

Note to readers with disabilities: *EHP* strives to ensure that all journal content is accessible to all readers. However, some figures and Supplemental Material published in *EHP* articles may not conform to [508 standards](#) due to the complexity of the information being presented. If you need assistance accessing journal content, please contact ehp508@niehs.nih.gov. Our staff will work with you to assess and meet your accessibility needs within 3 working days.

Supplemental Material

The Effects of Chronic Exposure to Ambient Traffic-Related Air Pollution on Alzheimer's Disease Phenotypes in Wildtype and Genetically Predisposed Male and Female Rats

Kelley T. Patten, Anthony E. Valenzuela, Christopher Wallis, Elizabeth L. Berg, Jill L. Silverman, Keith J. Bein, Anthony S. Wexler, and Pamela J. Lein

Table of Contents

Materials

Table S1. Antibodies and conditions used in immunohistochemistry (IHC) analyses.

Table S2. Summary of regional analysis of ThioS staining in 15 month-old TgF344-AD rats.

Table S3. Summary of major effects of TRAP by age and endpoint.

Table S4. Summary of numeric data in Figure 1.

Table S5. Summary of numeric data in Figure 2.

Table S6. Summary of numeric data in Figure 3 and Figure S2.

Table S7. Summary of numeric data in Figure 4 and Figure S3.

Table S8. Summary of numeric data in Figure 5.

Table S9. Summary of numeric data in Figure 6.

Figure S1. Photo montage of the various systems comprising the Tunnel Exposure Facility.

Figure S2. Effects of TRAP on cued fear conditioning and neuronal cell loss in the hippocampus. (A-B) Cued fear conditioning was performed on-site at 9.5 or 14.5 month old animals for the 10 and 15 month-old cohort. An increased average motion index indicates impaired cognitive behavior. (C-D) To assess neuronal cell loss, brain sections were immunostained for NeuN, a biomarker of neurons, and the number of NeuN-immunopositive cells per mm² were counted in the CA1. (E) Densitometric analyses of total tau relative to GAPDH in the crude pellet fraction of cortical tissue. All data presented as the mean \pm SD (n=10-12 animals per group for A-D; n=5-6 animals per group for E). Data were analyzed by three-way ANOVA using sex, genotype, and exposure as factors (A-B, E) or two-way ANOVA using genotype and exposures as factors (C, D) with *post-hoc* Sidak's test; *p<0.05. Circles represent individual animals (for C-E, each circle is an average of 4 technical replicates). M=male; F=female; WT=wildtype; Tg=TgF344-AD. Summary values are available in Table S6.

Figure S3. Effects of TRAP on A β deposition by brain region, and on guanidine-HCL soluble brain extracts. (A) Analyses of ThioS⁺ plaques by brain regions in TgF344-AD rats (DG=dentate gyrus; EC=entorhinal cortex; Thal=thalamus; Cer=cerebellum) (B) Guanidine-HCL-soluble ratios of A β 42:40, as measured by ELISA in cortical samples and normalized to A β levels in 3-month-old WT female rats. All data presented as the mean \pm SD (n=5-6 animals per group). Circles represent individual animals. Four brain sections were measured per animal in A, and two technical replicates were performed for each animal in B. M=male; F=female. Data were analyzed by three-way ANOVA using sex, genotype, and exposure as factors, with *post-hoc* Sidak's test. *p<0.05. Summary data are available in Table S7.