$ and TAU58/*Lamp5*$^{\Delta/\Delta}$ mice (*left*), quantification of spontaneous hyperexcitatory discharges (=spikes) per hour (*middle*) and occurrence of artifact-corrected spike trains during 24 hour EEG recordings (*right*). (**e**) Interictal cross-frequency coupling (CFC) of theta phase and gamma amplitude was disrupted in *Lamp5*$^{\Delta/\Delta}$, TAU58/Lamp5$^{\Delta/+}$ and more so in TAU58/Lamp5$^{\Delta/\Delta}$ mice (*left*). Phase amplitude blots (*middle*) and Modulation index of CFC (*right*) was compromised in TAU58/Lamp5$^{\Delta/\Delta}$ mice.

CONCLUSION

- *Lamp5* is a modifier gene of neuronal hyperexcitation
  - Collaborative Cross (CC) forward genetics mouse platform identified *Lamp5* as a vulnerable gene
  - Gene-dosage dependent increase of seizure severity in $Lamp5^{\Delta/+}$ and $Lamp5^{\Delta/\Delta}$ mice compared to $Lamp5^{+/+}$
- Loss of LAMP5 interneurons in AD brains and models
  - Decrease of LAMP5 expression in AD and FTLD-tau human brains
  - Loss of LAMP5 in APP23, APP/PS1 and TAU58 mouse models
  - Genetic reduction of LAMP5 led to the degeneration of LAMP5 interneurons
- LAMP5 reduction augmented functional deficits and neuronal network hypersynchronisity in Aβ- or tau-driven AD mouse models.

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