

A C57BL/6NJ Congenic Background Delays Neurodegeneration, Gliosis and Synaptic Loss in the PS19 Tauopathy Model

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Abstract

Background

The PS19/B6.C3F1 mouse model overexpressing human *P301S* tau is widely used in tauopathy research. However, its pathological progression is significantly modulated by its genetic background, limiting the translatability of preclinical findings. To isolate the *P301S* transgene effects from confounding variables, we characterized the PS19 line on a immunometabolically intact C57BL/6NJ background.

Methods

We performed regional mapping of tau pathology, gliosis, and synaptic integrity using immunohistochemistry, sarkosyl fractionation, Western blotting, and ELISA in PS19-NJ mice from 13 to 39/40 weeks of age. Tau lesions, microglial, astrocytic, and synaptophysin immunoreactivity were quantified.

Results

Tau pathology emerged at 26 weeks in the amygdala, hippocampus CA3/CA4, piriform cortex, and substantia nigra; by 32 weeks in CA1, entorhinal cortex, hypothalamus, locus coeruleus, red nucleus, and pontine reticular formation, with a marked increase by 39/40 weeks. Sarkosyl-insoluble tau was detectable at 32 weeks. Microglial activation increased at 32 weeks in parallel with tau burden, whereas astrogliosis was restricted to CA4 and CA1. Synaptophysin immunoreactivity indicated pronounced synaptic loss by 39/40 weeks.

Conclusions

Detailed analysis of temporal progression of disease pathology in metabolically intact C57BL/6NJ genetic background show delayed onset and progression of tau pathology, gliosis, and synaptic loss in PS19-NJ mice, providing greater temporal resolution than the model it was derived from, and eliminating confounding factors.

Introduction

Tauopathies comprise a diverse group of neurodegenerative diseases defined by the accumulation of pathological tau aggregates in the central nervous system, including Alzheimer's disease, Pick's disease, progressive supranuclear palsy, corticobasal degeneration and frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) [1, 2]. Tau normally stabilizes microtubules and supports axonal transport, but disease-associated post-translational modifications and pathogenic

MAPT mutations promote tau misfolding, microtubule dissociation and aggregation [3–7]. Among these mutations, P301S increases tau aggregation propensity and causes familial FTDP-17, providing the basis for the widely used PS19 mouse model, which overexpresses human 1N4R tau bearing the *P301S* mutation under the *Prnp* promoter [8, 9]. PS19 mice develop early synaptic dysfunction and microglial activation (starting at 3 months of age), followed by fibrillar tau pathology and cognitive decline (6 months), neuronal loss and regional brain atrophy (9 months), thereby modelling selected pathological features of human tauopathy [9, 10].

Despite its broad use, the original PS19 line was generated and maintained on a mixed C57BL/6×C3H F1 background and has been maintained as a B6;C3 hybrid stock. Genetic background composition and colony-maintenance practices may therefore contribute to heterogeneity and attenuated background-dependent immune and metabolic responses that could modify disease progression readouts and limit reproducibility [11, 12]. This variability has practical consequences for preclinical studies, with substantially larger cohorts required to detect therapeutic effects in mixed-background PS19 mice than in more genetically defined P301S models [13]. C57BL/6-backcrossed PS19 lines have since been developed and are now used alongside the original mixed-background line, reflecting the field's need for genetically standardized versions of this model [14]. However, C57BL/6 substrains are not equivalent, and background effects can reshape neurodegenerative phenotypes [15–17]. Therefore, a congenic PS19 line carrying the human *MAPT* P301S transgene in C57BL/6NJ background (which is expected to have low variability) was generated by Jax laboratory. Here, we characterize disease progression in these mice across brain regions and ages to determine whether this background preserves, attenuates or exacerbates the canonical PS19 phenotype, and to determine for which use cases the model is most suitable. Using immunohistochemistry for multiple tau phospho-epitopes and conformational states, biochemical analysis of sarkosyl-insoluble tau, quantification of total and phosphorylated tau, and assessment of gliosis, synaptic loss and neurodegeneration, we define the impact of the C57BL/6NJ background on the PS19 phenotype. Our findings establish a reference pathological profile for this congenic line, and provide a detailed temporal resolution for the emergence and accumulation of pathological changes.

Methods

Animals

The aim of this study was to characterize the PS19-NJ transgenic mouse model (JAX stock #024841) across disease progression. A total of 60 animals were included and distributed across five age groups: 13, 19, 26, 32, and 39/40 weeks ($n = 12$ per time point; 8 transgenic mice & 4 WT control mice), (3, 4.5, 6, 7.5 and 9 months respectively). These time points were selected to capture early, intermediate, and advanced stages of pathology. All animal procedures were performed in accordance with institutional and national guidelines for animal care and use and were approved by the relevant ethics committee and/or Institutional Animal Care and Use Committee. Jax laboratory performed the sacrifice and collected the right half hemisphere for biochemistry and the left half for immunohistochemistry analysis.

Tissue collection and allocation

Animals were deeply anesthetized with inhalant isoflurane prior to sacrifice. Initial perfusion with PBS was performed to remove blood. Brains were rapidly removed, hemisected sagittally and immediately frozen in dry ice and stored at -80°C. One hemisphere was assigned to immunohistochemistry and the contralateral hemisphere to biochemical analysis. For histological analysis, right hemispheres were frozen to be processed for cryo-embedding and sectioning. For biochemical analysis, frozen left hemispheres were retained at -80°C until homogenization and fractionation.

Tissue embedding and cryosectioning

Pre-frozen brain hemispheres were embedded in prechilled Killik O.C.T. embedding medium. Cryomolds and forceps were also prechilled on dry ice and embedding medium and 1× PBS were cooled on ice before use. Frozen hemispheres were maintained on dry ice during handling. To release tissue from the supporting cork, a small volume of prechilled PBS was applied. Hemispheres were oriented in cryomolds along the frontal-caudal axis and fully covered with embedding medium. Blocks were frozen on dry ice until the medium became opaque and were then transferred to - 80°C until sectioning. Before sectioning, embedded tissue blocks were equilibrated in the cryostat chamber for approximately 30–45 min. Coronal sections were cut at 10 µm thickness at - 20 to - 22°C and mounted onto charged glass slides. Slides were air-dried for 60 minutes at room temperature before staining. To ensure systematic and unbiased sampling across anatomically defined regions, a rotational sectioning strategy was employed.

For hippocampal and cortical analysis, sections were collected spanning Bregma - 1.70 mm to - 3.18 mm, defined based on anatomical and cytoarchitectural landmarks. For brainstem analysis, sections were collected from Bregma - 3.80 mm to - 6.30 mm, encompassing midbrain to medulla regions.

For each region, a total of 60 serial sections were collected per animal. Sections were mounted onto 30 slides, with three sections per slide. Sections were distributed in a rotational manner, such that consecutive sections on a slide were spaced at intervals of 30 sections, ensuring representative sampling across the rostro-caudal axis while minimizing regional bias.

Immunohistochemistry, immunofluorescence, and thioflavin-S staining

Sections were fixed in 4% paraformaldehyde for 10–15 min, washed three times in 1× PBS, and permeabilized in 0.3% Triton X-100 in PBS for 5 min. After blocking for 1 h at room temperature in a humidified chamber, sections were incubated overnight at 4°C with primary antibodies diluted in blocking solution.

Tau pathology was assessed using antibodies against phospho-tau epitopes Ser202/Thr205 (AT8), Thr212/Ser214 (AT100), Thr231 (AT180), Ser262, Ser396, and Ser422. Neuroinflammatory changes were evaluated using Iba1 and GFAP immunostaining to label microglia and astrocytes, respectively. Synaptic integrity was assessed using synaptophysin immunostaining. For selected analyses, double

immunofluorescence was performed using AT8 in combination with either Iba1 or GFAP to examine the spatial relationship between tau pathology and glial activation (**Supplementary Table S1**).

Following primary antibody incubation, sections were washed three times in PBS and incubated for 1 h at room temperature in the dark with fluorophore-conjugated secondary antibodies. Sections were then washed again thrice in PBS and mounted using an aqueous DAPI-containing mounting medium. Coverslips were sealed after mounting.

Fibrillar tau pathology was evaluated on designated sections by Thioflavin-S staining using previously published methods.

To ensure consistent analysis across animals and age groups, a predefined staining scheme was applied. Slides containing three rotationally distributed sections were assigned to either single-label or double-label immunofluorescence. Single-label staining was performed for AT100, AT180, phospho-tau Ser262, phospho-tau Ser396, phospho-tau Ser422, and synaptophysin. Double-label staining was performed for AT8/Iba1 and AT8/GFAP. Additional slides were reserved for Thioflavin-S staining, secondary-only controls, isotype controls, and repeat staining where required for technical validation.

Confocal microscopy

All stained sections were imaged on a ZEISS LSM710 confocal microscope using Zeiss Plan-Apochromat 20×/0.8 M27 objective. Raw image data were acquired using identical settings within each staining series. High-resolution acquisitions were performed at 1024 × 1024 pixels with a field size of 425.13 × 425.13 μm. For each staining series, low-magnification tiled images were first acquired to document the overall regional distribution of pathology, followed by high-resolution imaging of predefined regions of interest. Images were stored in the original .lsm file format.

Image analysis

Quantitative image analysis was performed using ImageJ/FIJI cross 12 region-of-interest areas: CA1, CA3, CA4, the amygdala, hypothalamus, entorhinal cortex, piriform cortex, thalamus, red nucleus, substantia nigra, pontine reticular nucleus, and locus coeruleus. For each antibody and anatomical region, analysis parameters were defined on a representative section and subsequently applied uniformly across the entire dataset. Phospho-tau and other diffuse immunoreactive signals were quantified by positive signal measurements, reported as signal positive area fraction (%). Iba1 and GFAP immunoreactivity were similarly quantified as signal positive area fraction as an index of glial responsiveness. For all analyses, image acquisition and quantification were performed using standardized settings across samples within each staining series. Where feasible, investigators were blinded to genotype and age group during image acquisition and downstream analysis. Values obtained from multiple sections for each animal were averaged to generate a single biological replicate.

Preparation of soluble and insoluble tau fractions

Frozen brain assigned to biochemical analysis was used to prepare pooled brain lysate fractions (S1 + S2), sarkosyl-soluble fractions (S3), and sarkosyl-insoluble fractions (3P). Tissue was homogenized for 30 s in a tenfold weight excess of ice-cold extraction buffer containing 20 mM Tris, pH 7.4, 800 mM NaCl, 1 mM EGTA, 1 mM EDTA, and 10% sucrose supplemented with protease inhibitors. Homogenization was performed in two steps. Tissue was first homogenized in half the final buffer volume and centrifuged, after which the pellet was re-homogenized using the same procedure. The resulting supernatants were pooled to generate the S1 + S2 fraction. Brain homogenates were centrifuged at $20,000 \times g$ for 20 min at 2°C. The pooled supernatants (S1 + S2) were transferred to fresh tubes. Sarkosyl was added to a final concentration of 1%, and samples were stirred for 1 h at room temperature. Samples were then centrifuged at $100,000 \times g$ for 1.5 h at room temperature. The supernatants were collected as the sarkosyl-soluble fraction (S3). Pellets were gently rinsed with a sarkosyl extraction buffer and centrifuged again at $100,000 \times g$ for 20 min at room temperature. The final pellet, corresponding to the sarkosyl-insoluble fraction (3P), was resuspended either in 6 M guanidine hydrochloride for ELISA or in $1\times$ loading buffer for western blot analysis to a final volume corresponding to 1/25 of the S1 + S2 fraction. Samples were sonicated for 5 min and stored at -20°C until analysis. Extraction of sarkosyl-insoluble tau was based on the method described by Greenberg and Davies [18].

Sandwich ELISA

Protein concentration of soluble fractions was determined by the Bradford method immediately after preparation of the pooled S1 + S2 fraction. These measurements were used to normalize input for downstream biochemical analyses. Then quantitative biochemical analysis of tau was performed on S1 + S2, S3, and 3P fractions using sandwich ELISA. Phospho-tau was measured using an AT8/DC190-HRP assay, whereas total tau was measured using a DC25/DC190-HRP assay. Ninety-six-well ELISA plates were coated overnight at 4°C with 2 µg/ml AT8 or DC25 antibody in PBS. Plates were washed five times with PBS containing 0.075% Tween-20 and blocked for 1 h at room temperature. Sarkosyl-insoluble fractions prepared in guanidine hydrochloride were diluted 1:50 in PBST and loaded in duplicate. Recombinant pathogenic tau 151–391/4R, phosphorylated in vitro and affinity-purified, was used to generate standard curves by twofold serial dilution in PBST. Plates containing standards and samples were incubated for 90 min at 37°C and then washed five times. DC190-HRP detection antibody was applied at a dilution of 1:15,000 in PBST and incubated for 60 min at 37°C. After washing, TMB substrate was added and plates were incubated for 20 min in the dark. The reaction was stopped with 0.25 M H₂SO₄, and absorbance was measured at 450 nm. Concentrations of phospho-tau and total tau were calculated by interpolation from the standard curve. Quantitative ELISA analyses were performed on soluble tau fractions and insoluble tau fractions.

Western blotting

Western blot analysis was performed on S1 + S2, S3, and 3P fractions. Samples were mixed with SDS sample loading buffer and heated at 95°C for 5 min. Proteins were separated on 5–20% gradient SDS-

polyacrylamide gels in a Tris-glycine-SDS buffer system for 40 min at 25 mA and transferred to nitrocellulose membranes.

Membranes were blocked in 5% fat-free dry milk in PBS for 1 h at room temperature and then incubated with primary antibodies against total tau (DC25), structurally modified tau (DC11), and phospho-tau species including AT8, pS396, pS262, and pS422. After washing, HRP-conjugated goat anti-mouse or goat anti-rabbit secondary antibodies diluted 1:3000 in PBS were applied. Blots were developed using chemiluminescence and imaged on an iBright imaging system. Soluble tau fractions (pooled S1 + S2 and S3) and insoluble tau fractions (3P) were analysed separately. Six tau isoforms were used as molecular weight controls, and Alzheimer's disease brain samples (Braak stage 6, Thal stage 5) were included as positive controls.

Statistical analysis

Statistical analyses were performed using RStudio and plotted with Graphpad Prism 11 software. Data are presented as median $\pm$ IQR unless otherwise stated. Comparisons across age groups and between transgenic and control animals were performed using non-parametric Kruskal-Wallis test (FDR (False Discovery Rate) or Benjamini-Hochberg (BH)-adjusted $p < 0.05$), as it is used to compare three or more independent groups, the data were non-normally distributed, contained outliers, and the observations were not repeatedly measured from the same subjects.

Results

1. Age-dependent accumulation of sarkosyl-insoluble tau in PS19NJ mice

The phenotype of the original PS19 model may be influenced by its mixed B6C3F1-derived genetic background. Subsequent congenic conversion onto defined C57BL/6 substrains reduces genetic-background heterogeneity but may also alter the timing, severity, and variability of the phenotype (Fig. 1a). The PS19NJ line characterized here was derived by backcrossing the original *Prnp*-MAPT*P301S PS19 line to C57BL/6NJ, resulting in a C57BL/6NJ-enriched PS19 colony (Fig. 1b, d). Given the limited characterization of PS19 mice on a C57BL/6NJ-enriched background (Fig. 1c), we examined male and female PS19NJ mice at 13, 19, 26, 32 and 39/40 weeks of age using complementary biochemical and histopathological approaches, with non-transgenic C57BL/6NJ mice serving as controls (Fig. 1e).

Biochemical analysis demonstrated a pronounced age-dependent redistribution of tau into the sarkosyl-insoluble fraction. Total tau and phosphorylated tau were quantified using DC25 and AT8 ELISAs, respectively, in total brain lysate and in sarkosyl-soluble and sarkosyl-insoluble fractions (Fig. 2a, c, e, g, h, i). The most prominent age-associated changes were confined to the insoluble pool. Insoluble total tau concentrations measured by DC25 remained comparatively low between 13 and 26 weeks, increased at

32 weeks and reached a median of 2900 ng/mL at 39/40 weeks, compared with 735 ng/mL at 13 weeks ($H(4) = 20.22$, $q = 0.0014$) (Fig. 2e). Insoluble AT8-positive tau followed a similar but steeper course; it was present at very low concentrations at 13 weeks, increased progressively through 19 and 26 weeks, became more variable among animals at 32 weeks and rose sharply by 39/40 weeks (Fig. 2k). Median concentrations increased from 0.445 ng/mL at 13 weeks to 476.880 ng/mL at 39/40 weeks ($H(4) = 29.18$, FDR-adjusted $q < 0.0001$). This trajectory indicates that the initial accumulation of insoluble phospho-tau was followed by a marked late-stage transition between 32 and 39/40 weeks. The divergent accumulation profiles of DC25- and AT8-reactive tau indicate that expansion of the total sarkosyl-insoluble tau pool was accompanied by pronounced enrichment of AT8-reactive tau, rather than a proportional increase in total and phosphorylated tau. In contrast, DC25 concentrations in total brain lysate and the sarkosyl-soluble fraction did not change significantly across age (Fig. 2a,c). Sarkosyl-soluble AT8 also remained stable, while the comparatively modest age effect for AT8 in total brain lysate did not withstand correction for multiple comparisons (Fig. 2g, h). Thus, disease progression in PS19NJ mice was not characterized by a generalized age-dependent increase in brain tau expression. Instead, it reflected the progressive sequestration of total and phosphorylated tau into the detergent-insoluble fraction. At 39/40 weeks, this biochemical phenotype clearly distinguished PS19NJ mice from C57BL/6NJ controls. Median insoluble AT8 reached 476.880 ng/mL in PS19NJ mice but remained undetectable in controls, producing complete separation between the groups. Median insoluble DC25 was similarly elevated at 2900 ng/mL in PS19NJ mice compared with 110 ng/mL in controls. Although terminal PS19NJ mice also exhibited higher concentrations of tau than controls in the total and sarkosyl-soluble fractions, these compartments did not increase progressively with age within the PS19NJ cohort. These differences are therefore consistent with transgene-associated tau expression, whereas expansion of the sarkosyl-insoluble pool represents the progressive pathological component of the phenotype.

To assess whether the increase in insoluble AT8 immunoreactivity was proportional to the accumulation of total insoluble tau, an apparent AT8-to-DC25 immunoreactivity ratio was calculated for each animal by dividing the AT8 concentration by the corresponding DC25 concentration. The median ratio increased from 0.000757 at 13 weeks to 0.00661, 0.01795, 0.03733 and 0.174 at 19, 26, 32 and 39/40 weeks, respectively (Kruskal-Wallis $H(4) = 30.17$, $P = 4.53 \times 10^{-6}$). At 39/40 weeks, the ratio was significantly higher than at each younger age (all Holm-adjusted $P = 6.22 \times 10^{-4}$), representing an approximately 230-fold increase relative to 13 weeks. Thus, accumulation of sarkosyl-insoluble tau was accompanied by a disproportionate increase in AT8 immunoreactivity.

Qualitative immunoblotting supported the temporal pattern identified by ELISA (Fig. 2b, d, f, j–l). DC25-reactive tau was detectable in the total and sarkosyl-derived fractions throughout the age course, consistent with expression of the human tau transgene (Fig. 2b, d, f). AT8 immunoreactivity was also observed in the total and sarkosyl-derived fractions of PS19NJ mice (Fig. 2j, k). In the most highly insoluble 3P fraction, however, DC25 and AT8 immunoreactivity was absent or below visual detection at

early ages and became clearly detectable at 32 and 39/40 weeks (Fig. 2f, l). Corresponding 3P signals were not evident in C57BL/6NJ controls.

Immunoblotting for additional phospho-tau epitopes showed a similar age-dependent redistribution (**Fig. S1a–c**). pSer262-, pSer396- and pSer422-reactive tau was detectable in the total brain lysate and sarkosyl-soluble fractions of PS19NJ mice across the age series. In contrast, immunoreactivity in the sarkosyl-insoluble 3P fraction was absent or below visual detection at early ages and became clearly detectable at 32 and 39/40 weeks. Corresponding 3P signals were not evident in C57BL/6NJ controls. Consistent with the biochemical findings, terminal-age PS19NJ brain sections showed immunoreactivity for AT8, AT100, AT180, pSer396 and pSer422, together with Thioflavin S-positive deposits and phospho-tau-positive neuronal profiles (**Fig. S1d,e**). This concordant appearance of multiple phospho-tau epitopes in the highly insoluble fraction and brain tissue supported the development of advanced multisite tau pathology (**Fig. S1a–e**).

2. Progressive and regionally selective phospho-tau pathology in PS19NJ mice

To determine how tau pathology develops anatomically in PS19NJ mice, phospho-tau immunoreactivity was examined across 12 brain regions using five antibodies recognizing distinct phosphorylation sites: AT8 (pSer202/pThr205), AT100 (pThr212/pSer214), AT180 (pThr231), pSer396 and pSer422. The regions were selected to encompass interconnected hippocampal, cortical, limbic, diencephalic, midbrain and brainstem structures implicated in the development and propagation of tau pathology (Fig. 3a,b). At the animal level, all five phospho-tau markers increased significantly with age after correction for multiple testing (all FDR-adjusted $q \leq 1.25 \times 10^{-4}$). Immunoreactivity was generally low at 13 weeks, became detectable at intermediate ages in selected regions and increased markedly by 39/40 weeks. For each antibody, terminal phospho-tau burden exceeded that observed at every younger age. The convergence of these markers at the terminal stage indicates that advanced pathology in PS19NJ mice is characterized by phosphorylation of tau at multiple epitopes rather than by the isolated appearance of a single phospho-tau species.

AT8 immunoreactivity was initially sparse and regionally restricted but became progressively more extensive with age. The complete age-by-region image series illustrates the emergence of AT8-positive neuritic and somatic profiles across the PS19NJ disease course (**Fig. S2**). Initial AT8 immunoreactivity is observed most prominently in the amygdala, and to a lesser degree in CA4 and EC at 26 weeks. At 39/40 weeks, abundant AT8 immunoreactivity was evident in hippocampal regions, including CA1, CA3 and CA4, as well as in the amygdala, hypothalamus, entorhinal cortex, substantia nigra, pontine reticular nucleus and locus coeruleus (Fig. 3c). Quantification confirmed that the terminal increase was not confined to a single anatomical system but involved structures distributed throughout the rostrocaudal neuraxis (Fig. 3d–f,h–p). The regional heatmap demonstrates that the magnitude and timing of AT8 accumulation differed between structures, with particularly prominent terminal involvement of CA4, CA1,

amygdala and pontine reticular nuclei (Fig. 3g). Despite this coordinated temporal progression, the anatomical distribution differed among phosphorylation sites. AT8 showed significant age-dependent progression in 11 of the 12 regions examined, encompassing CA1, CA3, dentate gyrus, entorhinal and piriform cortices, amygdala, hypothalamus, thalamus, red nucleus, locus coeruleus and substantia nigra (Fig. 3d–p). Overall, AT8 pathology extended from hippocampal and cortical regions into limbic and diencephalic structures and several vulnerable midbrain and brainstem nuclei.

AT100 and AT180 each demonstrated significant age effects in 11 regions (**Figs. S3 and S4**, respectively). Both markers showed broad involvement of hippocampal, cortical and limbic structures together with diencephalic, midbrain and brainstem nuclei. Their extensive regional distributions closely paralleled the widespread terminal AT8 phenotype, supporting the emergence of a multisite phospho-tau state during advanced disease.

The pSer396 epitope also showed broad progression, with significant age effects in 10 regions (**Fig. S5**). These included CA1, CA3, dentate gyrus, entorhinal and piriform cortices, amygdala, hypothalamus, locus coeruleus, pontine reticular nucleus and substantia nigra. The pSer396 pattern therefore spanned forebrain and brainstem structures but was somewhat restricted compared to the patterns observed with AT8, AT100 and AT180.

In contrast, pSer422 displayed the most anatomically selective progression (**Fig. S6**). Significant age effects were detected in CA1, dentate gyrus, amygdala, hypothalamus, locus coeruleus and pontine reticular nucleus. The restricted pSer422 distribution, despite its pronounced terminal increase at the animal level, indicates that individual phosphorylation events do not occur uniformly throughout the affected neuroanatomical network. Rather, the regional biochemical environment or cellular composition may influence the phosphorylation state acquired by accumulating tau.

Phospho-tau burdens measured with the five antibodies were positively correlated across animals. The strongest unadjusted relationships involved AT8, AT100, AT180 and pSer396, consistent with the coordinated accumulation of multiple phosphorylation sites as pathology advanced. Most associations were substantially attenuated after adjustment for age, indicating that the covariance among markers was driven primarily by their shared progression through disease stages rather than by a strong age-independent relationship between epitopes.

Exploratory analyses suggested sex-associated differences in AT8 and AT180 burden and an age-by-sex interaction for pSer396. The pSer396 interaction reflected greater terminal immunoreactivity in females, whereas the AT8 sex difference was driven predominantly by higher staining in males at early and intermediate ages, with similar terminal burdens in both sexes. Because these patterns were not consistent across antibodies and the individual age-by-sex groups were small, they were interpreted as hypothesis-generating rather than as evidence of a robust sex-dependent phenotype.

3. Widespread Iba1-positive microgliosis accompanies tau progression in PS19NJ mice

To determine whether the development of phospho-tau pathology was accompanied by a microglial response, Iba1-positive area coverage was quantified across the same 12 brain regions and five age groups as examined for tau pathology. At week 39/40, PS19NJ mice demonstrated prominent Iba1 immunoreactivity in hippocampal, cortical, limbic, hypothalamic, midbrain and brainstem structures (Fig. 4a). The complete age-by-region image series showed the progressive appearance of Iba1-positive profiles throughout the disease course (Fig. S7).

At the animal level, Iba1-positive area remained comparatively low between 13 and 26 weeks, increased markedly by 32 weeks and remained elevated at 39/40 weeks (FDR-adjusted $q = 1.25 \times 10^{-4}$). Regional quantification demonstrated significant age-dependent changes in all 12 regions examined: CA1, CA3, dentate gyrus, amygdala, hypothalamus, thalamus, entorhinal cortex, piriform cortex, red nucleus, substantia nigra, pontine reticular nucleus and locus coeruleus (Fig. 4b–m). Microgliosis was therefore not restricted to regions with the greatest phospho-tau burden but developed across a broad neuroanatomical network.

The timing and magnitude of this response differed among regions. In several structures, including CA1, CA3, amygdala, hypothalamus, thalamus, entorhinal cortex, piriform cortex, red nucleus, substantia nigra and locus coeruleus, Iba1-positive area increased prominently by 32 weeks (Fig. 4b–k,m). Within the pontine reticular nucleus, the microglial response emerged by week 26 and exhibited a monotonic accumulation that reached its zenith at 39/40 weeks, appearing to precede pathological AT8 + tau accumulation in this region (Fig. 4l). Some regions showed substantial inter-animal variability at 32 and 39/40 weeks, indicating heterogeneity in the magnitude or timing of the microglial response during the transition to advanced pathology.

At 39/40 weeks, the median animal-level Iba1-positive area was 4.401% in PS19NJ mice compared with 0.620% in C57BL/6NJ controls ($q = 0.012$). Significant terminal elevations were detected in eight regions, including CA1, dentate gyrus, amygdala, hypothalamus, piriform cortex, thalamus, pontine reticular nucleus and substantia nigra (Fig. 4b–m). Across animals, the Iba1-positive area was strongly associated with the phospho-tau burden. Iba1 and AT8 immunoreactivity were positively correlated in the unadjusted analysis (Spearman $\rho = 0.71$, FDR-adjusted $q = 2.5 \times 10^{-6}$), consistent with the parallel expansion of microgliosis and phospho-tau pathology across disease stages. This association was substantially attenuated after adjustment for age and sex, indicating that it was driven primarily by their shared temporal progression rather than by a stable relationship independent of disease stage. Their temporal profiles were nevertheless not identical: microgliosis became prominent by 32 weeks, whereas the largest increase in insoluble and tissue AT8 burden occurred between 32 and 39/40 weeks. The microglial response thus plateaued in some regions, while phospho-tau burden continued to accumulate.

4. Astrogliosis is more regionally restricted than microgliosis in PS19NJ mice

Astroglial response was evaluated by quantifying GFAP-positive area coverage across the same 12 brain regions examined for phospho-tau and Iba1 pathology. Representative images from 39/40-week PS19NJ mice demonstrated prominent GFAP-positive astrocytic processes in selected hippocampal, limbic and subcortical regions (Fig. 5a). The complete age-by-region image series further illustrated the spatially heterogeneous development of GFAP immunoreactivity across the disease course (**Fig. S8**). At the animal level, GFAP-positive area remained comparatively stable between 13 and 26 weeks, increased by 32 weeks and remained elevated at 39/40 weeks. The overall age effect was significant after correction across the IHC markers (FDR-adjusted $q = 0.0106$). The timing of this increase broadly coincided with the emergence of pronounced microgliosis, placing the astroglial response within the transition from intermediate to advanced tau pathology. In contrast to the widespread Iba1-microglia response, however, significant age-dependent GFAP-astrocytes changes were detected in only four of the 12 regions examined: CA1, the dentate gyrus/CA4 region, amygdala and substantia nigra (Fig. 5b,d,e,k). These regions represent anatomically distinct hippocampal, limbic and midbrain compartments, indicating that astrogliosis was not confined to a single neural system. Nevertheless, the absence of significant age effects in the remaining regions demonstrates that the astroglial response was considerably more selective than the microglial response, which progressed significantly in all 12 regions. The regional trajectories also differed in their timing and magnitude. GFAP-positive area increased prominently at 32 weeks in CA1, the dentate gyrus/CA4 region and amygdala and remained elevated, although variable, at 39/40 weeks (Fig. 5b,d,e). In the substantia nigra, the increase was most evident at the terminal age (Fig. 5k). By comparison, CA3, hypothalamus, thalamus, entorhinal cortex, piriform cortex, pontine reticular nucleus and locus coeruleus showed substantial baseline or inter-animal variability without a statistically supported age-dependent trajectory (Fig. 5c,f–i,l,m). This heterogeneity indicates that increasing tau pathology did not elicit a uniform astrocytic response throughout the brain and may reflect a feed-forward interaction in which tau pathology and microgliosis promote astrocytosis, which could further exacerbate tau pathology. At 39/40 weeks, median GFAP-positive area was 4.946% in PS19NJ mice compared with 3.436% in C57BL/6NJ controls. The terminal genotype difference remained significant after correction across the three non-tau IHC markers, although the separation was modest (FDR-adjusted $q = 0.048$). Regionally, significant terminal elevations were detected in the substantia nigra and red nucleus (Fig. 5j,k). The red nucleus therefore showed a terminal genotype difference despite the absence of a significant overall age trajectory, suggesting a late or heterogeneous astroglial response that was not adequately represented by a monotonic age-dependent pattern. GFAP and Iba1 burdens were positively correlated across animals in the unadjusted analysis (Spearman $\rho = 0.67$, FDR-adjusted $q = 1.3 \times 10^{-5}$), consistent with the emergence of astrocytic and microglial responses during the same broad phase of disease. This association weakened after adjustment for age and sex, indicating that much of their covariance reflected shared progression across disease stages. Their markedly different anatomical distributions further demonstrate that astroglial and microglial changes were not equivalent components of a uniform inflammatory response.

5. Synaptophysin immunoreactivity declines as tau and glial pathology increase in PS19NJ mice

To assess whether the progression of tau and glial pathology was accompanied by disruption of the presynaptic compartment, synaptophysin-positive area was quantified across the same 12 brain regions. Synaptophysin is an integral synaptic-vesicle protein enriched in presynaptic terminals, and reduced tissue immunoreactivity may therefore reflect loss of presynaptic terminals, depletion of synaptic vesicles or altered synaptophysin expression within surviving terminals. Representative images from 39/40-week PS19NJ mice showed reduced and spatially heterogeneous synaptophysin labelling across hippocampal, cortical, limbic, hypothalamic, midbrain and brainstem regions (Fig. 6a). The complete age-by-region image series demonstrated the progressive development of this phenotype (**Fig. S9**).

Synaptophysin immunoreactivity declined progressively with age. Median animal-level synaptophysin-positive area decreased from 28.829% at 13 weeks to 26.150% at 19 weeks, 22.948% at 26 weeks, 20.723% at 32 weeks and 18.134% at 39/40 weeks. The overall age effect remained significant after correction across the IHC markers (FDR-adjusted $q = 0.0014$). The monotonic decline indicates that presynaptic abnormalities developed gradually across the PS19NJ disease course rather than appearing only at the terminal stage.

Significant age effects were detected in 11 of the 12 regions examined: CA3, dentate gyrus/CA4, amygdala, hypothalamus, thalamus, entorhinal cortex, piriform cortex, red nucleus, substantia nigra, pontine reticular nucleus and locus coeruleus (Fig. 6c–m). CA1 was the only region without a statistically supported age-dependent decline (Fig. 6b). The synaptophysin phenotype was therefore anatomically widespread and encompassed forebrain and brainstem structures, closely matching the broad distribution of phospho-tau pathology and microgliosis.

The regional trajectories nevertheless differed in magnitude and timing. Progressive reductions were especially apparent in CA3, the dentate gyrus/CA4 region, amygdala, hypothalamus, thalamus, entorhinal and piriform cortices, substantia nigra, pontine reticular nucleus and locus coeruleus (Fig. 6c–i,k–m). In several regions, lower synaptophysin immunoreactivity was already evident at 26 or 32 weeks, before the maximal terminal accumulation of AT8-positive tau. This temporal pattern suggests that presynaptic compromise develops during the intermediate stages of disease and becomes progressively more widespread as tau and glial pathology advance.

At 39/40 weeks, median synaptophysin-positive area was 18.134% in PS19NJ mice compared with 31.409% in C57BL/6NJ controls. All terminal PS19NJ values were below the corresponding control values, resulting in complete separation between the groups (median difference – 13.274 percentage points, 95% CI – 16.462 to – 10.023; rank-biserial $r = -1.00$; FDR-adjusted $q = 0.012$). Significant terminal reductions were detected in 10 regions: CA3, dentate gyrus/CA4, amygdala, hypothalamus, thalamus, entorhinal cortex, red nucleus, substantia nigra, pontine reticular nucleus and locus coeruleus (Fig. 6c–

h,j-m). Together, the longitudinal and terminal comparisons establish reduced synaptophysin immunoreactivity as a robust component of the PS19NJ phenotype.

Across animals, synaptophysin and Iba1 immunoreactivity followed opposing trajectories and were inversely correlated (Spearman $\rho = -0.64$, FDR-adjusted $q = 3.5 \times 10^{-5}$). Animals with greater microglial burden therefore tended to exhibit lower synaptophysin immunoreactivity. The relationship was attenuated after adjustment for age and sex ($\rho = -0.34$) and did not remain significant after correction across the cross-marker comparisons. Thus, the association largely reflects the concurrent progression of microgliosis and presynaptic pathology across disease stages and does not establish that microglia directly caused the synaptophysin reduction.

AT8 and synaptophysin immunoreactivity showed a similar inverse cross-sectional association ($\rho = -0.45$, $q = 0.0053$), which was also abolished after accounting for age and sex. At the age-group level, however, median AT8 burden increased as median synaptophysin immunoreactivity declined. Importantly, the synaptophysin reduction was apparent at intermediate ages, before the sharp terminal increase in AT8-positive tau. Presynaptic abnormalities therefore accompanied the developing tauopathy and were not restricted to animals with maximal end-stage phospho-tau accumulation.

Discussion

The PS19 mouse has been one of the most widely used tauopathy models since its original description on a hybrid B6C3H/F1 background [9], but the genetic heterogeneity of that stock introduces uncontrolled phenotypic variability driven by C3H-derived modifier alleles unrelated to the tau transgene [13]. These include a loss-of-function *Tlr4* allele that attenuates innate immune tone [19] and metabolic modifiers that confer strong resistance to diet-induced obesity [20], confounding any metabolic challenge applied to the hybrid. The existing B6J congenic line eliminates C3H heterogeneity but introduces a distinct problem: the B6J substrain carries a multi-exon deletion in *Nnt*, impairing mitochondrial NADPH regeneration and antioxidant defence [21], and shows metabolic and neuroimmune characteristics that differ substantially from B6NJ [22]. For studies interrogating the interaction of tau pathology with metabolic stress the two substrains are not interchangeable. The PS19NJ line described here addresses this gap by providing a fully defined B6NJ-congenic tauopathy model compatible with the growing ecosystem of B6NJ-background transgenic and dietary paradigms.

PS19NJ mice develop a progressive tauopathy that faithfully recapitulates the hallmark features of the parental model. Sarkosyl-insoluble tau accumulated across all phospho-epitopes examined, pS202, pT205, pS262, pS396, pS422 with a more than 1,000-fold increase in insoluble AT8 phospho-tau between 13 and 39/40 weeks by ELISA, alongside a stable soluble tau pool throughout, confirming progressive sequestration of human tau into the detergent-insoluble aggregate compartment. Phospho-tau species was most consistently found in the amygdala, hippocampal subfields, piriform cortex, and brainstem nuclei, recapitulating the regional distribution reported for the original line [9]. The pathological onset was, however, temporally attenuated relative to the hybrid: sarkosyl-insoluble tau was absent at 13

weeks and emerged robustly only from 32 weeks onward, compared to the 16–20-week onset reported in B6C3H/F1 PS19 mice [9]. A comparable right-shift has been noted in the B6J congenic line [23], pointing to C3H modifier removal as the primary driver rather than any specific B6NJ property. The attenuation extends the pre-tangle and early-tangle window, widens the therapeutic interval for intervention studies, and facilitates dissection of early pathological events from end-stage neurodegeneration, features that complement rather than undermine the model's utility relative to the more aggressive hybrid or rTg4510 lines.

Ser262, phosphorylated by MARK kinases within the KXGS motif of the microtubule-binding domain, promotes tau detachment from microtubules and is considered an early event in the transition to free cytoplasmic tau [24]. Its restriction to the insoluble pool in PS19NJ mice suggests that once tau enters the aggregate pathway, MARK-mediated Ser262 phosphorylation becomes stably incorporated into the fibrillar core, and the temporal emergence of insoluble pS262 paralleling pS202, pT205 and preceding maximal pS396 and pS422 supports its use as a marker of intermediate aggregate maturation in this model.

The neuroinflammatory phenotype of PS19NJ mice is characterised by an association between microglial activation and tau pathology burden: Iba1 reactivity reached significance in all 12 sampled regions, including areas with modest tau load, with regions such as CA4, amygdala, or locus coeruleus showing pronounced microglial response by week 32. The broad microglial response likely reflects activation driven by soluble tau oligomers and axonal pathology rather than insoluble tangles per se [25, 26], which aligns with the observation that Iba1 and AT8 reactivity are not proportional across regions. Meanwhile, statistically significant GFAP elevation compared to wild-type controls was restricted to the CA4, SN, red nucleus, and thalamus at end-stage; this spatially restricted astrogliosis possibly mirrors the closer coupling of reactive astrocytes to frank neurodegeneration reported in human tauopathies [27, 28]; another possible explanation is the differential reactivity of rodents' astrocytes with anti-GFAP antibodies, with most hippocampal astrocytes but only a small subset of their cortical counterparts staining positive for GFAP [29]. Time-dependent synaptophysin loss was observed across 11 of the 12 regions examined; CA1 alone exhibited synaptophysin values indistinguishable from controls at w39/40, distinguishing PS19NJ from the original hybrid line where hippocampal synaptic loss is detectable by six months [30]. Statistically significant differences from controls were observed in brainstem regions (the red nucleus and PRN), consistent with the high tau burden in LC and PRN projection neurons and the early axonal pathology characteristic of PS19 brainstem. Numerical differences were observed in all other examined regions besides the CA1; formal significance testing was limited by the number of data points in the WT control group (n = 4). The sequence of pathological changes in this model is i) tau aggregation driven by a pro-aggregant mutation [8], ii) tau hyperphosphorylation, iii) neuroinflammation and synaptic loss. This is likely not an unidirectional cascade, but rather a positive feedback loop, with pathological tau stimulating neuroinflammation, and microglial responses triggering increased phosphorylation in turn [25].

The above characteristics make the model well suited for the evaluation of the links between tau pathology, neuroinflammation, and synaptic loss. It is especially suited for research into primary tauopathies, but also serves as a model 'snapshot' of a part of the AD neurodegenerative cascade centered around the tau aggregation-neuroinflammation loop that is likely to play a critical role in the progression of tau pathology in AD. It is likely well-suited for the evaluation of therapeutics aimed at these pathophysiological mechanisms in the absence of confounding factors.

The healthy genetic background makes the model also a suitable 'base stock' for the creation of hybrid lines. Key limitations of this study are the absence of lifespan data and longitudinal behavioural assessments as well as body weight, both of which could provide a more comprehensive characterisation of the phenotype. The original PS19 line experiences ~ 80% mortality by 12 months, with deaths occurring from 9 months of age [9]. Due to the slower disease progression in this model, longer survival times could be expected, though we have not tested this hypothesis in the present study. Lifespan expectations need to be established for the C57BL/6NJ model to inform the design of studies with survival as the primary endpoint.

Finally, the attenuated disease progression allows for a more granular observation of the disease course, and supports the assessment of therapeutics whose effects develop over longer timespans, such as active immunotherapies. The window between onset of pathology and terminal stage is sufficiently wide to allow a degree of pathology to develop before applying an intervention, as opposed to models with a more aggressive disease course that push experimental designs towards prevention studies.

The above mentioned attributes of the model make it a valuable addition to the arsenal of preclinical models of AD and non-AD tauopathies.

Conclusion

The C57BL/6NJ model bearing the P301S tau mutation develops progressive tau aggregation, tau hyperphosphorylation at all assessed AD-relevant tau epitopes, triggering neuroinflammation (microgliosis and astrogliosis) and synaptic loss. The progression is slowed compared to the original PS19 strain, offering a greater temporal resolution of pathophysiological changes.

Developing the model on this genetic background eliminates important previously reported immunological and metabolic confounders, supporting unbiased evaluation of interventions targeting tau pathology and neuroinflammation.

Limitations

In this model, the most pronounced changes occur during the period between Week 32 and Week 39/40. The absence of a Week 36 group limits the ability to assess the dynamics of changes in this important period. The present study does not include a survival analysis, and does not include a histological or biochemical analysis of the terminal stage. The 39/40w time point was intentionally chosen so that none

of the mice were terminal stage yet, for both ethical and practical reasons. As this study was focused on histological and biochemical characterization, behavioral assessments were not performed. The wildtype control group is small, limiting comparisons to transgenic animals in some analyses.

Declarations

Availability of data and materials

Data supporting these analyses are available from the corresponding author upon reasonable request.

Ethics approval

The Jackson Laboratory's Institutional Animal Care and Use Committee (IACUC) approved the animal use protocol #IACUC-03001-1 JMCRS Breeding Services – Bar Harbor.

Competing interests

All authors affiliated with Roche have received payments from Roche or one of its subsidiaries.

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Authors' contributions

NB and SUZ performed data acquisition, analysis and wrote the manuscript.

GS performed data analysis, wrote the manuscript

JW performed study design, tissue processing and co-ordinating the animal experiments with Jackson Laboratory.

PN, NZ, SK conceptualized, designed and supervised the study, performed data analysis & co-authored the manuscript.

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Figures

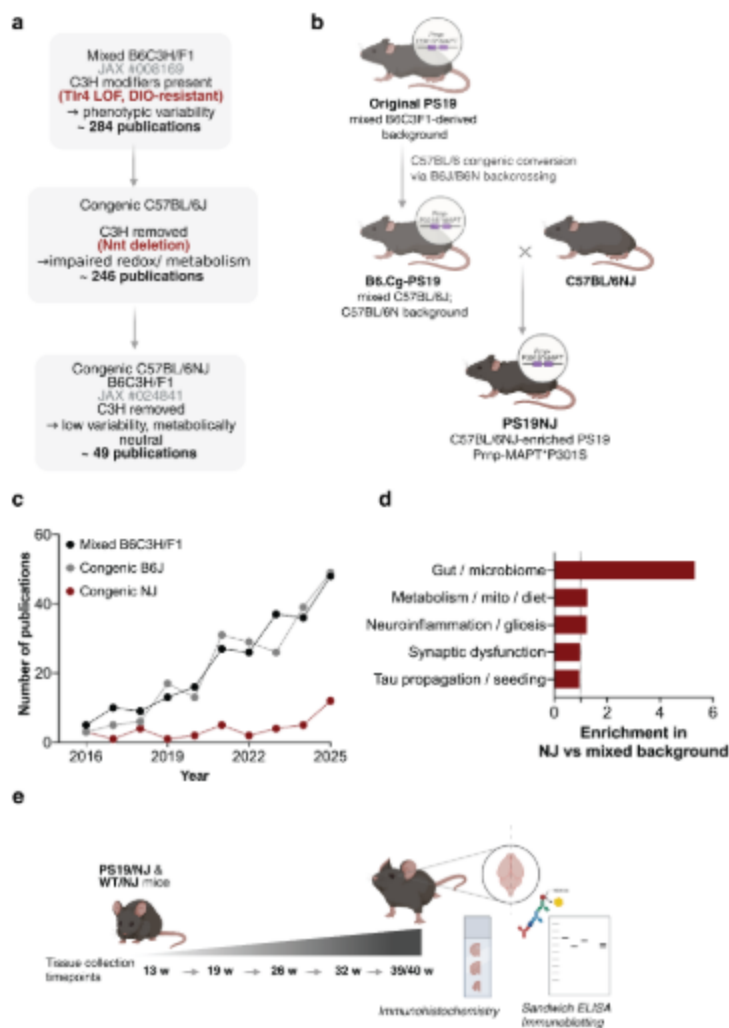


Figure 1

Generation of the PS19NJ mouse line, its use in the literature, and study design. **a**, Generation of the mixed B6C3H/F1, congenic C57BL/6J, and congenic C57BL/6NJ PS19 mouse backgrounds through selective breeding and backcrossing, highlighting key genetic characteristics and approximate literature usage. **b**, Breeding strategy used to generate the PS19NJ mouse line from B6.Cg-PS19 and C57BL/6NJ

mice. **c**, Annual publication trends (2016–2025) for studies using the mixed B6C3H/F1, congenic C57BL/6J, and congenic C57BL/6NJ PS19 backgrounds. **d**, Topic enrichment in studies using the congenic C57BL/6NJ background relative to the mixed B6C3H/F1 background. The dashed line indicates parity (enrichment = 1). **e**, Experimental design showing tissue collection at 13, 19, 26, 32, and 39/40 weeks for immunohistochemistry, sandwich ELISA, and immunoblotting. Note: Subfigure a, c and d are derived with the help of ONA AI.

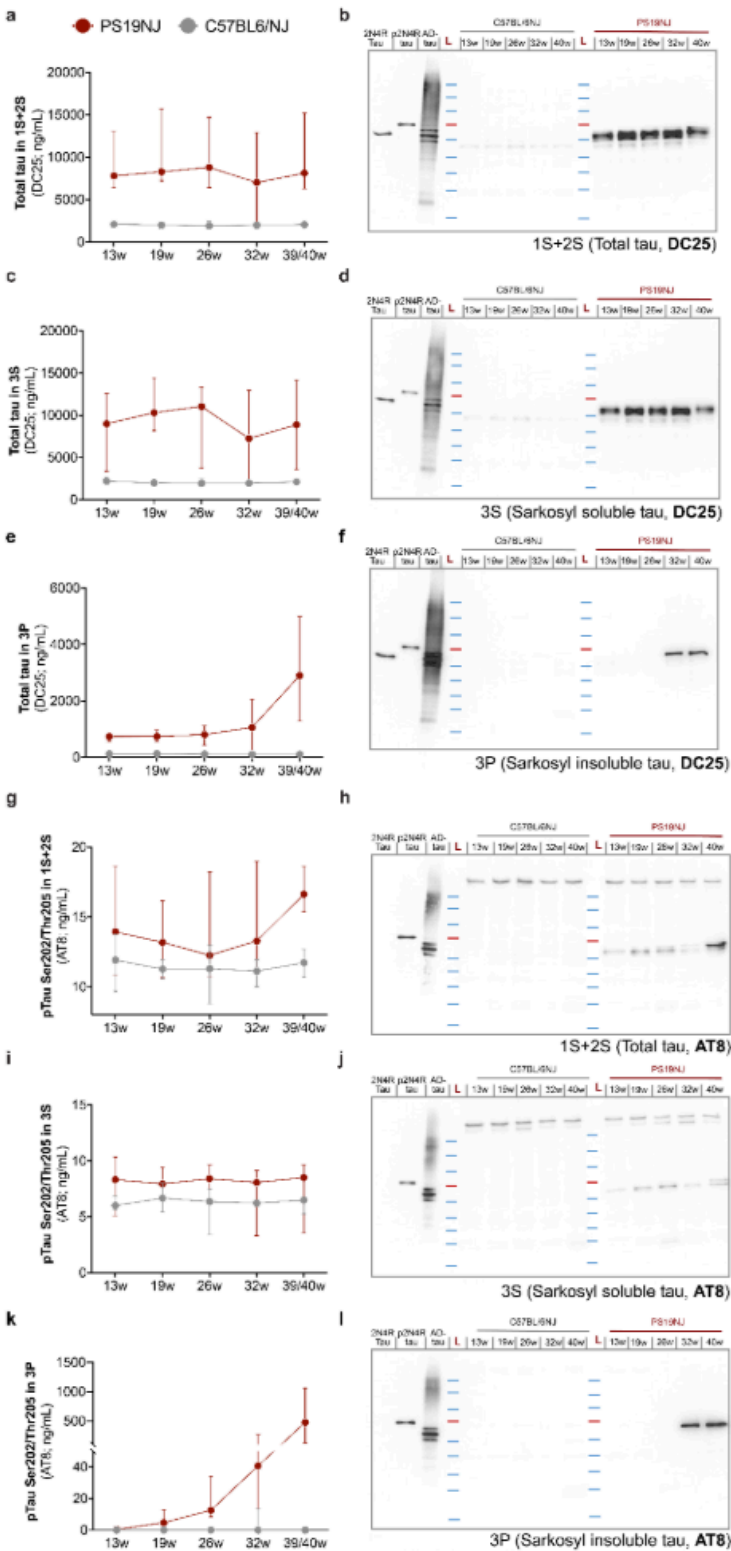


Figure 2

Age-dependent accumulation of soluble and insoluble tau species in PS19NJ mice. Tau concentrations measured by ELISA and representative immunoblots are shown for the combined soluble (1S + 2S), sarkosyl-soluble (3S) and sarkosyl-insoluble pellet (3P) fractions from C57BL/6NJ and PS19NJ mice aged 13, 19, 26, 32 and 39–40 weeks. **a, c, e** Total tau concentrations measured by DC25-based ELISA in the 1S + 2S, 3S and 3P fractions, respectively. **b, d, f** Corresponding fractions immunoblotted with DC25. **g, i, k** Ser202/Thr205-phosphorylated tau concentrations measured by AT8-based ELISA in the 1S + 2S, 3S and 3P fractions, respectively. The discontinuous y-axis in **i** accommodates the increased concentration at 39–40 weeks. **h, j, l** Corresponding fractions immunoblotted with AT8. Sarkosyl-insoluble total and phosphorylated tau accumulated with age in PS19NJ mice but remained negligible in C57BL/6NJ controls. Recombinant 2N4R tau, phosphorylated recombinant 2N4R tau (p2N4R tau) and Alzheimer's disease-derived tau (AD-tau) were included as immunoblot controls. L indicates the prestained molecular-weight ladder; marker positions correspond to 170, 130, 95, 72, 55, 43, 34, 26 and 17 kDa. ELISA data are presented as mean $\pm$ SD.

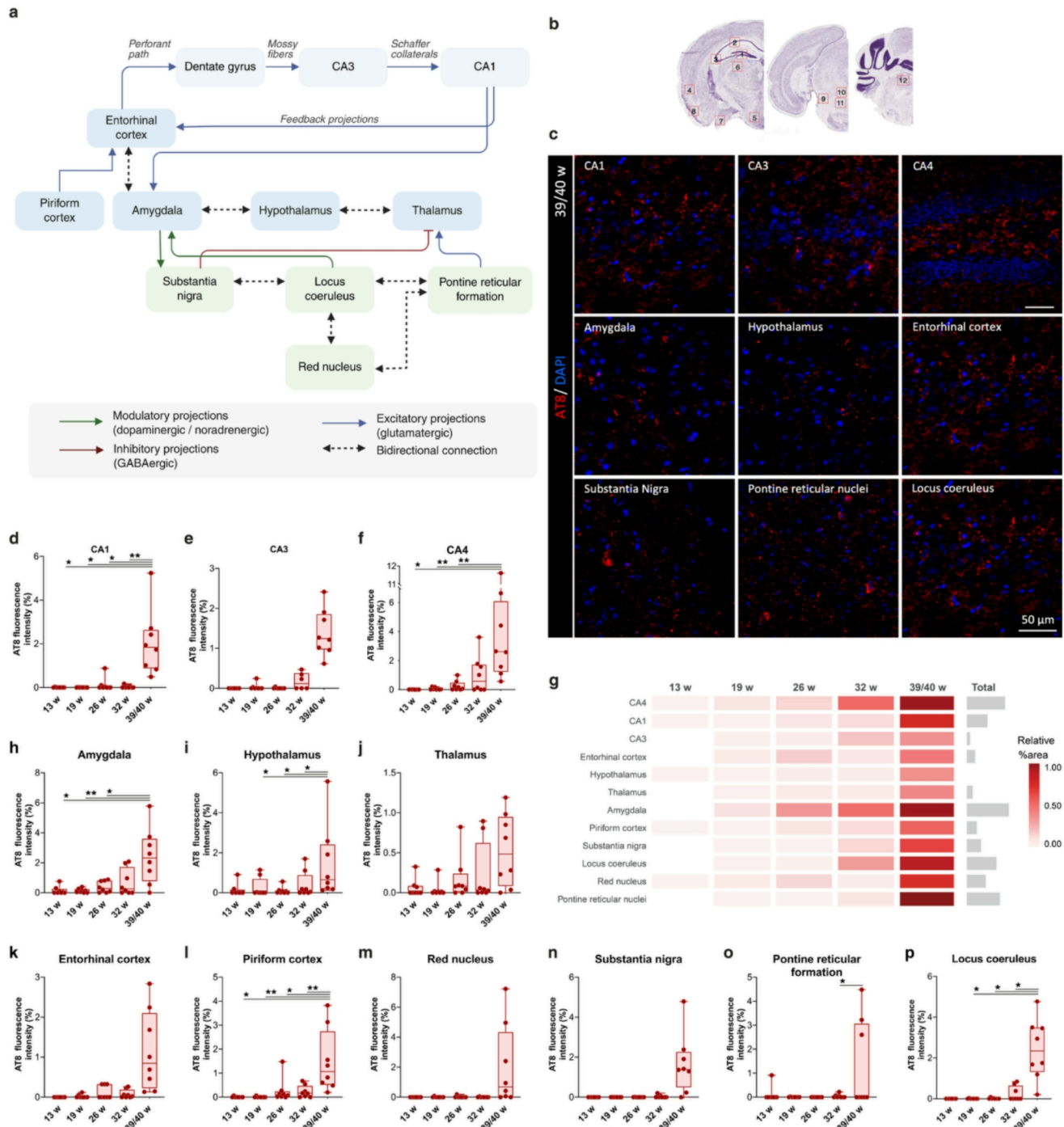


Figure 3

Spatiotemporal progression of AT8-positive tau pathology across interconnected brain regions in PS19NJ mice. **a**, Regions of interest. **b**, Schematic of the anatomical connectivity between the analysed brain regions.: (1) CA1, (2) CA4/dentate gyrus, (3) CA3, (4) piriform cortex, (5) amygdala, (6) thalamus, (7) hypothalamus, (8) entorhinal cortex, (9) substantia nigra, (10) locus coeruleus, (11) pontine reticular nuclei and (12) red nucleus. **c**, Representative immunofluorescence images of AT8-positive

phosphorylated tau with DAPI nuclear counterstain in selected brain regions. *Scale bar, 50 μ m.* **d-p,** Quantification of AT8 positive area fraction across the regions **g**, Heatmap showing the relative AT8-positive area across the analysed brain regions at 13, 19, 26, 32 and 39/40 weeks of age, with the adjacent bar indicating the cumulative relative AT8-positive area across all time points. Significance was assessed using two-sided Mann–Whitney U tests with Benjamini–Hochberg FDR-adjusted *P* values; $P < 0.05$, $P < 0.01$, and $P < 0.001$.

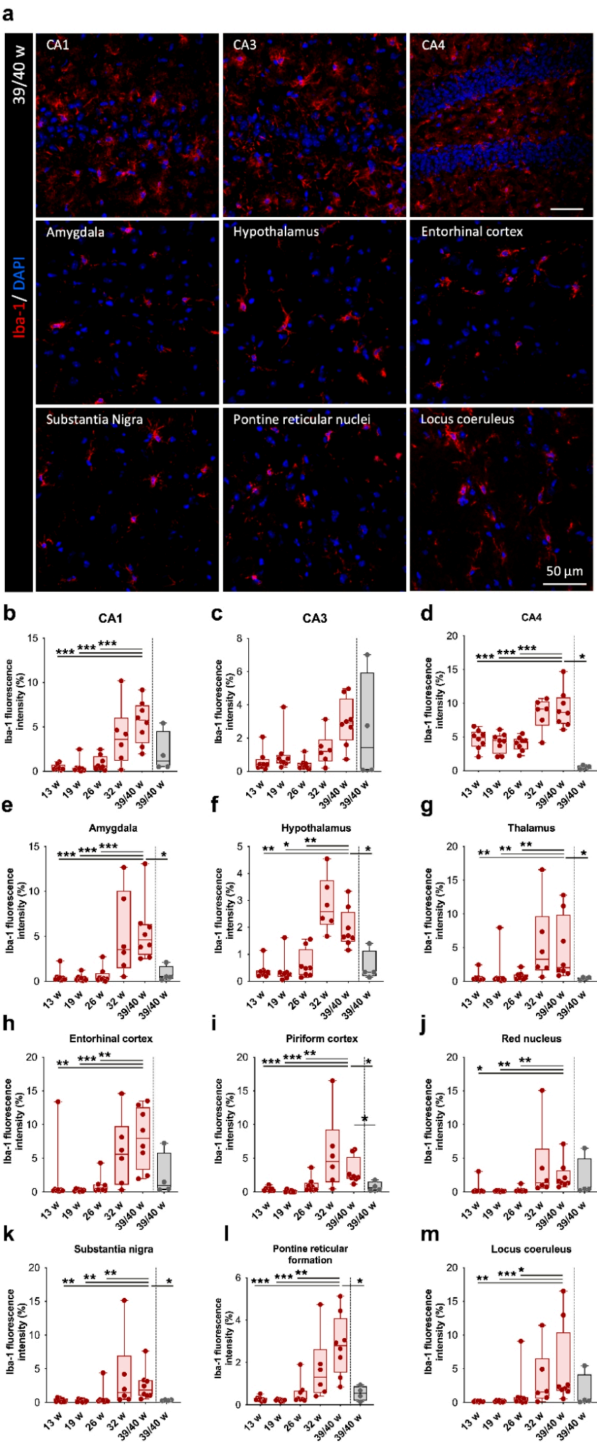


Figure 4

Progressive microglial response to tau aggregation across brain regions in PS19NJ mice. (a)

Representative immunofluorescence images of Iba-1 (red) and DAPI (blue) nuclear counterstain in the CA1, CA3, CA4, amygdala, hypothalamus, entorhinal cortex, substantia nigra, pontine reticular nuclei, and locus coeruleus at 39/40 weeks of age. Scale bar, 50 μ m. **(b-m)** Quantification of Iba-1 positive area fraction (%) across the indicated 12 brain regions at 13, 19, 26, 32, and 39/40 weeks of age. Data are presented as median $\pm$ IQR (n = 8 mice per group). Significance was assessed using two-sided Mann–Whitney U tests with Benjamini–Hochberg FDR-adjusted *P* values; *P*<0.05, **P**<0.01, and **P**<0.001.

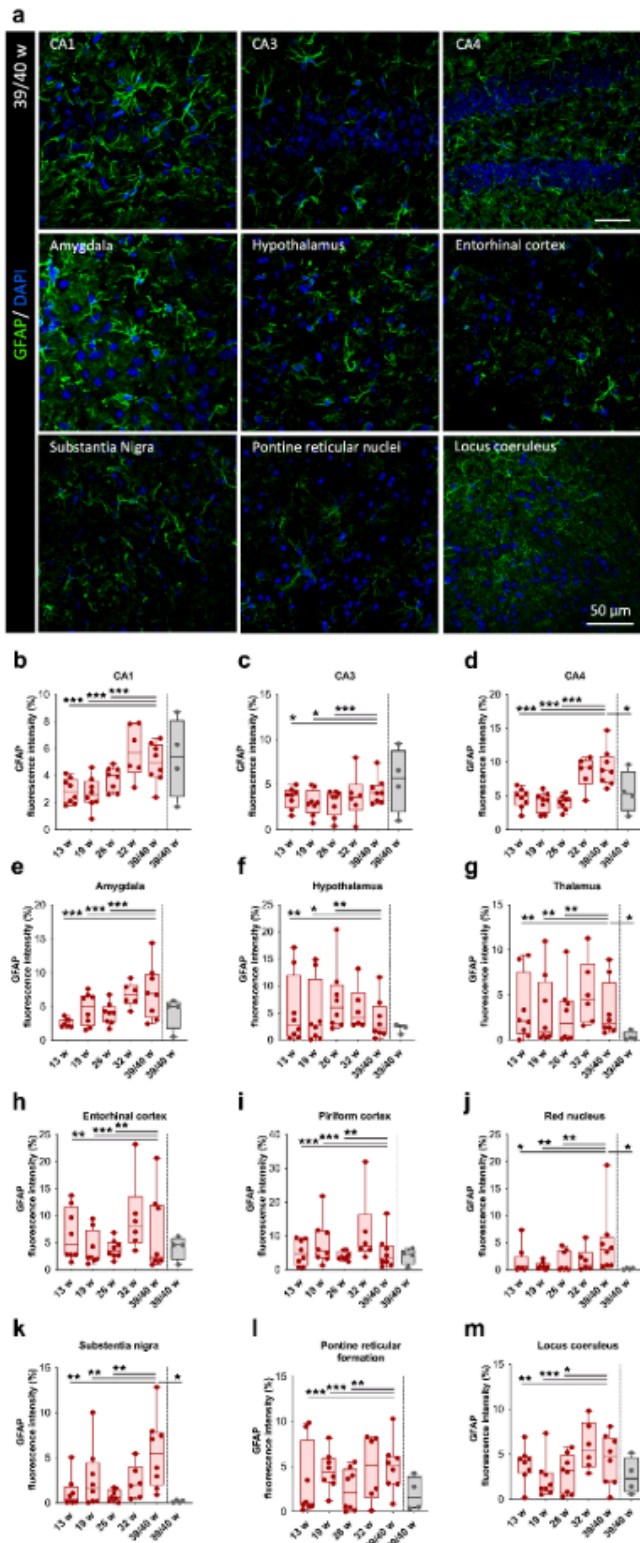


Figure 5

Age-dependent astrogliosis in the hippocampus of PS19NJ mice. Representative immunofluorescence images of GFAP (green) and DAPI (blue) nuclear counterstain in the dentate gyrus (DG), CA1, and CA3 hippocampal subregions at 13, 19, 26, 32, and 39/40 weeks of age. PS19NJ mice exhibit progressive astrocytic hypertrophy and increased GFAP immunoreactivity, with notable process thickening evident by 32 and 39/40 weeks compared to younger time points. Scale bar, 50 μ m. (n = 8 mice per group; age-

matched 39/40-week-old controls, n = 4). Significance was assessed using two-sided Mann–Whitney U tests with Benjamini–Hochberg FDR-adjusted *P* values; *P*<0.05, ***P***<0.01, and ***P***<0.001.

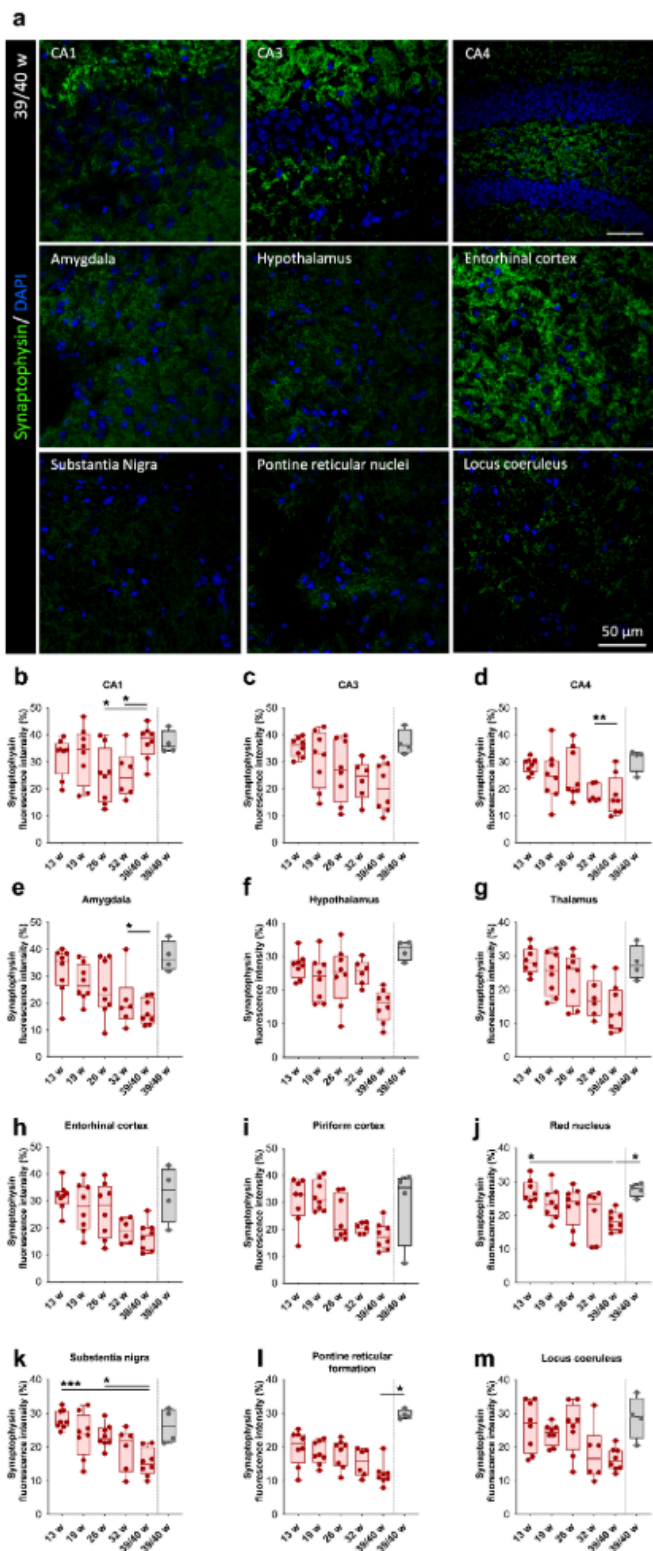


Figure 6

Progressive loss of presynaptic integrity in PS19NJ mice. Representative immunofluorescence images of synaptophysin (green) and DAPI (blue) nuclear counterstain in the CA1, CA3, CA4, amygdala,

hypothalamus, entorhinal cortex, substantia nigra, pontine reticular nuclei, and locus coeruleus at 39/40 weeks of age. PS19NJ mice demonstrate reduced synaptophysin immunoreactivity across sampled brain regions relative to age-matched controls at the terminal time point. Scale bar, 50 μm . ($n = 8$ mice per group). Significance was assessed using two-sided Mann–Whitney U tests with Benjamini–Hochberg FDR-adjusted P values; $P < 0.05$, **$P < 0.01$** , and **$P < 0.001$** .

Supplementary Files

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