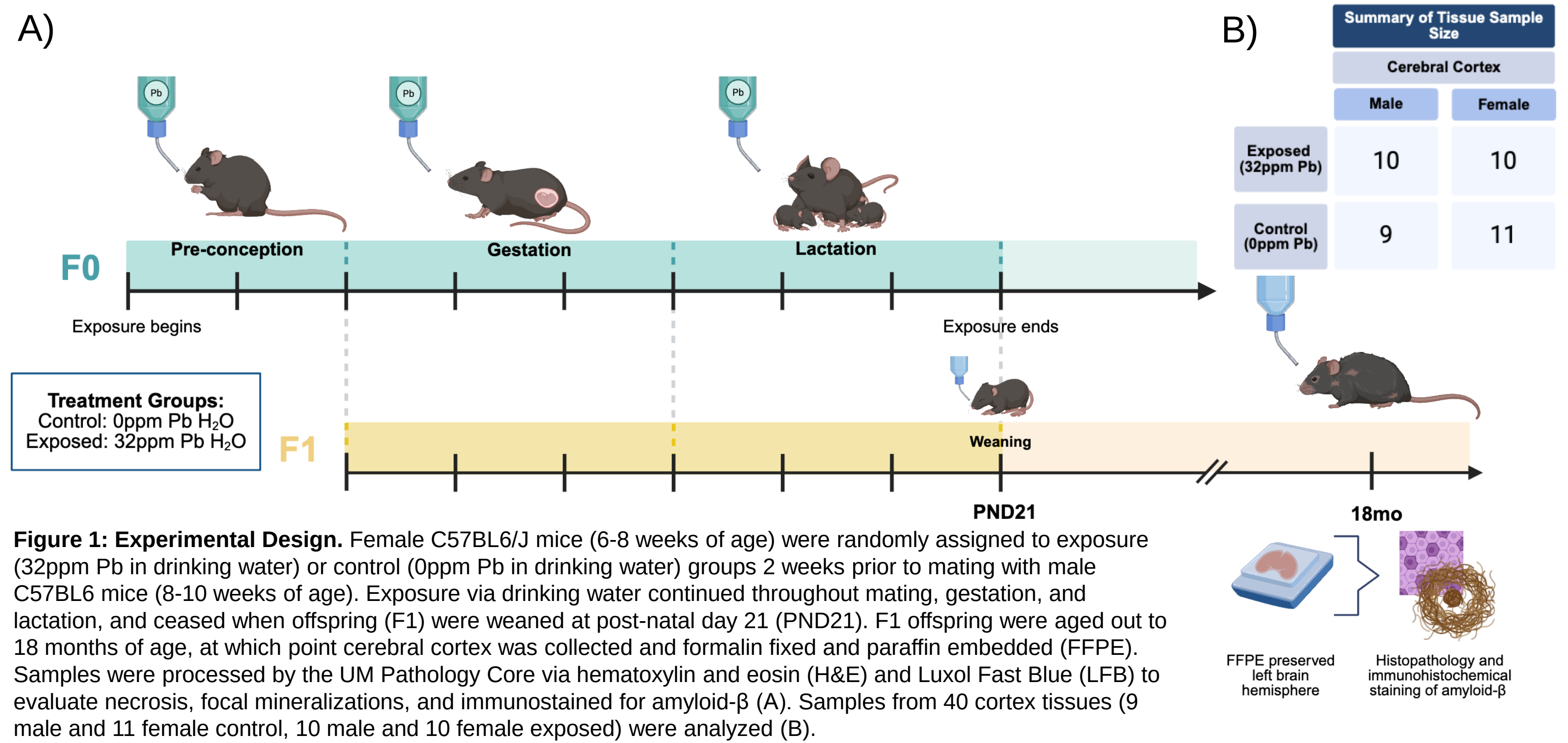


BACKGROUND & FINDINGS

Exposure to neurotoxicants during early development can have long-lasting implications for cognition and health. Continued work is needed to understand the impact of environmentally relevant lead (Pb) exposure during early periods of development and whether exposure correlates with pathological changes in the brain during old age. Previous work assessing this question at the metabolomic level found distinct profiles of metabolites with perinatal Pb exposure in both plasma and cortex for male and female mice during late life, particularly those relevant to fatty acid metabolism, suggesting Pb exposure may perturb lipid biosynthesis and function. Given its relevance to neurodegenerative diseases such as Alzheimer's, and its dependence on lipid metabolism, we were next interested in whether perinatal Pb exposure is associated with pathological changes in the aging mouse (C57/B6) brain such as amyloid- β . To address this, we evaluated the left hemisphere of the brain in 18-month-old mice that were exposed to Pb during perinatal development (gestation through the first three weeks of life) via dam drinking water. We observed no gross changes in brain morphology associated with Pb exposure such as necrosis or significant pockets of mineralization, quantified via hematoxylin & eosin (H&E) and Luxol Fast Blue (LFB) staining. We evaluated amyloid- β presence via an antibody targeting monomeric, oligomeric, and fibrillar forms of the amyloid- β 1-42 peptide. Low to moderate amyloid- β immunolabeling was present diffusely across the grey matter of the cerebrum and thalamus but was largely absent in white matter tracks. Mean amyloid- β intensity across the entire tissue was higher in exposed female samples relative to controls, and this trend was also present in males but did not reach statistical significance. There were significantly more amyloid- β positive cells in the exposed samples versus control for both males and females. Amyloid- β plaques, as visualized in the positive control sample from a mouse model of Alzheimer's disease, were not present in any samples.

METHODS



RESULTS

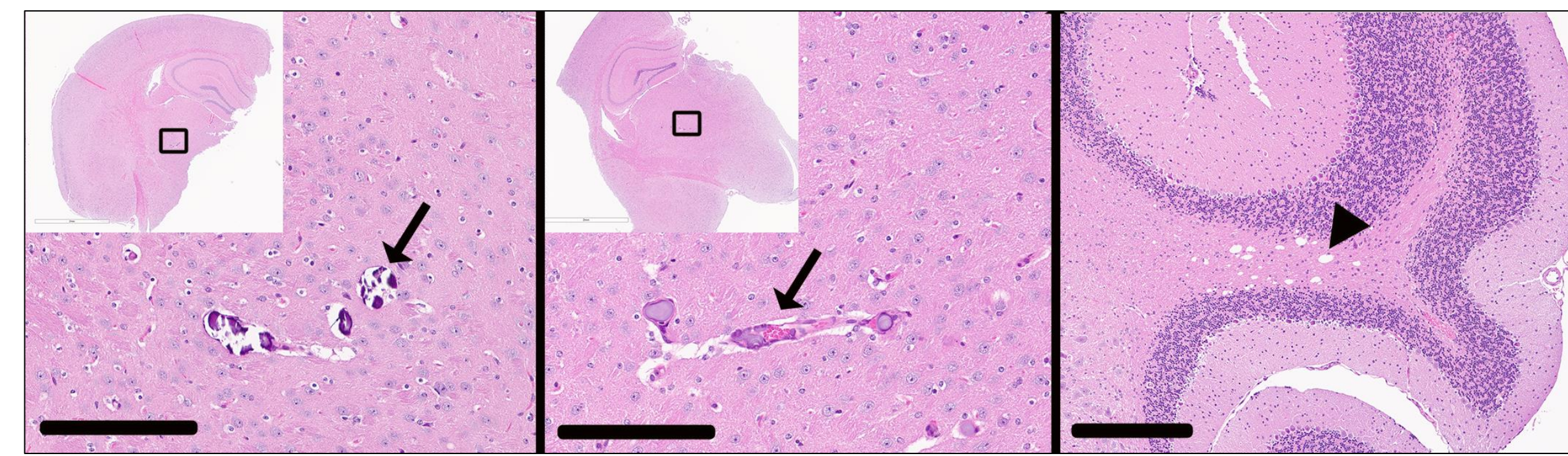


Figure 2: Representative images from male control (A) and male exposed (B, C) samples showing thalamic mineralization (A,B) and cerebellar artifactual vacuolation (C). Insets in A, B show low power overview of location of thalamic mineralization. H&E. bars 200 μ m (A,B), 300 μ m (C).

	H&E Mineralization Present		LFB Mineralization Present	
	Female	Male	Female	Male
Exposed (32ppm Pb)	1/10	2/10	1/10	3/10
Control (0ppm Pb)	2/11	4/9	2/10	2/11
Chi-Squared p-value	1	0.62	1	1

Table 1: Prevalence of Mineralization in Exposed versus Control Samples. Data on the occurrence of foci mineralization, in either H&E slides or LFB slides, are presented as a ratio of the number of samples with foci mineralization over the total number of samples in that group. Comparisons between occurrence between exposed (32ppm Pb) and control (0ppm Pb) for a given group (males or females) achieved via a Fisher's exact test and significance is denoted by p-value (significance accepted at $p < 0.05$).

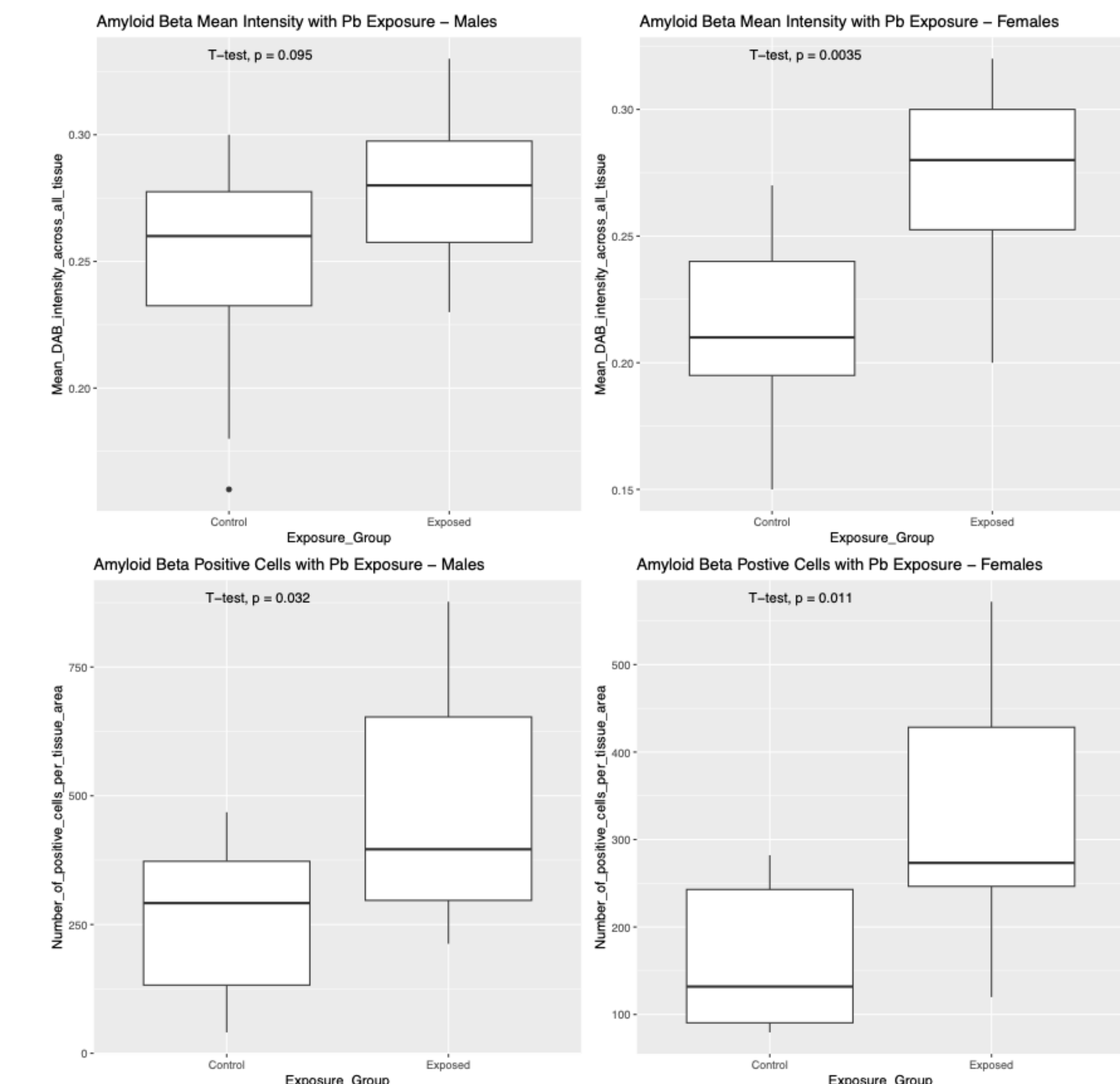


Figure 3: Quantification of diffuse amyloid- β staining in males (A) and females (B) and amyloid- β -positive cells in males (C) and females (D). Statistical comparisons achieved using two-sided t-test (Significance accepted at $p < 0.05$).

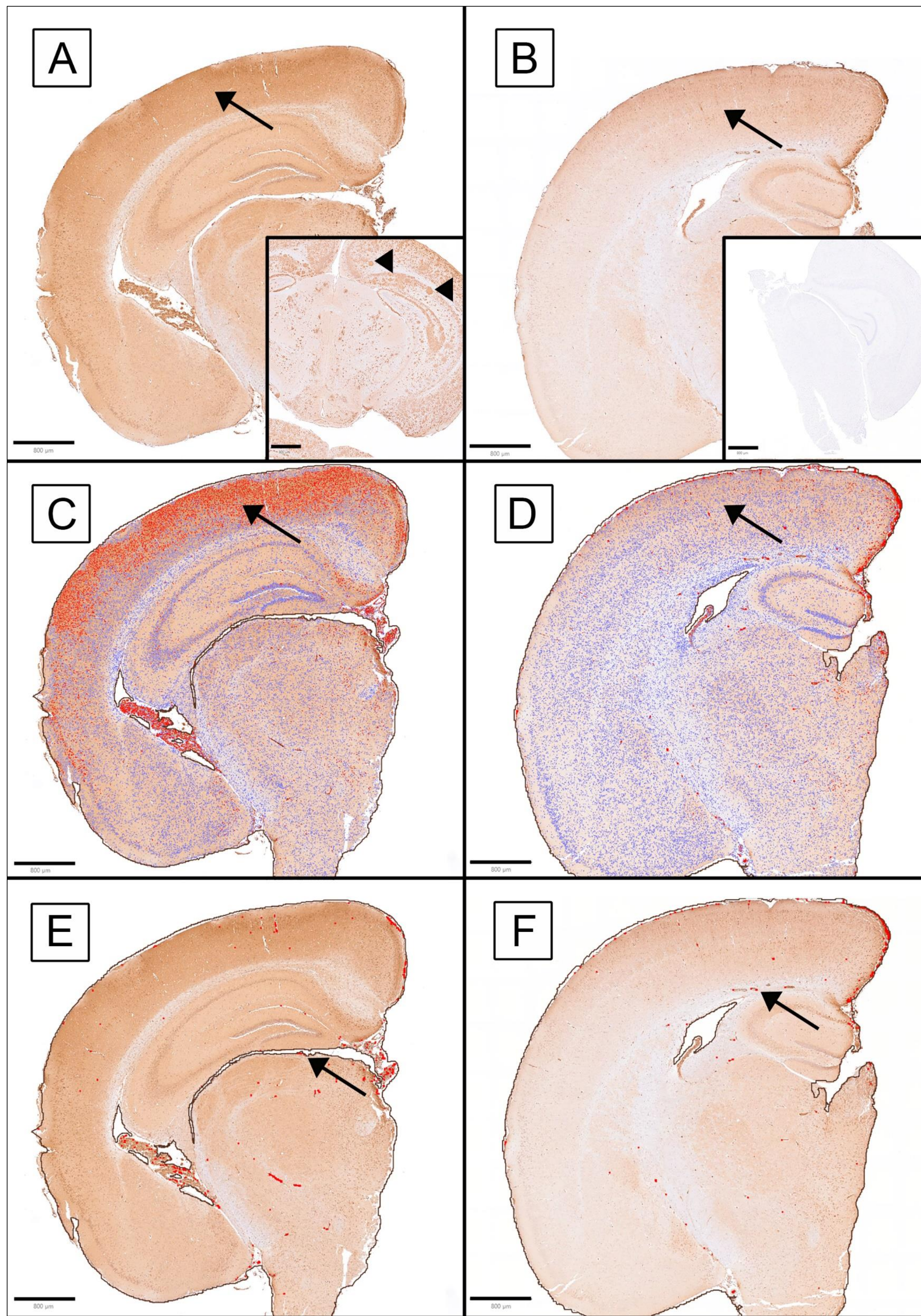


Figure 4: Representative low magnification images of amyloid- β immunostained Exposed Female Sample 1 (A,C,E) and Control Female Sample 2 (B,D,E). Sample 1 showed diffuse moderate to high intensity staining in grey matter (A) and Sample 2 showed diffuse low to moderate intensity staining in the same areas (B). A positive control brain section from a murine Alzheimer's disease model (A, inset) and negative control (B, inset) are also shown. Digital overlays showing positive (red) and negative (blue) cells are shown in C, D and digital overlays of the highest intensity pixel areas are shown in E,F. Bars 1 mm.

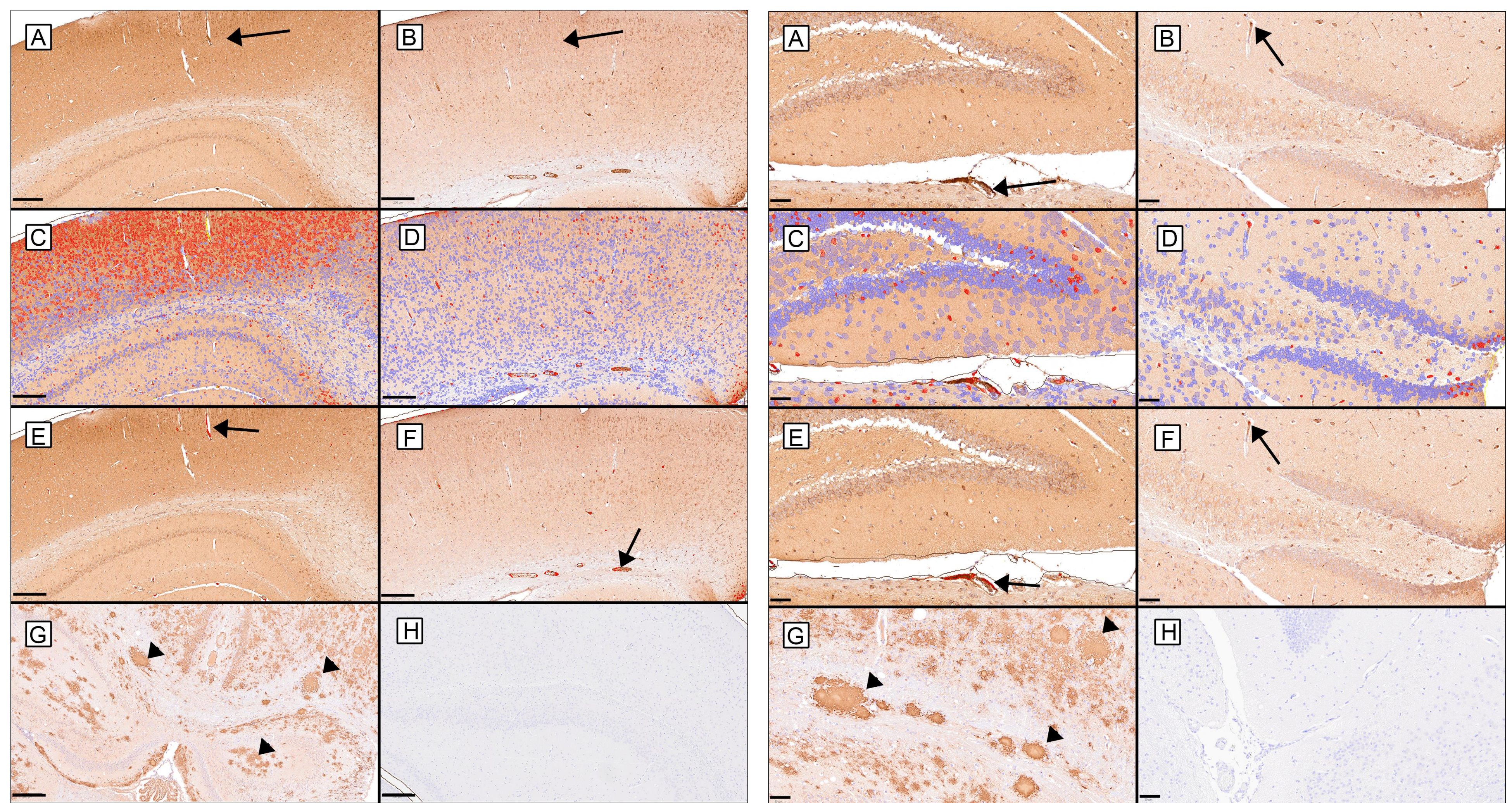


Figure 5: Representative images of amyloid- β immunostained Female Exposed Sample 1 (A,C,E), Female Control Sample 2 (B,D,F), a positive control brain section from a murine Alzheimer's disease model (G), and a negative control (H). Sample 1 showed diffuse moderate to high intensity staining in grey matter (A) and Sample 2 showed diffuse low to moderate intensity staining in the same areas (B). Both samples showed highest intensity staining in vascular walls (arrows, A, B). Digital overlays of positive (red) and negative (blue) cells are shown in C, D and digital overlays of the highest intensity pixel areas (arrows) are shown in E,F. The positive control (G) shows numerous extracellular beta amyloid plaques (arrowheads) and also amyloid- β staining in vascular walls (arrow). Negative control shows no staining (H). Bars 200 μ m.

Figure 6: High magnification representative images of amyloid- β immunostained Female Exposed Sample 1 (A,C,E), Female Control Sample 2 (B,D,F), a positive control brain section from a murine Alzheimer's disease model (G), and a negative control (H). Sample 1 showed diffuse moderate to high intensity staining in grey matter (A) and Sample 2 showed diffuse low to moderate intensity staining in the same areas (B). Both samples showed highest intensity staining in vascular walls (arrows, A, B). Digital overlays of positive (red) and negative (blue) cells are shown in C, D and digital overlays of the highest intensity pixel areas (arrows) are shown in E,F. The positive control (G) shows numerous extracellular beta amyloid plaques (arrowheads) and also amyloid- β staining in vascular walls (arrow). Negative control shows no staining (H). Bars 50 μ m.

CONCLUSIONS & FUTURE DIRECTIONS

- Perinatal Pb exposure led to an increase in diffuse amyloid- β and amyloid- β -positive cells in 18-month murine brain.
- Lack of amyloid- β plaques may reflect C57/B6 resistance to dementia pathology, which has been characterized previously.
- Ongoing work is quantifying the presence of other dementia hallmarks, including phosphorylated tau, neuroinflammation, and metabolic alterations in the same tissues along with behavioral phenotyping of learning and memory.
- We are also assessing the role of perinatal Pb exposure in the 5xFAD mouse model, which robustly presents dementia hallmarks, to characterize interactions between genetic risk and Pb exposure.

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