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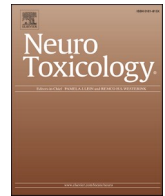
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Comparing effects of gaseous vs. particulate components of traffic-related air pollution on Alzheimer's disease biomarkers in a genetically susceptible rat model[☆]

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ABSTRACT

Epidemiological data link traffic-related air pollution (TRAP) to increased risk of Alzheimer's disease (AD) and AD-related dementia (ADRD), and while experimental animal studies corroborate this association, most utilized exposure paradigms that failed to recapitulate the complexity of current, real-world TRAP exposure. Furthermore, the specific components of TRAP that promote AD pathogenesis remain unknown. This study assesses AD-relevant pathology in a transgenic rat model of AD (TgF344-AD) following chronic exposure to the gas phase vs. particulate matter (PM) components of TRAP in emissions from light-duty only (LDV) vs. combined light- and heavy-duty (LDV+HDV) vehicles. Male and female TgF344-AD rats were transported to the Caldecott Tunnel Exposure Facility (CTEF) at 1 month of age after being randomly assigned to one of six exposure groups: (1) filtered air (FA), (2) LDV+HDV gases only; (3) LDV PM only; (4) LDV+HDV PM only; (5) LDV PM and gases; and (6) LDV+HDV PM and gases. AD-relevant endpoints were quantified in 4-, 9-, 12-, and 15-month-old animals. Cortical A β levels at the 4, 9, and 15-month timepoints were significantly influenced by sex-by-exposure interactions. Amyloid plaque accumulation in the entorhinal cortex and hippocampus exhibited significant sex-dependent effects at 9 and 12 months so males and females were analyzed separately across all timepoints. At 9 months, females exposed to LDV+HDV PM had increased amyloid plaques in the hippocampus, and at 15 months, both females and males exposed to LDV PM and females exposed to LDV PM and gases exhibited significantly increased plaque levels in the entorhinal cortex. The rate of plaque accumulation over all four time points was accelerated in the entorhinal cortices of both sexes exposed to LDV and LDV+HDV PM alone or in combination with gases. There were no exposure effects on phosphorylated tau or Thioflavin S staining; however, the rate of neuronal cell loss with increasing age was significantly increased by exposure to LDV emissions in both females and males. Collectively, these findings demonstrate that chronic exposure to ambient TRAP in real-time promoted AD-relevant outcomes in the TgF344-AD rat with effects that varied according to age, sex, and

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brain region. TRAP effects occurred more prominently in groups exposed to LDV PM. Further research is needed to determine the mechanism(s) by which sex-dependent TRAP effects exacerbate AD pathogenesis so that effective interventions can be developed to improve the health of the aging brain.

1. Introduction

Numerous epidemiological studies across diverse geographical locations have reported a positive association between traffic-related air pollution (TRAP) and increased risk for dementia, including Alzheimer's disease (AD) (Casey et al., 2024, Christensen et al., 2024, Fu and Yung, 2020, Kim et al., 2025, Laccarino et al., 2021, Livingston et al., 2020, Zhu et al., 2025). Experimental animal studies largely corroborate these data (Jew et al., 2019, Kilian et al., 2023, Sahu et al., 2021); however, most of these studies have used relatively high concentrations of specific components of TRAP that do not accurately represent human-relevant TRAP exposure. Several studies have exposed mice to ambient air in environments with heavily polluted air, including metropolitan Mexico City (Calderón-Garcidueñas et al., 2020), a highway tunnel in Boston (Bos et al., 2012) and ambient city air in China (Liu et al., 2018). These studies showed accumulation of particulate matter (PM) in the olfactory bulbs and hippocampus of exposed animals coincident with AD-relevant biomarkers, including upregulated expression of proinflammatory proteins, accelerated neuronal cell loss, and increased phosphorylated-tau in relevant brain regions.

To address this data gap, we designed an exposure facility immediately adjacent to a major roadway tunnel system in northern California (Bein et al., 2022) – the Caldecott Tunnel Exposure Facility (CTEF) – to study the effects of chronic exposure to ambient TRAP in wild-type Fischer 344 and transgenic TgF344-AD rats that express two human risk genes for AD (Cohen et al., 2013). Unlike most of the existing data, the PM_{2.5} concentrations in this study were at or below the EPA National Ambient Air Quality Standards and recapitulated the diurnal patterns of TRAP to which humans living near major roadways are exposed (Bein et al., 2022). Relative to controls housed in the same tunnel facility, but exposed to filtered air (FA), TRAP sex-dependently accelerated tauopathy and cognitive dysfunction in wildtype (WT) rats, increased neuroinflammation and neuronal cell loss in WT and TgF344 rats, and accelerated amyloid plaque deposition and brain uptake of TRAP-associated particulate matter (PM) in the transgenic rats (Patten et al., 2021).

TRAP is a complex mixture of tailpipe-emitted gases (CO, NO_x, volatile organic compounds), fugitive evaporation gaseous emissions, and particulate matter (PM) emitted from roadway, brake and tire wear and tailpipe exhaust. It remains unclear which of these TRAP components promote AD-relevant pathology and increased dementia. The answer is critical for identifying high-risk components in order to implement actionable policy changes. While it is established in the literature that PM_{2.5} is strongly associated with AD, gaseous components of TRAP such as NO₂/NO_x, CO, and SO₂ are also linked to increased AD risk (Chen et al., 2017, Fu and Yung, 2020, Tzivian et al., 2015). A population-based cohort study conducted in Ontario found that increased incidence of dementia in relation to adults living near major roadways was strongly associated with PM_{2.5} and NO₂ (Chen et al., 2017). Another randomized, double-blind crossover study found that PM_{2.5} depletion from acute diesel-exhaust exposure did not protect against TRAP effects, but instead increased NO₂ and exacerbated allergen-induced loss of lung function in human subjects (Wooding et al., 2019). Given the difficulties of dissociating particulate vs. gaseous components in epidemiological studies, there is a need for rigorous experimental studies designed to examine the relative contributions of these components to TRAP's effects on the brain.

To address this data gap, we leveraged the CTEF to expose male and female TgF344-AD rats to TRAP collected from tunnel bores trafficked by either light-duty vehicles (LDV) only or both light- and heavy-duty

vehicles (LDV+HDV). These vehicular sources exhibit substantial size and composition differences in emissions. TRAP collected independently from Bore 1 (LDV+HDV) or Bore 2 (LDV only) was delivered to animals in real-time as either unfractionated TRAP (PM + gases) or following fractionation into PM only vs. gases only. AD biomarkers, including Aβ levels, amyloid plaques, neurofibrillary tangles, phosphorylated tau (pTau), and neuronal cell loss, were quantified at varying ages up to 15 months of age, corresponding to 14 months of exposure.

2. Methods

2.1. The caldecott tunnel exposure facility (CTEF)

The CTEF has been described in detail in prior publications (Bein et al., 2022, Edwards et al., 2020, Patten et al., 2021). Animals in the TRAP exposure groups were continuously exposed to air drawn directly from one of two bores at the CTEF: Bore 1 (B1), which services both light-duty and heavy-duty vehicles (LDV+HDV), or Bore 2 (B2), which services light-duty vehicles (LDV) only. Air was delivered to exposure chambers in which rats were housed either unchanged (PM+Gas) or fractionated into PM only or gases only. The negative control group was exposed to filtered air (FA), which was generated onsite from ambient air collected outside the vivarium then filtered using a combination of emissions control technologies prior to delivery to the exposure chamber housing the animals as previously described (Patten et al., 2021). These emission control technologies included coarse filtration for removing dust and large debris, in-line activated carbon for removing volatile organic compounds, and a 6-port ultrahigh-efficiency particle filtering system for removing ultrafine, fine and coarse PM. The air exchange rate through all the exposure chambers was maintained at roughly 15 per hour, consistent with IACUC requirements.

2.2. TRAP characterization

To quantify differences in exposure dynamics between the two bores, particle size distribution, particle number and particle concentration were measured using a scanning mobility particle sizer (TSI model 3938 water-based SMPS with a model 3088 soft X-ray neutralizer) while PM₁, PM_{2.5}, PM₄, and PM₁₀ mass concentrations were measured using a multi-channel optical particle counter (TSI DustTrak model 8533 DRX). These instruments were operated semi-continuously throughout the exposures and randomly alternated in parallel between the two bores. Temporal resolution was 90-sec for particle size distribution and 10-sec for particle concentrations. A statistical snapshot of each metric was obtained by binning all data in 10-min intervals over a 24-h day for each day of the week (Sunday-Saturday) and then averaging across each interval to provide an average weekly trend, as well as the variance in that trend, for each bore independently.

2.3. Animals

All animal experiments adhered to protocols approved by the University of California, Davis (UCD) Institutional Animal Care and Use Committee. Animals were obtained from a breeding colony of TgF344-AD rats established at UCD in 2016 from male hemizygous TgF344-AD rats (Cohen et al., 2013) originally acquired from Emory University (Atlanta, Georgia). TgF344-AD rats overexpress two human transgenes, APP^{swe} and PSEN1^{ΔE9}, each of which is independently linked to early-onset familial AD (Giau et al., 2019, Hoogmartens et al., 2021). Unlike the more widely used transgenic mice engineered to overexpress

amyloid- β ($A\beta$) peptides, mutant presenilin and/or mutant tau, the TgF344-AD rat recapitulates the progression of key clinical features of AD, including age-dependent neuroinflammatory responses, cerebral amyloidosis that precedes tauopathy, loss of neurons in the cerebral cortex and hippocampus and cognitive disturbances (Anckaerts et al., 2019, Bazzigaluppi et al., 2018, Cohen et al., 2013, Joo et al., 2017, Leplu et al., 2019, Rorabaugh et al., 2017, Smith and McMahon, 2018).

The APPswe and PSEN1 Δ E9 transgenes are linked and inherited in a simple Mendelian fashion. Male and female rats hemizygous for these transgenes develop the full AD phenotype (Cohen et al., 2013). All animals used in this study (including breeders) were genotyped by polymerase chain reaction (PCR) gel electrophoresis using primers specific to the human AD risk genes. Male hemizygous TgF344-AD rats were bred with female WT F344 rats purchased from Charles River Laboratories (West Sacramento, California). We synchronously mated 112 females to produce 104 litters, generating 976 age-matched pups. Between postnatal day 2–4 (P2–P4), pups were tattooed on their paws using nontoxic animal tattoo ink (Ketchum Manufacturing, Lake Luzerne, New York) according to coded identification numbers. To identify pups expressing the AD transgenes, tail-clippings were obtained between P7–P10 and genotyped as previously described (Rutkowski et al., 2024). Pups were weaned and sexed on P21. To obtain a sample size of 9 animals per exposure group per timepoint per sex, 216 male and 216 female TgF344-AD rats were randomly selected for this study and within each sex, randomly assigned to one of six exposure groups and one of 4 timepoints per exposure group. At P28, the 432 TgF344-AD rats were transported to the CTEF.

The vivarium was maintained under controlled environmental conditions (20–26°C, 12:12 light:dark cycle) with food (2018 Teklad Global 18% Protein Rodent Diet, Teklad, Madison, Wisconsin) and filtered water provided *ad libitum* as previously described (Berg et al., 2020, Edwards et al., 2020, Patten et al., 2020, Patten et al., 2021). Rats were housed in Allentown (Allentown, NJ) rat cages (18.75" x 10.25" x 8") with wire lids and glass water bottles. The cages were filled with TEK-Fresh™ bedding (Teklad). Rats were socially housed to provide enrichment (2–3 per cage) with other pups of the same sex within the same exposure group. In the rare cases where a rat was temporarily single housed (e.g., attrition, odd number of rats remaining in group), it was given a Rat Retreats™ shelter (Bio-Serv, Flemington, NJ).

At 4, 9, 12 and 15 months of age, which corresponded to 3, 8, 11, and 14 months of chronic exposure, respectively, 9 male and 9 female rats from each exposure group (for a total of 108 rats per timepoint) were transported back to an IACUC-approved rodent vivarium at the UCD Center for Health & the Environment (CHE) where they were euthanized over the course of the next four days to collect tissues. The husbandry conditions at the CHE were the same as those at the CTEF, except for the absence of tunnel traffic noise and vibration and all animals were exposed to standard room air. For tissue collection, animals were anesthetized using 5% isoflurane (Isospire, Dechra, Northwich, United Kingdom) and pure oxygen (Airgas, Sacramento, California) delivered at a rate of 1.5 L/min. Once anesthetized, blood was collected by cardiac puncture with ~2.5 mL of whole blood collected into plasma tubes (Becton-Dickinson, Milpitas, California) and a separate ~2.5 mL aliquot of whole blood collected into serum separator tubes (Becton-Dickinson). Plasma tubes were centrifuged within 30-min of collection for 15-min at 2000 X g, while serum was centrifuged after standing for 30-min at room temperature. Following blood collection, rats were transcardially perfused with 200-mL of cold 0.1 M phosphate buffered saline (PBS; pH 7.2; 137 mM NaCl, 10 mM sodium phosphate dibasic, 1.8 mM potassium phosphate monobasic) at a rate of 15 mL/min using a Masterflex peristaltic pump (Cole Parmer, Vernon Hills, Illinois). Brains were removed from the skull and bisected sagittally using a stainless-steel rat brain matrix (Zivic Instruments, Pittsburgh, Pennsylvania); the left hemisphere was microdissected to isolate the hippocampus, whole cortex, and cerebellum, which were flash frozen in liquid nitrogen and then stored in cryotubes at –80°C until processed for subsequent biochemical

analyses.

2.4. Biochemical analyses

Plasma samples were diluted two-fold in sterile PBS before being sent out to Eve Technologies Corporation (Calgary, Canada) for analysis using a human amyloid beta and tau 4-plex Discovery Array (Luminex 200, Catalog #HDA4T4), which was previously validated in a pilot test using our rat plasma samples. This assay measured $A\beta_{1-40}$, $A\beta_{1-42}$, pTau 181, and total Tau. Frozen cortical tissue (~100-mg) was processed using a two-step extraction method to obtain the detergent-soluble (Triton-soluble) and insoluble (guanidine hydrochloride; GHCl) fractions of amyloid-beta peptides as previously described (Johnson-Wood et al., 1997, Patten et al., 2021). Levels of $A\beta_{1-40}$, $A\beta_{1-42}$ were measured in the Triton-soluble and GHCl extracts of cortical tissue using sandwich enzyme-linked immunosorbent assays raised against human amyloid peptides (Invitrogen, Waltham, Massachusetts, Catalog #KMB3481 and KMB3441 respectively) and normalized to total protein levels determined using a bicinchoninic acid (BCA) assay (Thermo Scientific-Pierce, Waltham, Massachusetts, Catalog #23225), both according to manufacturer's instructions.

2.5. Immunohistochemical analyses

The right hemisphere of the perfused rat brain was cut into 2-mm coronal sections and fixed in 4% w/v paraformaldehyde (PFA; Sigma Chemical, St. Louis, Minnesota) in 0.1 M phosphate buffer for 24-h at 4°C. Sections were then transferred to 30% w/v sucrose (ThermoFisher Scientific) in PBS for at least 3 days before being embedded in Optimal Cutting Temperature (OCT) compound (ThermoFisher Scientific) and flash frozen in a methanol and dry ice bath. Cryoblocks were sectioned at 10- μ m and mounted onto slides. Two sections from the hippocampus of each rat (Bregma –3.3 to –4.2; validated using *The Rat Brain in Stereotaxic Coordinates* (Paxinos and Watson, 2014)) were immunostained as previously described (Patten et al., 2020).

Primary antibodies were diluted in blocking buffer (0.03% triton PBS, 10% normal goat serum, 1% BSA). Primaries included: OC anti-amyloid fibril antibody (1:750, Millipore, Burlington, Massachusetts, Ab2286, RRID:AB_1977024), NeuN (1:500, Millipore, MAB377, RRID:AB_2298772), and phosphorylated Tau (pTau) serine 422 (1:500, Abcam, Waltham, Massachusetts, Ab79415, RRID:AB_1603345). To visualize primary antibody:antigen complexes, sections were reacted with secondary antibodies that recognized the primary antibodies. Secondaries, which were diluted in triton-PBS at 1:1000, included: goat anti-rabbit IgG conjugated to Alexa Fluor 568 (Life Technologies, Eugene, Oregon, A11036, RRID:AB_10563566) and goat anti-mouse IgG conjugated to Alexa Fluor 488 (Invitrogen, A11017, RRID:AB_2534084). TrueBlack (Biotium, Fremont, California) was used according to manufacturer's instructions to quench lipofuscin autofluorescence, then sections were mounted in ProLong Gold with DAPI (ThermoFisher Scientific, P36930). Negative controls, which were included in each batch stain, were incubated with secondary antibodies only after incubation with blocking buffer that did not include any primary antibody.

For Thioflavin S (ThioS) analyses, sections were stained with 0.02% w/v Thioflavin S (Sigma Chemical) in water for 8 min, then de-stained with 50% w/v ethanol in milli-Q water (ThermoFisher Scientific) for 3 $\times$ 1 min, as described previously (Patten et al., 2021). Slides were mounted in Prolong Gold without DAPI (ThermoFisher Scientific) and cover slipped with borosilicate coverslips 0.16 mm thick (Corning, Sunnyvale, California).

2.6. Fluorescence imaging and analysis

Fluorescent images were acquired using a 20x long working distance, air-immersion objective (0.45 NA) on an ImageExpress MicroXLS High-

Content Analysis System (Molecular Devices, San Jose, California) using MX6 Software [version 6.2] (Molecular Devices). Positive staining was identified as fluorescence intensity at least 2x that of background fluorescence. For the entorhinal cortex and hippocampus, 16 or 32 overlapping image tiles, respectively, were stitched together using MX6 software to produce the final images used for image analysis. All images were analyzed using ImageJ [version 1.8.0; National Institutes of Health], and data were averaged for each animal ($n = 5-6$ per group) across 2 imaged sections per animal per brain region. Outcomes that were quantified within regions of interest included the area immunopositive for OC or pTau or stained with ThioS, as well as number and area of A β deposits (OC immunostaining) and plaques (ThioS staining). The number of neurons per unit area in regions of interest were quantified as the number of cells with colocalized NeuN immunoreactivity and DAPI staining using ImageJ.

2.7. Statistical analyses

GraphPad Prism statistical software (version 10.0; GraphPad

Software, San Diego, California) and RStudio IDE (version 2023.12.1 +402; Posit Software, Boston, Massachusetts) were used for statistical analyses. Our primary goal was to identify significant differences in AD-relevant histopathological phenotypes between exposure groups. Because of known sex differences in outcomes (Ferretti et al., 2018, Kim et al., 2019), male and female data were analyzed separately. For data generated in the biochemical analyses, simple linear regression was used to determine significant exposure differences; all other data were analyzed using two-way analysis of variance (ANOVA) with exposure and age as factors, and post-hoc Dunnett's test with correction for multiple comparisons by timepoint within sex. A p-value < 0.05 was considered statistically significant, and a p-value < 0.1 was noted to have potential biological relevance.

3. Results

3.1. TRAP exposure characterization

Average weekly trends in particle concentration at 10-min resolution

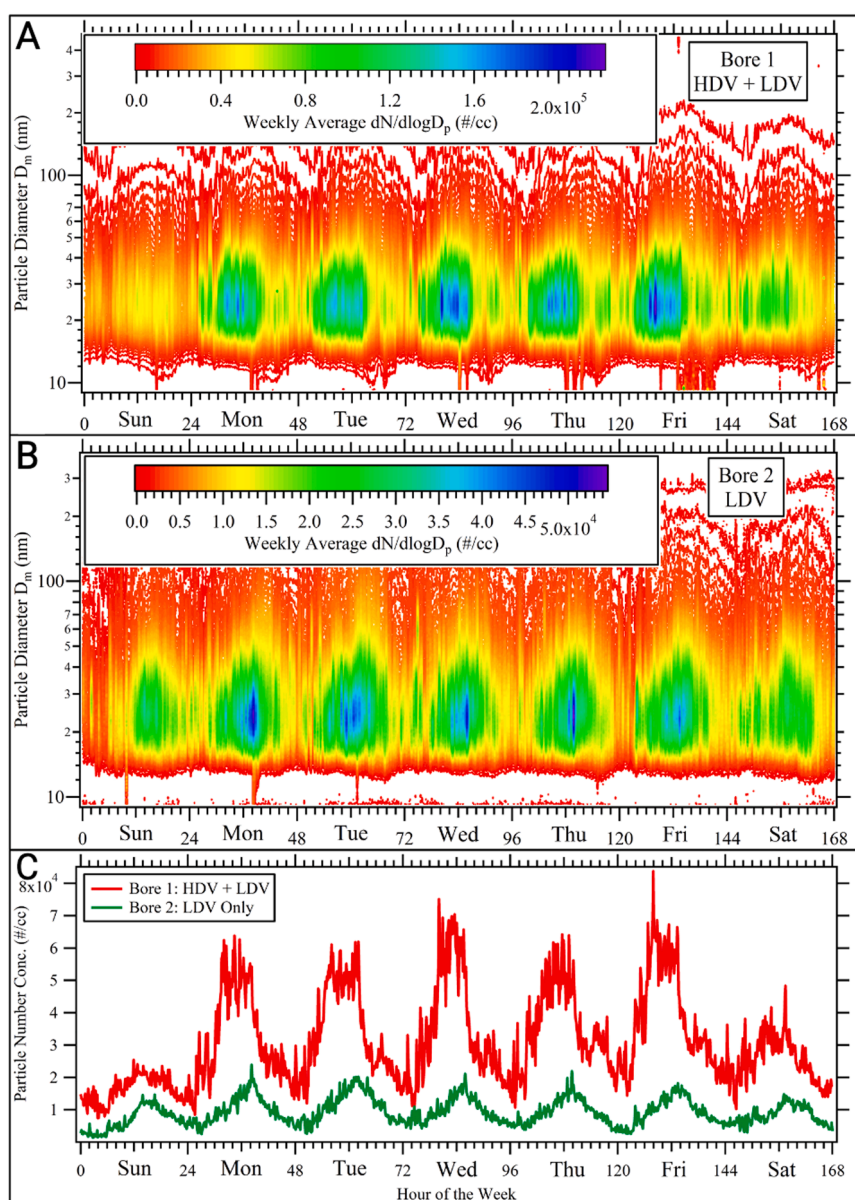


Fig. 1. The size distribution of weekly average particle number concentration measured in (A) bore 1 and (B) bore 2, as well as (C) the total particle number concentration measured in bore 1 and 2.

were used to provide a statistical snapshot of exposure dynamics for each bore. Fig. 1 shows the size distribution of particle number concentration for B1 (LDV+HDV) (Fig. 1A) and B2 (LDV) (Fig. 1B), as well as the total integrated particle number concentration for B1 and B2 (Fig. 1C). Identical plots for the variance in these variables are included in Supporting Information (Figure S1).

At cursory glance, the temporal trends are similar between B1 and B2 in several important ways: (1) strong diurnal dosing due to daily commuter traffic patterns over the 5-day work week; (2) lower concentrations on the weekends due to reduced traffic volume; (3) daily peak exposure duration of ~6 h with moderate to low concentrations otherwise; (4) particle size distribution dominated by ultrafine particles ($D_p < 100$ nm) with daily peak concentrations around 25-nm but shifting to wider distributions peaking at larger particle diameters when transitioning to moderate and low exposures as traffic subsides and the influence of the baseline ambient air pollution increases; and (5) temporal trends in variance that mirror the average with standard deviations similar in magnitude to the average, but positively skewed and highly leptokurtic, suggesting high exposure periods show greater variability with more frequent and extreme outlier events.

Quantitatively, however, exposure dynamics are strikingly different

between the bores in several key measures. To help elucidate this, the data in Fig. 1C are recast in Fig. 2A as the weekly average ratio of total particle number concentration between B1 and B2 (black trendline) as well as the rate of change in particle number concentration in B1 (red trendline) and B2 (green trendline) determined using a 60-min rolling average. Collectively, these data show that: (1) particle concentrations are routinely a factor of 2–10 times greater in B1 than B2 (average = 3.6 ± 1.6); (2) the timing of peak concentration is out of phase by about 4 h between the bores with B1 peaking mid-morning and B2 in the mid-afternoon; (3) the rate of change in particle concentration between successive max and min exposures is significantly greater in B1; (4) secondary peaks are observed nightly in B1 around 20:00 but not in B2; and (5) mean particle diameter is significantly larger in B2 (52-nm) than B1 (40-nm). Table 1 provides a side-by-side statistical comparison between the primary PM metrics measured in B1 versus B2. Despite a complex array of tunnel variables, it can be shown explicitly that these differences are attributable to differences in HDV traffic between the bores, as shown in Supporting Information (Supplemental Methods).

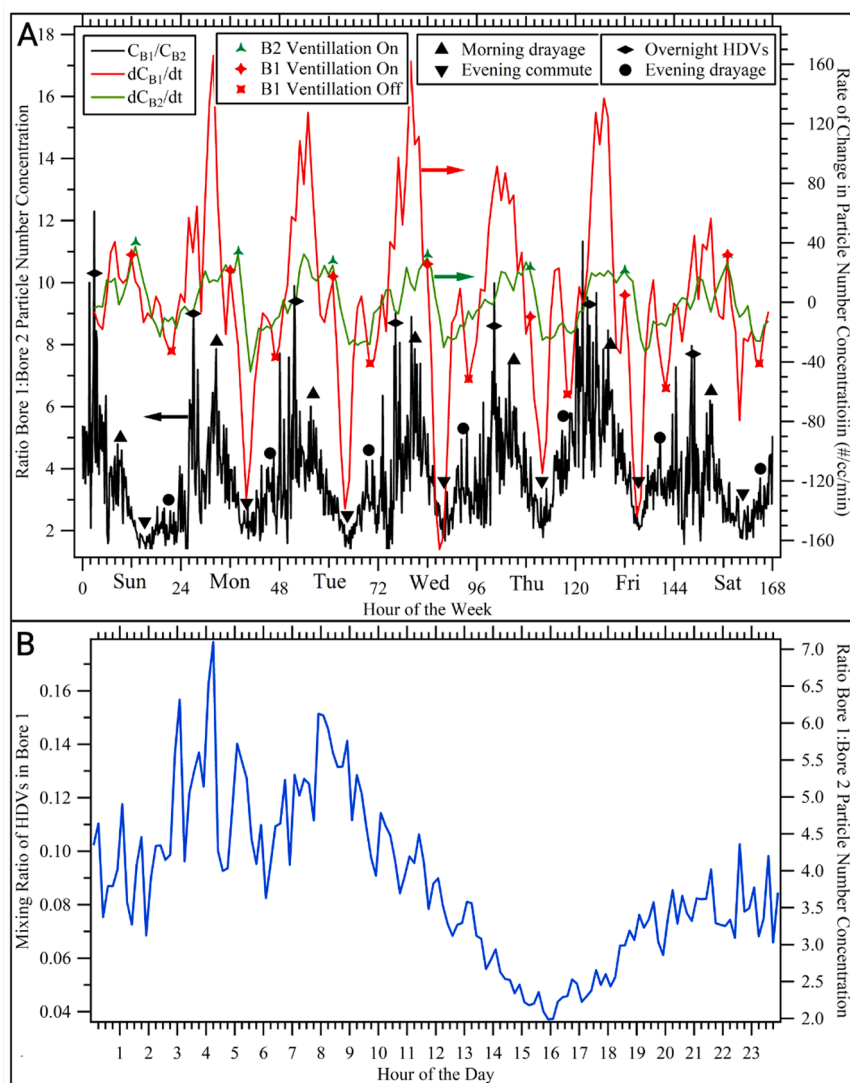


Fig. 2. The weekly average (A) ratio of total particle number concentration measured in Bore 1 versus Bore 2 ($\bar{C}_{B1}/\bar{C}_{B2}$; black trendline) and rate of change in particle number concentration for Bore 1 (dC_{B1}/dt ; red trendline) and Bore 2 (dC_{B2}/dt ; green trendline). (B) The daily average mixing ratio of HDVs in Bore 1 $\bar{\chi}_{HD}$ as a function of hour of the day; see text for discussion.

Table 1
Statistical comparison between particle metrics measured in Bore 1 versus Bore 2.

Instrument	Measurement	Bore 1: HDV + LDV		Bore 2: LDV		Bore 1: Bore 2 P-values	Filtered Air	
		Average	Std. Dev.	Average	Std. Dev.		Average	Std. Dev.
TSI DustTrak 8533 DRX Optical Particle Counter	PM ₁ Mass Conc. (µg/m ³)	10.3	2.2	10.0	2.2	0.0081	0.8	0.5
	PM _{2.5} Mass Conc. (µg/m ³)	10.8	2.2	10.4	2.2	0.0028		
	PM ₄ Mass Conc. (µg/ /m ³)	10.9	2.2	10.6	2.2	0.0014		
	PM ₁₀ Mass Conc. (µg//m ³)	11.1	2.3	10.8	2.2	0.0016		
	TSP Mass Conc. (µg/m ³)	11.2	2.3	10.8	2.3	0.0017		
TSI 3938 Scanning Mobility Particle Sizer	Median D _p (nm)	29.8	2.5	34.5	4.6	< 0.0001	43	13
	Mean D _p (nm)	40.3	3.6	52	8.1	< 0.0001	51	12
	Geometric Mean Dp (nm)	32.5	2.4	39.1	4.7	< 0.0001	43	11
	Mode D _p (nm)	23.9	1.9	25.6	3.2	< 0.0001	37	17
	Particle Number Conc. (#/cc)	3.1E+ 04	1.5E+ 04	9.2E+ 03	4.1E+ 03	< 0.0001	28	22
	PM _{0.3} Mass Conc. (µg//m ³)	3.9	1.3	2.5	0.8	< 0.0001	1.8E-02	7.0E-03

HDV = heavy duty vehicle; LDV = light duty vehicle, D_p = Particle diameter, Conc. = Concentration, TSP = Total suspended particles

3.2. Effects of TRAP on Aβ levels and Aβ proteinopathies

Amyloid precursor protein (APP) processing, in which APP is cleaved into Aβ peptides of varying lengths with specific physiological and pathological properties, is dysregulated in AD (Zheng and Koo, 2011). The ratio of Aβ₁₋₄₂ to Aβ₁₋₄₀ is commonly used as a clinical measure to predict cognitive status (Fandos et al., 2017), thus, we quantified Aβ₄₂:Aβ₄₀ in the plasma and in the triton-soluble (T-S) and guanidine

hydrochloride (GHCl)-soluble fractions of cortical tissue. In the plasma, there were no statistically significant effects of TRAP exposure (Fig. 3), though it is notable that in the 4-month males, the Gas(LDV+HDV) exhibited a lower ratio compared to the FA (p < 0.1) (Fig. 3C). Overall levels of Aβ₄₂:Aβ₄₀ in the triton-soluble (T-S) fraction trended upwards from 4 to 12 months and decreased slightly at 15 months in males and females (Fig. 4A-B). There were no significant effects of exposure on the T-S fraction at any age (Fig. 4C-F). In the G-HCl fraction, overall levels of

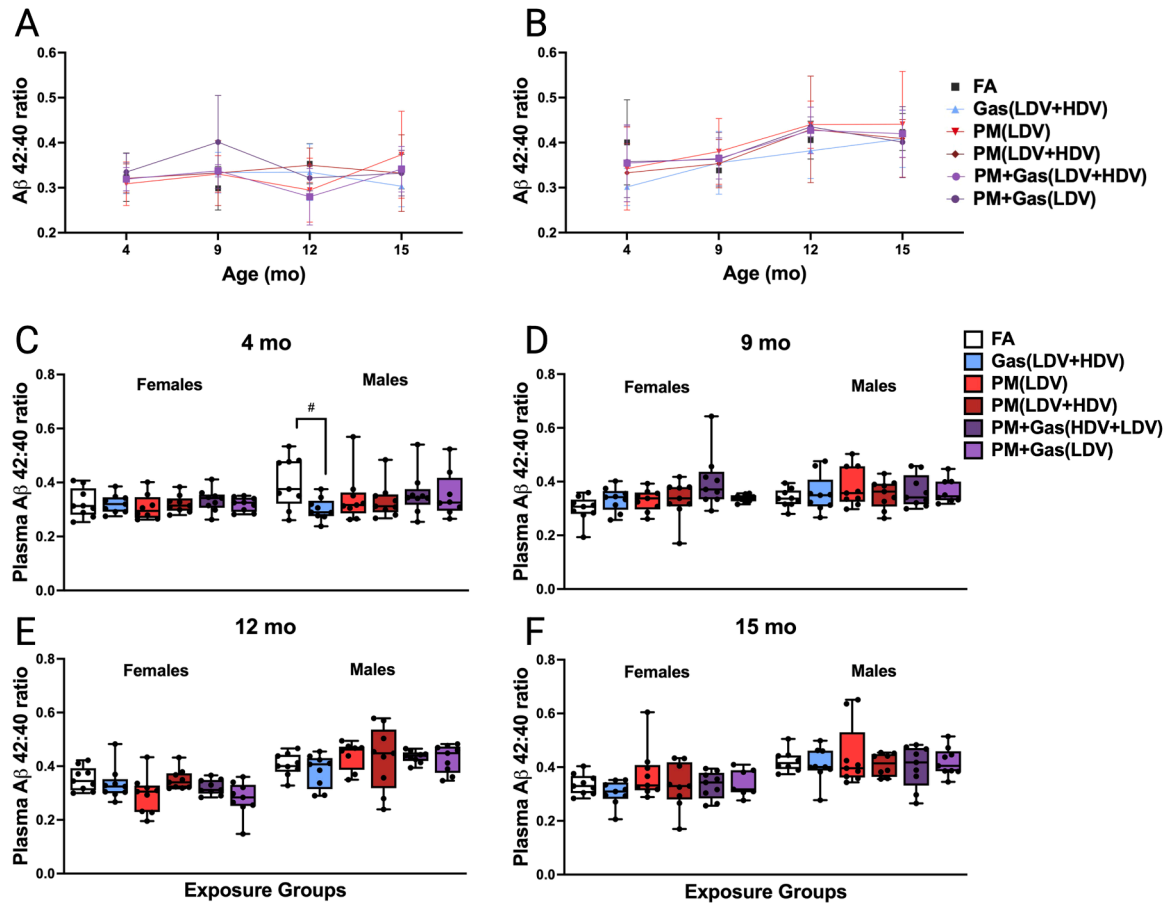


Fig. 3. Effect of different TRAP exposures on the ratio of Aβ₄₂:40 oligomers in the plasma of TgF344-AD rats. The concentration of amyloid-β (Aβ-42 and Aβ-40) were measured in plasma using a 4-Plex assay and data are presented as the ratio of Aβ₄₂:40. (A-B) Trendlines of mean Aβ₄₂:Aβ₄₀ ratios ± SD in the plasma as a function of age in females (A) and males (B). (C-F) Aβ₄₂:40 ratios in plasma at 4 months (mo) of age (C), 9 mo (D), 12 mo (E) and 15 mo (F). Data are shown as box and whisker plots in which the horizontal line in the box represents the median; lower and upper limits of the box, 1st and 3rd quartile, respectively; whiskers, the minimum and maximum; and dots, values obtained for individual animals (n = 8–9 animals per group per sex). *Indicates significantly different at p < 0.05; # at p < 0.1 as determined using simple linear regression with FDR correction. Females and males were analyzed separately due to known sex differences.

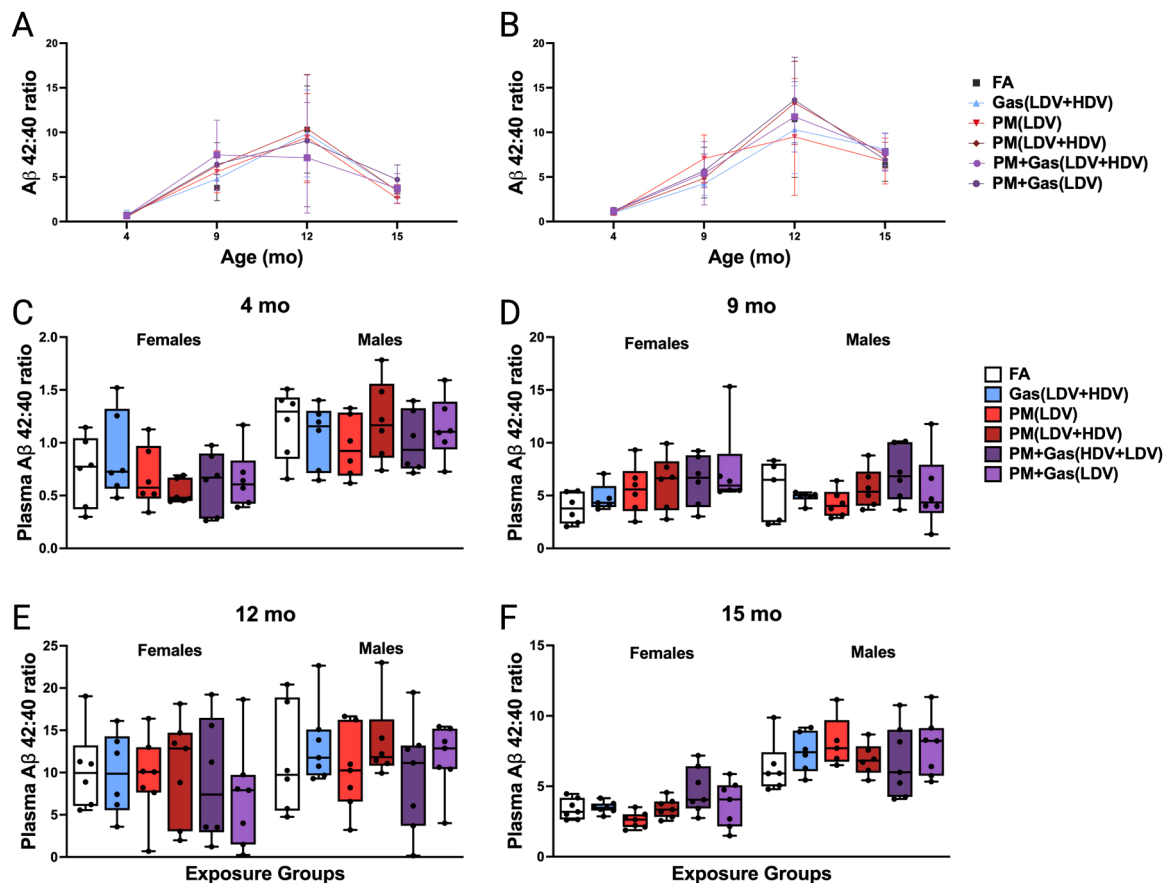


Fig. 4. Effect of TRAP exposures on A β 42:40 ratio in the triton-soluble fraction of cortical tissue from TgF344-AD rats. The concentrations of A β -42 and A β -40 were measured in the triton-soluble (T-S) fraction of cortical tissue, which contains soluble amyloid oligomers, by ELISA. Data are presented as the ratio of A β 42:40. (A–B) Age-related changes in the mean A β 42:A β 40 ratios $\pm$ SD in the T-S fraction of cortical tissue from females (A) vs. males (B). (C–F) A β 42:40 ratios in the T-S fraction of cortical tissue at 4 months (mo) of age (C), 9 mo (D), 12 mo (E) and 15 mo (F). Data are shown as box and whisker plots in which the horizontal line in the box represents the median; lower and upper limits of the box, 1st and 3rd quartile, respectively; whiskers, the minimum and maximum; and dots, values obtained for individual animals ($n = 5$ –7 animals per group per sex). *Indicates significantly different at $p < 0.05$; # at $p < 0.1$ as determined using simple linear regression with FDR correction. Females and males were analyzed separately due to known sex differences.

A β ₄₂: A β ₄₀ increased from 4 to 9 months, decreased at 12 months and increased slightly at 15 months (Fig. 5A–B). There was a significant effect of exposure in the 4-month females where A β ₄₂: A β ₄₀ ratios were decreased in all exposure groups compared to FA (Fig. 5C). PM+Gas(LDV+HDV), Gas(LDV+HDV), PM+Gas(LDV) and PM(LDV) were all significantly decreased ($p < 0.05$), and PM(LDV+HDV) was also decreased ($p < 0.1$). In contrast, 4-month males exhibited a higher ratio of A β ₄₂: A β ₄₀ in the PM(LDV+HDV) group ($p < 0.1$) (Fig. 5C). No significant differences between exposure groups were observed at the 9- and 12-month timepoints (Fig. 5D–E), but in the 15-month males the PM+Gas(LDV+HDV) group had a higher ratio compared to FA ($p < 0.1$) (Fig. 5F).

Amyloid beta plaques are pathological hallmarks of AD observed in the TGF-344 AD rat model (Cohen et al., 2013). We measured amyloid beta deposition using quantitative immunohistochemistry (qIHC) with the anti-amyloid antibody clone OC (Kayed et al., 2007), which recognizes fibrillar A β . qIHC analyses were performed on brain regions implicated in AD pathology, specifically, the entorhinal cortex and the hippocampus (Thal et al., 2002). Within the hippocampus, the CA1, CA3, and DG subregions of the hippocampus were analyzed separately. To quantify plaque accumulation, we measured both mean plaque size, as well as total amyloid deposition, which was assessed as the percentage of total area immunopositive for OC within each brain region. We detected a significant effect of sex at 9- and 12-month timepoints as determined by three-way ANOVA with region (EC and DG), sex, and exposure as factors [15 mo: $p = 0.0908$, 12 mo: $p = < 0.0001$, 9 mo:

$p = 0.0009$, 4 mo: $p = 0.7044$]. Therefore, males and females were separated for further analysis, using a two-way ANOVA with both age and exposure as factors (Figs. 6 and 7).

Post-hoc analysis using Dunnett's test demonstrated that compared to sex- and age-matched FA controls, amyloid deposition in the entorhinal cortex at 15 months was significantly increased in females exposed to PM(LDV) and PM+Gas(LDV) [$p = 0.027$, $p = 0.0222$, respectively] (Fig. 6A), and in males exposed to PM(LDV) [$p = 0.0076$] (Fig. 7A). In contrast, there were no significant effects of exposure on amyloid deposition in any of the hippocampal subregions at 15 months. Amyloid plaque size was increased by TRAP exposure in the CA3 region of 9-month females (Fig. 6H). However, these trends were flipped in the 12-month animals and TRAP exposure generally decreased plaque area compared to FA. In 12-month males, PM(LDV+HDV) decreased plaque area in the CA3 region [$p = 0.0348$] (Fig. 7D). In 12-month females, plaque area was decreased in the CA3 region in the Gas(LDV+HDV), [$p < 0.0001$], PM(LDV) [$p = 0.0011$], and PM+Gas(LDV+HDV) [$p = 0.021$] exposure groups, and also decreased in the DG in the PM (LDV) exposure group [$p = 0.0257$] (Fig. 6B, D).

Since A β levels typically increase with age, we also measured the progression of amyloid deposition to assess whether exposure increased the rate of amyloid deposition over time. Linear regression of each subregion was calculated separately to capture the progression of amyloid deposition across the four time points. Slopes of the resultant trendlines were compared between exposure groups by one-way ANOVA. In both males and females, there was a significant increase in

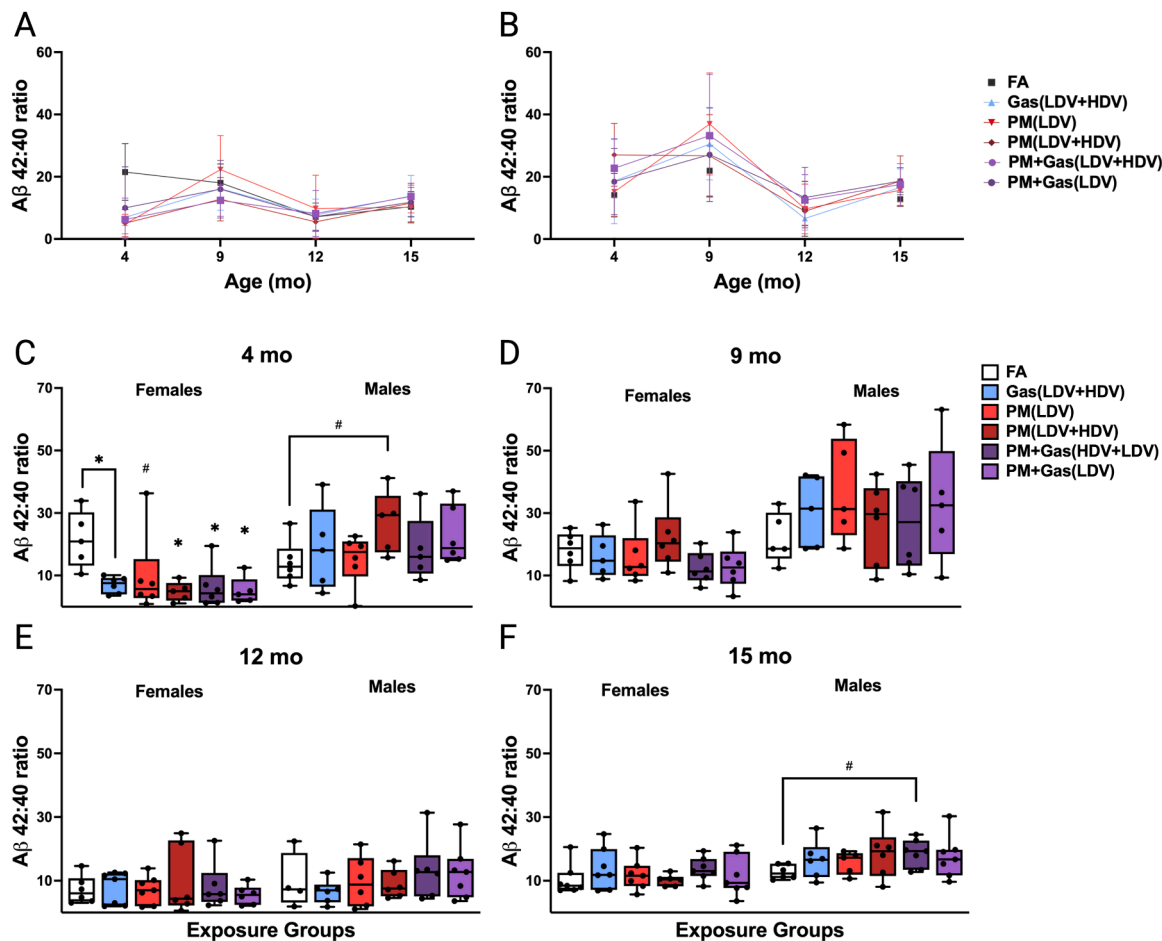


Fig. 5. Effect of different TRAP exposures on A β ratio in the guanidine hydrochloride fraction of cortical tissue from TgF344-AD rats. The concentrations of A β -42 and A β -40 were measured in the guanidine hydrochloride (GHCl) fraction of cortical tissue, which contains insoluble amyloid fibrils and aggregates, by ELISA. Data are presented as the ratio of A β 42:A β 40. (A–B) Age-related changes in the mean A β 42:A β 40 ratios $\pm$ SD in the GHCl fraction of cortical tissue from females (A) vs. males (B). (C–F) A β 42:A β 40 ratios in the GHCl fraction of cortical tissue at 4 months (mo) of age (C), 9 mo (D), 12 mo (E) and 15 mo (F). Data are shown as box and whisker plots in which the horizontal line in the box represents the median; lower and upper limits of the box, 1st and 3rd quartile, respectively; whiskers, the minimum and maximum; and dots, values obtained for individual animals ($n = 4$ –7 animals per group per sex). *Indicates significantly different at $p < 0.05$; # at $p < 0.1$ as determined using simple linear regression with FDR correction. Females and males were analyzed separately due to known sex differences.

progression of plaque deposition in the entorhinal cortex in exposed groups compared to FA (Fig. 8). For females, this was observed in the Gas(LDV+HDV), PM(LDV), PM(LDV+HDV), PM+Gas(LDV+HDV), and PM+Gas(LDV) exposure groups [$p < 0.0001$ across all exposure groups] (Fig. 8C). Males also exhibited increased accumulation of plaques over time in relative to FA when exposed to Gas(LDV+HDV), PM(LDV) and PM(LDV+HDV) [$p < 0.0001$] (Fig. 8d). No consistent effects of TRAP exposure were observed in hippocampal subregions, although there were altered trendlines across regions, especially increased slopes in PM (LDV) exposed males and females (see Figure S2 in Supporting Information).

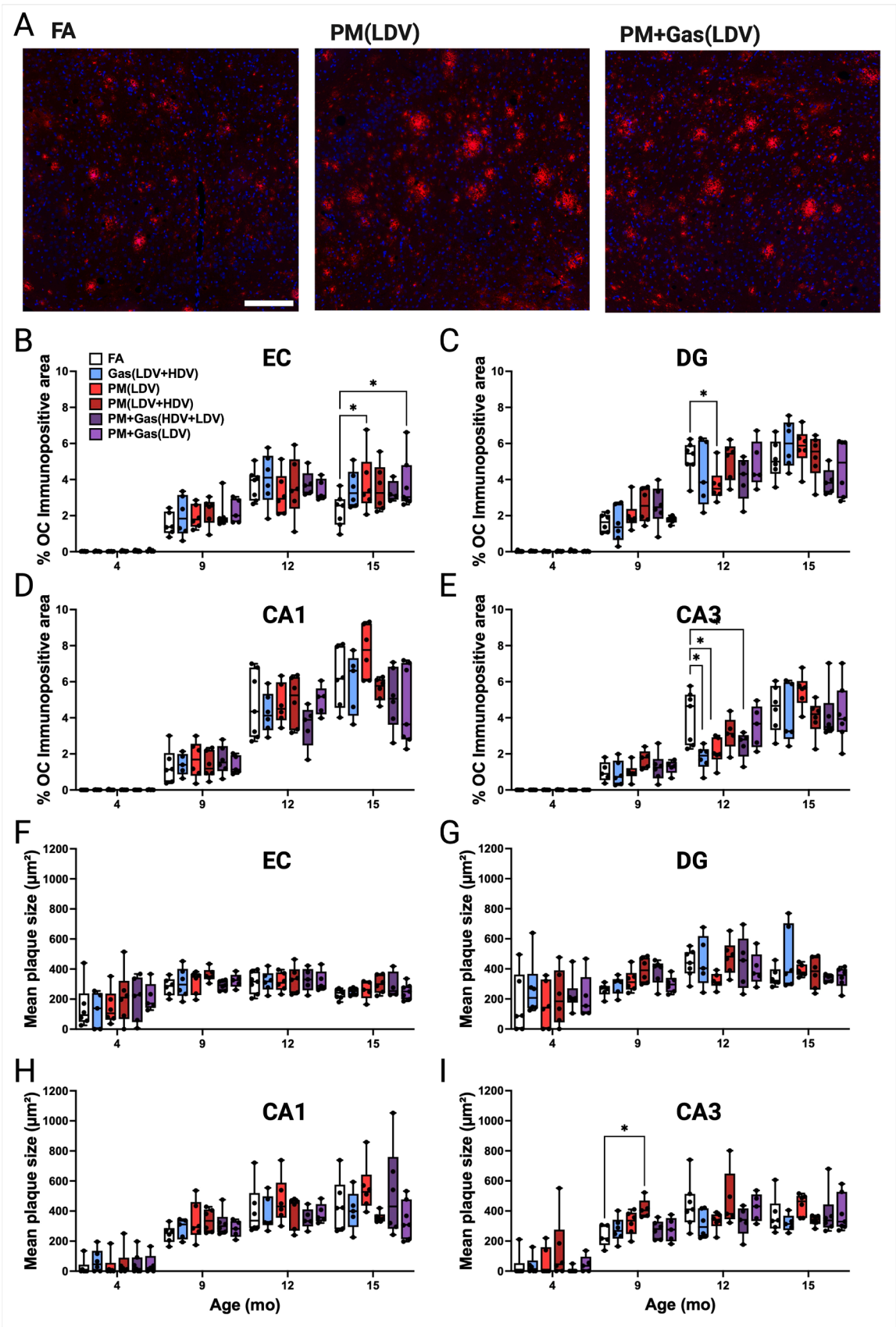
AD-relevant protein aggregations were also measured using Thioflavin S (ThioS) staining, which recognizes β -sheet rich structures, including amyloid plaques and neurofibrillary tangles (NFTs) (Bussière et al., 2004). As with OC analyses, males and females were analyzed separately according to subregion using a two-way ANOVA with exposure and age as factors. There were no exposure differences in either plaque size or percent area covered in plaques observed in either males or females across the different brain regions (Fig. 9). Linear regression-based analyses of plaque progression also did not yield conclusive increases across different regions or sexes (data provided in Supporting Information, Figure S3).

3.3. Effects of TRAP on phosphorylated tau

Hyperphosphorylated tau and NFTs have been observed in the TgF344-AD rat model (Cohen et al., 2013) and in wildtype F344 rats chronically exposed to ambient TRAP at the CTEF (Patten et al., 2021). To supplement the ThioS staining data, we specifically assessed levels of phosphorylated tau, which cause NFTs and are a critical pathological component of AD (Rawat et al., 2022). qIHC was performed using an anti-phosphorylated tau (phosphor ser422) antibody that is well established in neurodegeneration (Augustinack et al., 2002) and is an early indicator of tau pathogenesis (Miao et al., 2019, Voss et al., 2011). In line with previous observations (Morrone et al., 2020, Patten et al., 2021), pTau immunostaining was detected adjacent to amyloid plaques. Although pTau immunoreactivity was detected as early as 9 months in these animals, no significant exposure effects on pTau expression were observed in the brain via qIHC (Fig. 10 and Supporting Information, Figure S4) or in plasma pTau:total Tau ratios (Supporting Information, Figure S5).

3.4. Effects of TRAP on neuronal cell loss

Decreased neuronal cell density is also characteristic of AD (DeTure and Dickson, 2019). In the TgF344 rat model, increased neuronal cell loss relative to age-matched wildtype rats occurs by 16 months of age



(caption on next page)

Fig. 6. Effect of different TRAP exposures on A β deposition in female TgF344-AD rats. Brain sections were immunostained with the OC anti-amyloid antibody to visualize A β deposition. (A) Representative images of OC immunoreactivity in the entorhinal cortex of 15-month-old females exposed to FA, PM(LDV) or PM+Gas (LDV). Sections were immunostained with OC (red) and counterstained with DAPI (blue). Bar= 200 μ m. A β deposition was quantified in the entorhinal cortex (EC, panels B and F), dentate gyrus (DG, panels C and G), and CA1 (panels D and H) and CA3 (panels E and I) subregions of the hippocampus as the area of OC immunostaining normalized to region area (B–E) and as mean size (μ m²) of OC-immunopositive deposits (F–I) as a function of age. Data are presented as box-and-whisker plots, in which the horizontal line in the box represents the median; lower and upper limits of the box, 1st and 3rd quartile, respectively; whiskers, the minimum and maximum; and dots, values obtained for individual animals ($n = 5$ –7 animals per exposure group). Data were analyzed by two-way ANOVA using exposure and time as factors, with post hoc Dunnett's test. *Significantly different; $p < 0.05$.

(Cohen et al., 2013). We quantified neuronal cell loss via qIHC analyses of NeuN, a neuronal cell marker (Gusel'nikova and Korzhevskiy, 2015) to measure the number of neurons per region in the EC, CA3, CA1, and DG. As with amyloid plaques, neuronal cell loss increased with increasing age (DeTure and Dickson, 2019). We observed a significant effect of age across all brain regions and in both sexes using a two-way ANOVA with age and exposure as factors (female EC: $p < 0.0001$, male EC: $p = 0.0281$, female CA3: $p < 0.0001$, male CA3: $p = 0.1251$, female CA1: $p = 0.0169$, male CA1: $p = 0.0476$, female DG: $p < 0.0001$, male DG: $p < 0.0001$). There were no significant exposure effects across brain regions, and *post-hoc* Dunnett's test did not demonstrate any exposure-dependent changes relative to FA within each subregion (Supporting Information, Figure S6). To assess the rate of neuronal cell loss over time, we used linear regression and compared slopes of TRAP exposure groups to FA within each subregion using a one-way ANOVA. *Post-hoc* Dunnett's test demonstrated that relative to sex-matched FA controls, exposure to LDV emissions that included PM, either alone or in combination with gases, increased neuronal cell loss over time in some brain regions in males and females (Fig. 11 and Supporting Information, Figure S7). Notably, in females, both PM(LDV) and PM+Gas(LDV) exposed groups showed increased neuronal cell loss in the EC (Fig. 11C), which is consistent with increased amyloid plaques observed in these groups in the same region and sex (Fig. 6B). However, across brain regions, multiple exposure groups had decreased neuronal cell loss compared to FA, which did not appear to follow any consistent patterns (Supporting Information, Figure S7).

4. Discussion

Our findings are consistent with previous studies demonstrating that ambient TRAP promotes AD histopathological phenotypes (Alemany et al., 2021, Calderón-Garcidueñas et al., 2002, Calderón-Garcidueñas et al., 2018, Calderón-Garcidueñas et al., 2004, Fu and Yung, 2020, Hullmann et al., 2017, Laccarino et al., 2021, Patten et al., 2021), while extending these findings to determine the effect of gas phase vs. particulate matter components of TRAP derived from LDV and HDV emissions. We demonstrate that TRAP increased amyloid plaques, evident as increased plaque size and rate of accumulation of amyloid deposits over time, although these effects were dependent on complex interactions between sex-, brain region-, and age. The rate of plaque accumulation over the four time points was accelerated in the entorhinal cortices of both sexes exposed to PM alone or in combination with gases from both LDV and HDV emissions. Comparisons within timepoints indicated that relative to FA, TRAP increased plaques in females at 9 months, and in both sexes at 15 months. Sex-dependent effects on amyloid plaque accumulation across both the entorhinal cortex and the hippocampus were also observed at both 9 and 12 months. In addition to qIHC, TRAP altered cortical A β 42:40 ratios at 4, 9, and 15 months with effects exhibiting significant sex-by-exposure interactions. We also observed that the rate of neuronal cell loss with increasing age was significantly increased by exposure to LDV emissions in both females and males. However, we did not observe significant effects of TRAP exposure on neurofibrillary tangles or phosphorylated tau levels across timepoints or regions, consistent with our previous observations of TgF344-AD rats (Patten et al., 2021).

The primary TRAP component associated with increased amyloid proteinopathies and rate of neuronal cell loss was PM. The exposure-

dependent increases in amyloid deposition, as determined by plaque levels at any given timepoint and the rate of accumulation over time, were observed in PM alone or PM+Gas groups. We did not detect significant changes in the group exposed only to gas, suggesting that PM plays a critical role in TRAP effects on amyloid proteinopathies. Indeed, PM has been linked to more severe AD pathology in numerous epidemiological and clinical studies (Christensen et al., 2024, Shi et al., 2020).

In addition to studying TRAP composition, we also investigated whether variations in the vehicular fleet differentially influenced AD-relevant pathology. This was achieved by drawing TRAP from different tunnel bores, with one bore used to obtain emissions from LDV+HDV vehicles (B1) and one for emissions from LDV vehicles only (B2). Somewhat unexpectedly, when we compared effects of LDV only vs. combined LDV+HDV emissions, the most robust effects on AD pathology were seen in LDV only (B2) exposure groups. Although some LDV+HDV exposure groups demonstrated TRAP effects on AD pathology, primarily at the 9-month timepoint, LDV exposure groups consistently demonstrated significant differences in OC immunoreactivity and amyloid deposition.

Differential outcomes based on the type of vehicular emissions likely reflect differences in the concentration and/or chemical composition of TRAP between emission types. HDVs are mostly diesel powered whereas LDVs are mostly gasoline powered (Kirchstetter et al., 1999). Both HDV and LDV emissions are a complex mixture of particles and gases, but their chemical composition and physical characteristics can vary substantially from vehicle to vehicle and even from the same vehicle depending on load, fuel, and engine type (Armas et al., 2008, Kisin et al., 2013). As discussed in Supporting Information (Supplemental Methods), differences in exposure dynamics between the bores are primarily attributed to HDV traffic. Although B1 allows both LDV and HDVs, the majority of the air pollution constituents are emitted by the HDVs because they emit orders of magnitude more than LDVs (Grosjean et al., 2001, Kirchstetter et al., 1999). Although previous studies have shown that during peak traffic hours, the traffic volume through B2 is greater than B1 (Allen et al., 2001, Ban-Weiss et al., 2008, Geller et al., 2005, Kirchstetter et al., 1999), PM characterization of our B1 vs. B2 exposures indicated similar PM levels between the bores, with B1 (LDV+HDV emissions) exhibiting only slightly higher PM concentration. Therefore, the increased effects associated with exposure to LDV only (B2) emissions compared to LDV+HDV (B1) emissions suggest that variances in PM composition rather than PM concentration drive the differential effects of these two vehicular emission sources on AD histopathological phenotypes. A recent study of southern California freeway TRAP demonstrated that HDV-dominated freeways exhibited increased diesel-related carbonaceous pollutants and metals associated with abrasion, road dust, and break and tire wear from heavier vehicles, while LDV emissions consisted predominantly of tailpipe emissions and contained relatively more secondary organic aerosols (Fazelvalipour et al., 2026). Determining the exact compositional differences of PM in vehicular emission in B1 vs. B2 in our exposure is a critical next step towards elucidating the differential emission effects observed in this study.

There is a known effect of sex on AD pathogenesis (Ferretti et al., 2018), with increased prevalence of disease and accelerated cognitive decline in women. Epidemiological studies of the association between air pollution and cognitive impairment also demonstrated sex-dependent changes (Kim et al., 2019, Zhang et al., 2018). Our

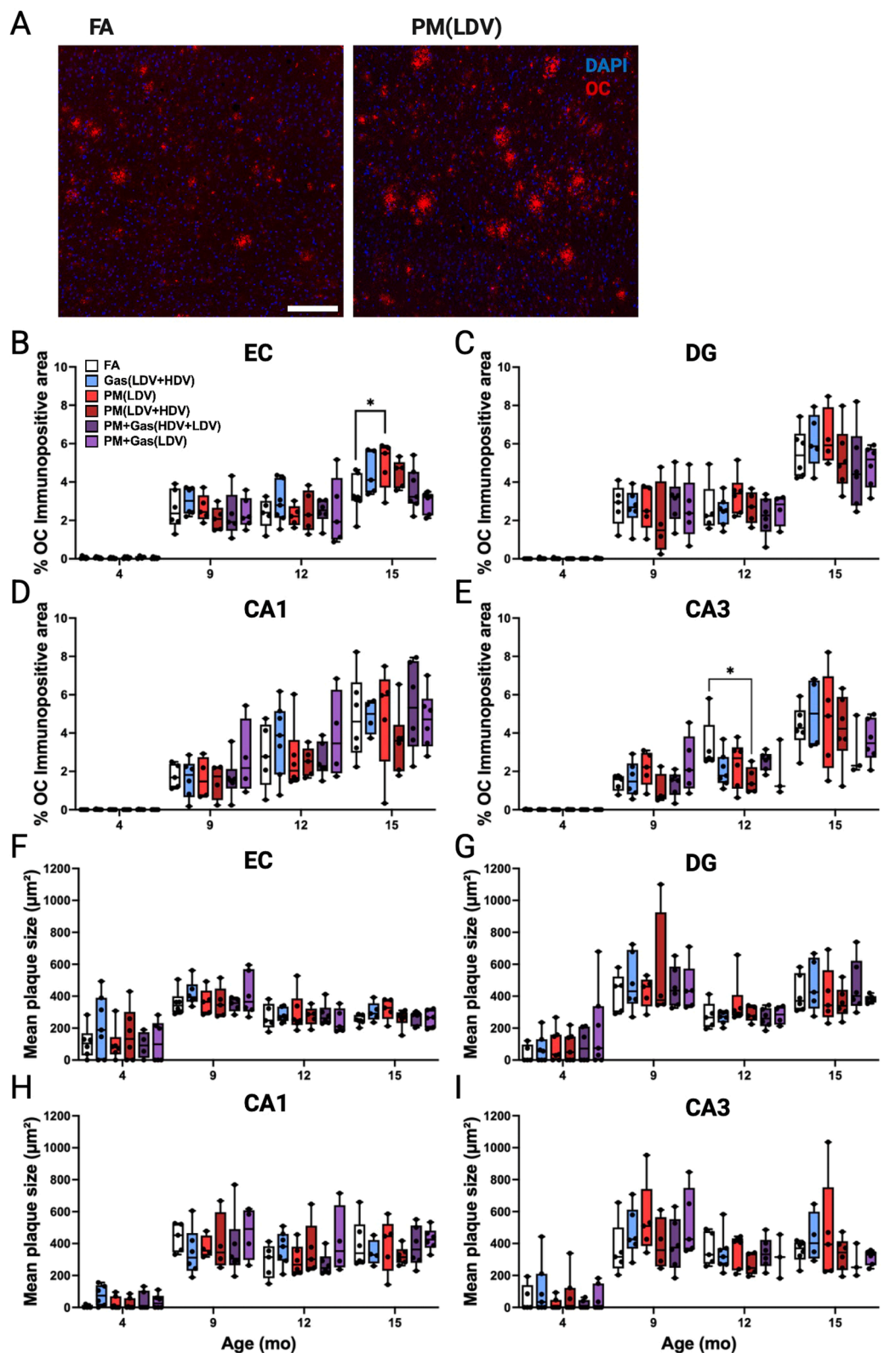


Fig. 7. Effect of different TRAP exposures on amyloid beta deposition in male TgF344-AD rats. (A) Representative images of the entorhinal cortex of 15-month-old male TgF344-AD rats stained with OC (red) and counterstained with DAPI (blue) from FA and PM(LDV) exposed animals. Bar = 200 μm . Plaque deposition was quantified from OC anti-amyloid IHC in the entorhinal cortex (B, F), DG (C, G), CA1 (D, H), and CA3 (E, I). Males and females were analyzed separately due to previously described sex differences, and data were analyzed separately for each subregion. (B-E) Quantification of OC immunopositive area normalized to region area. (F-I) Quantification of mean plaque size (μm^2). Data are presented as box-and-whisker plots, in which the horizontal line in the box represents the median; lower and upper limits of the box, 1st and 3rd quartile, respectively; whiskers, the minimum and maximum; and dots, values obtained for individual animals ($n = 5-7$ animals per exposure group). Data were analyzed by two-way ANOVA using exposure and time as factors, with post hoc Dunnett's test; * = $p < 0.05$.

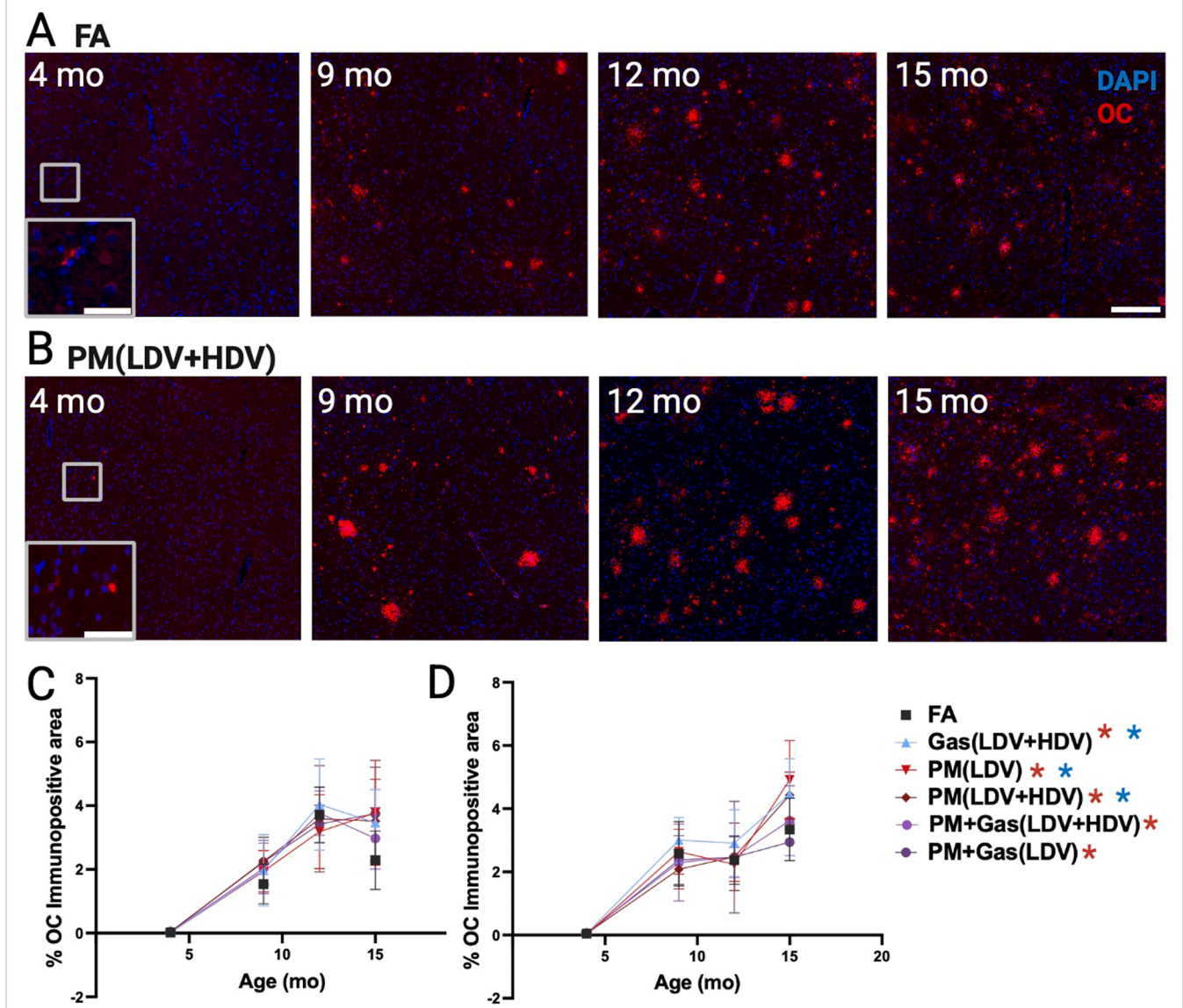


Fig. 8. Effect of different TRAP exposures on amyloid beta deposition over time in female and male TgF344-AD rats. Brain sections were immunostained with the OC anti-amyloid antibody to visualize A β deposition (red) in the entorhinal cortex; sections were counterstained with DAPI (blue). (A, B) Representative images of OC immunoreactivity in the entorhinal cortex of 4, 9, 12 and 15-month-old females exposed to FA (A) or PM(LDV+HDV) (B) starting when they were 1 month old. Bar = 200 μ m. Insets are enlarged images of the small box in the photomicrograph; bar = 50 μ m. (C, D) A β deposition quantified as the area of OC immunostaining normalized to region area in the entorhinal cortex of females (C) or males (D). Data are shown as the mean $\pm$ SD (n = 21–25 animals per group per sex); with lines for exposure group determined using linear regression. *Significantly different from FA at p < 0.05 as determined by one-way ANOVA and post-hoc Dunnett's multiple comparisons test. Red * indicate differences in females; blue *, significant differences in males.

findings similarly suggest important sex-based differences in TRAP effects on A β levels and amyloid plaque accumulation. Across brain regions, 9-month males showed increased plaque levels relative to females, while at 12 months, plaque levels were greater in females relative to males; cortical A β levels also differed by sex and the sex ratio varied with age. Since the TgF344 AD rat model has been reported to not show sex differences in amyloid pathology at any given age (Cohen et al., 2013), our observation of sex differences in the effects of TRAP on amyloid pathology suggests a potential role for environmental factors, such as TRAP, to contribute to potential sex-based differences in AD risk and disease progression observed in humans (Aggarwal and Mielke, 2023; Ferretti et al., 2018).

Our results also implicate TRAP in selectively modulating different stages of AD pathogenesis. We observed altered plaque levels by OC qIHC, but similar changes were not found with ThioS staining. While

both OC and ThioS are well-established tools for detecting amyloid deposition in the brain, they target different A β aggregation states. While both OC and ThioS label mature fibrils and plaques, OC also captures earlier aggregation states, such as soluble fibrillar oligomers and diffuse plaques (Kayed et al., 2007). The significant exposure effects we observed using OC IHC but not ThioS staining suggests that TRAP exposure promotes the formation of fibrillar oligomers and earlier-stage amyloid deposits that precede the formation of fibrillar plaques (Kayed et al., 2007; Sarsoza et al., 2009). Consistent with the possibility that TRAP primarily modulates earlier stages of AD pathogenesis, we did not observe exposure-dependent changes in absolute neuronal cell count or tau levels, which typically become evident after the initiation of amyloid pathology.

Similar to our previous study that used the same exposure facility (Patten et al., 2021) to expose rats to complete TRAP (PM+Gas) from

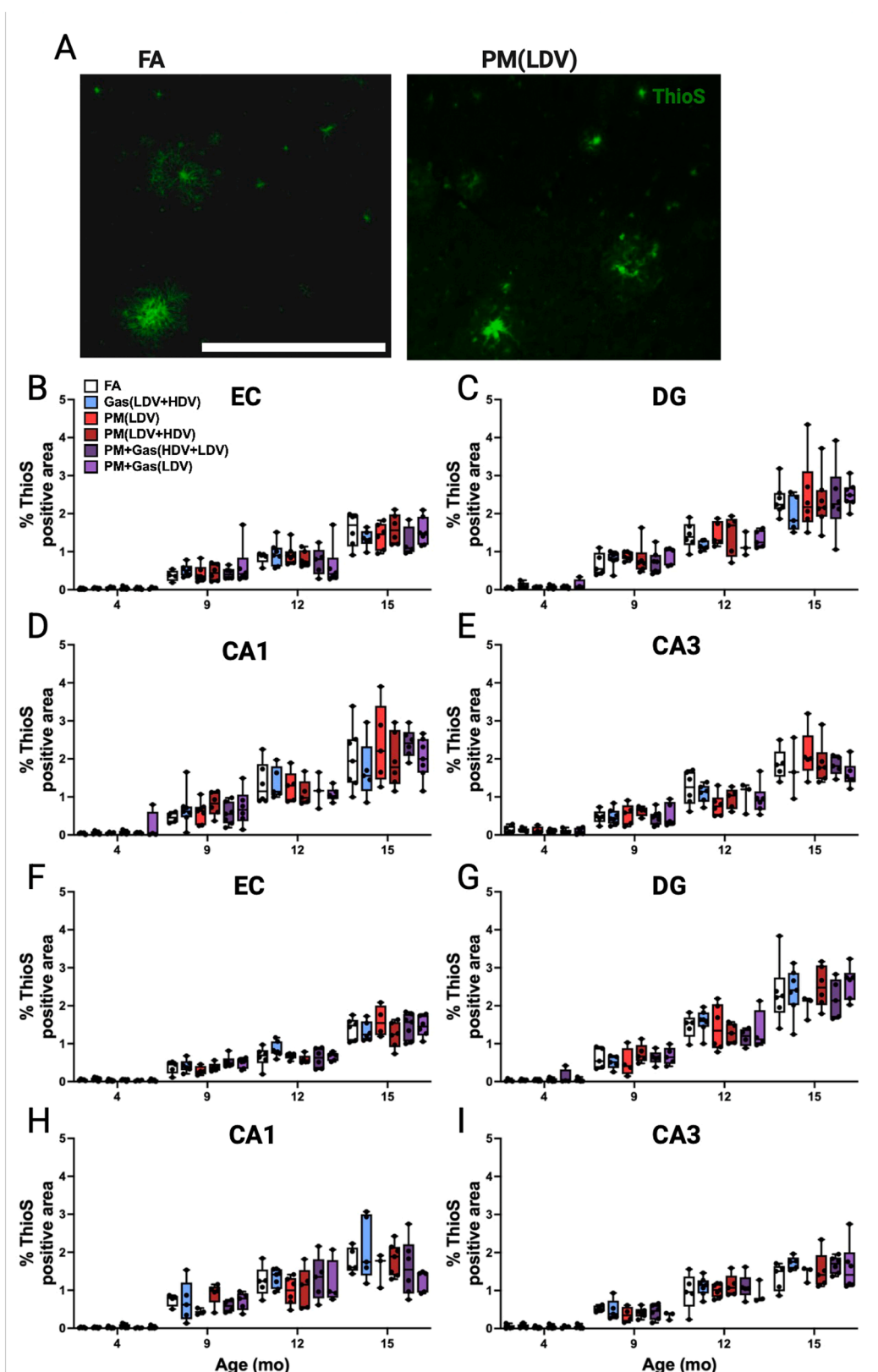


Fig. 9. Effect of different TRAP exposures on amyloid plaques in female and male TgF344-AD rats. Amyloid plaques were visualized by Thioflavin-S (ThioS) staining of brain sections. (A) Representative photomicrographs of ThioS staining (green) in the entorhinal cortex of 15 mo old female rats exposed to FA and PM+Gas(LDV+HDV) exposed animals. Bar= 200 μ m. (B-H) Amyloid plaques were quantified as the % area of ThioS staining in the entorhinal cortex (EC; panels B, F), dentate gyrus (DG; panels C, G), and CA1 (panels D, H) and CA3 (panels E, I) subregions of the hippocampus of females (B-E) and males (F-I). Data are presented as box-and-whisker plots, in which the horizontal line in the box represents the median; lower and upper limits of the box, 1st and 3rd quartile, respectively; whiskers, the minimum and maximum; and dots, values obtained for individual animals ($n = 5-7$ animals per exposure group). Data were analyzed by two-way ANOVA using exposure and time as factors, with post hoc Dunnett's test and a p value of 0.05. No significant differences were identified.

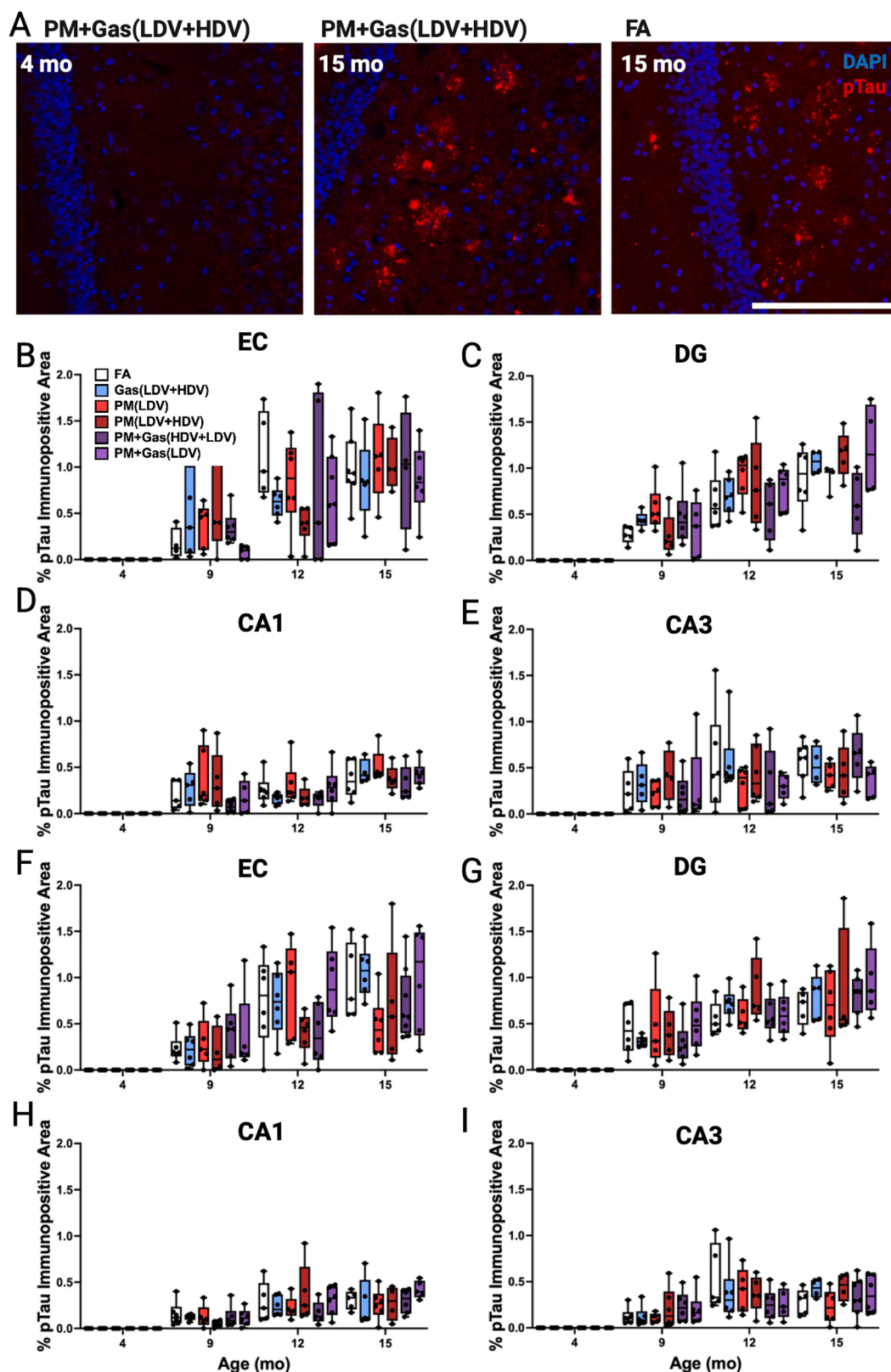


Fig. 10. Effect of different TRAP exposures on phosphorylated Tau in female and male TgF344-AD rats. To detect pathogenic phosphorylated tau (pTau), brain sections were immunostained for pTau (red) and counterstained with DAPI (blue). (A) Representative images of pTau immunoreactivity in the dentate gyrus (DG) of female rats. (B–I) The area of pTau immunoreactivity normalized to region area in mm^2 in the entorhinal cortex (EC, panels B, F), DG (panels C, G) and CA1 (panels D, H) and CA3 (panels E, I) in female (B–E) and male (F–I) rats. Data are presented as box-and-whisker plots, in which the horizontal line in the box represents the median; lower and upper limits of the box, 1st and 3rd quartile, respectively; whiskers, the minimum and maximum; and dots, values obtained for individual animals ($n = 5–7$ animals per exposure group). Data from each subregion were analyzed using one-way ANOVA with the p value set at 0.05; males and females were analyzed separately. No significant differences between exposure group were identified.

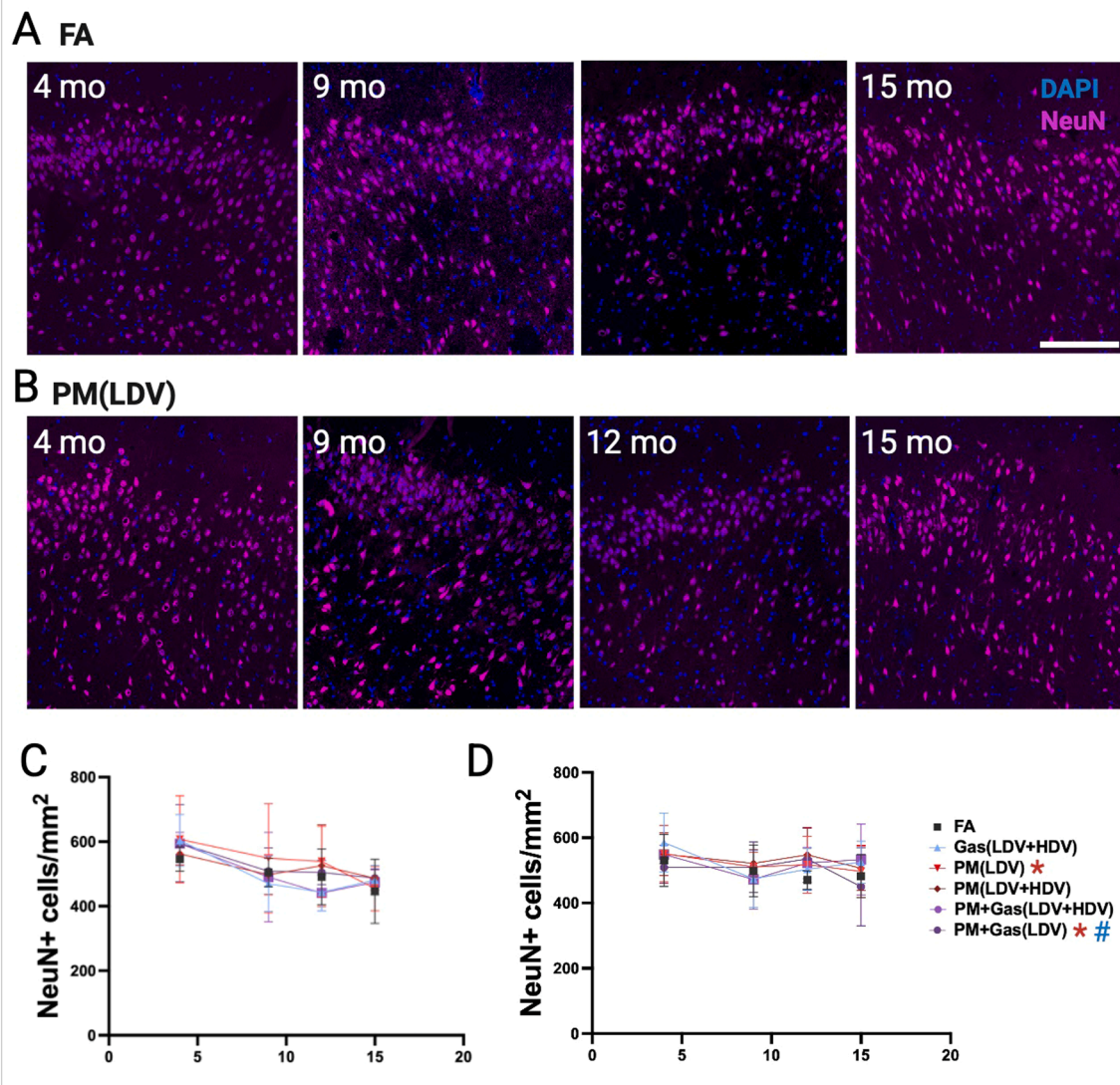


Fig. 11. Effect of different TRAP exposures on neuronal cell density over time in female and male TgF344-AD rats. Brain sections were immunostained for NeuN (magenta) to identify neuronal cells in the entorhinal cortex; sections were counterstained with DAPI (blue). (A, B) Representative images of NeuN immunoreactivity in the entorhinal cortex of 4, 9, 12 and 15-month-old females exposed to FA (A) or PM(LDV) (B). Bar = 200 μ m. (C, D) Neuronal cell density, quantified as the number of NeuN+ neurons to area in the entorhinal cortex of females (C) or males (D). Data are shown as the mean $\pm$ SD ($n = 21$ – 25 animals per group per sex); with lines for exposure group determined using linear regression. *Significantly decreased count from FA at $p < 0.05$ as determined by one-way ANOVA and post-hoc Dunnett's multiple comparisons test. # Significantly increased from FA at $p < 0.05$ as determined by one-way ANOVA and post-hoc Dunnett's multiple comparisons test. Red indicate differences in females (C) and blue indicate significant differences in males (D).

LDV+HDV emissions, we observed significant effects of TRAP exposure on OC amyloid fibrils across sexes at 15 months, sex effects on OC qIHC and cortical A β 42:40 ratios, and no TRAP dependent changes in pTau. In contrast to our earlier study, however, we did not observe significant TRAP exposure effects on ThioS+ plaques and tangles or on ELISA A β levels. Additionally, our previous study observed that TRAP exposure caused significant neuronal cell loss in the EC at 15 months. While we did not observe significant TRAP effects on cell counts of NeuN immunopositive cells at any given time point, we did observe accelerated neuronal cell loss in the EC over time. The less robust exposure-dependent effects observed in the current study compared to the previous study suggest that variations in TRAP exposure over time modified the nature and severity of TRAP impacts on the aging brain. Notably, comparison of PM levels recorded in the previous vs. current study indicates that this parameter was only slightly decreased between the studies. Another possible explanation for the different outcomes observed between these two studies is that the exposures in the previous

study began in the winter, whereas in the current study, exposures began in the summer. In addition to differences in summer- vs winter-blend fuels that alter TRAP composition, seasonal meteorological conditions may also alter PM dynamics and health effects (Hsu et al., 2017, Martinez-Soto et al., 2026, Rackow et al., 2025). PM_{2.5} concentrations from anthropogenic emissions in California have been shown to differ between summer and winter (Zhu et al., 2019). Seasonal rain and storms also remove and dilute PM and gaseous TRAP components (Wang et al., 2023). This washout effect also alters the particle sizes to which animals are exposed (Guo et al., 2016). However, whether seasonal variation is the explanation for the different outcomes observed in the previous vs. current study has yet to be determined.

In summary, the results from the current study largely corroborate earlier findings implicating TRAP as an AD risk factor and further suggest that PM is the critical component in TRAP responsible for the effects of TRAP on AD pathogenesis. Our findings also suggest that LDV and HDV emissions differentially impact the aging brain, with LDV emissions

causing more robust pathological responses than HDV emissions. The explanation for this somewhat counterintuitive observation has yet to be identified, but likely reflects differences in the chemical composition of PM emitted by LDV vs. HDV. Chemical speciation of the particulate matter found in the brains of these animals is the focus of ongoing studies in our laboratory. Other outstanding questions that demand additional investigation include the mechanism(s) by which TRAP promotes AD pathology, and how these mechanisms are influenced by sex and age. While there remain many key data gaps that need to be addressed to enact policies to protect the aging brain from TRAP, the fact we observed less robust effects on AD in this study relative to our previous study suggests the feasibility of significantly altering AD risk by changing emission composition through regulatory actions.

CRediT authorship contribution statement

Wexler Anthony Stein: Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Peter Andrew:** Writing – review & editing, Software. **Anthony Valenzuela:** Writing – review & editing, Project administration. **Heui Hye Park:** Writing – review & editing, Project administration, Data curation. **Mei-Yun Cheng:** Writing – original draft, Investigation. **Nathifa Nasim:** Writing – original draft, Investigation. **Lein Pamela J:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Keith Bein:** Writing – review & editing, Writing – original draft, Resources, Investigation, Funding acquisition, Conceptualization. **Laura S. Van Winkle:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Vidya Chandrasekaran:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Given her role as co-Editor-in-Chief of NeuroToxicology, Pamela J. Lein, had no involvement in the peer review of this article and had no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Remco Westerink, co-Editor-in-Chief of NeuroToxicology. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.neuro.2026.103472](https://doi.org/10.1016/j.neuro.2026.103472).

Data availability

Data will be made available on request.

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