Characterization of Tau Expressing P301S Mouse Model for Tauopathy – Longitudinal Brain Structural and Metabolic Profile

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1 INTRODUCTION & STUDY DESIGN

Background: P301S (B6;C3-Tg(Prnp-MAPT*P301S)PS19Vle/J) mouse is a widely used tauopathy model. Majority of the work described for the model focuses on the brain pathology after 6 months of age, when there has been reported more prominent tau pathology, neuronal cell loss and atrophy. As the early development of tauopathy, behavioral phenotype and both structural and metabolic profile of the brain in P301S (TG) model have not been well characterized, we sought to examine longitudinal phenotype of this model with MR anatomical imaging, 1H-spectroscopy, metabolic imaging (PET) and behavioral features, and comparing them to aged matched background strain mice.

2 METHODS

Animals Female (n=20) and male (n=19) P301S (B6;C3-Tg(Prnp-MAPT*P301S)PS19Vle/J, Jackson Laboratory, USA) and age matched B6CH3F1 back ground strain mice (n=20 for both genders) at the age of 7 weeks were enrolled to the follow-up study. Mice were kept in standard housing conditions on ad libitum food and water.

MR experiments: Imaging was performed at Bruker Biospec USR AVIII 11.7T magnet (Bruker Biospin GmbH, Ettlingen, Germany) in isoflurane anesthetized animals.

Structural T2-MRI & 1H-MR Spectroscopy: A Turbo-RARE with in-plane resolution of 78 microns and thirty-one 0.45 mm slices. Bilateral hippocampal voxel (1.2x4.0x1.5 mm³, 7.2 μl volume) with PRESS sequence (TE/TR = 10/2000 ms). Data as averaged 512 excitations, number of points 2048 and spectral width of 5 kHz. Peak areas for resolved metabolites were analyzed using LCModel (Stephen Provencher Inc., Oakville, Canada).

[18]FDG PET: At the age of 7 months male mice were fasted overnight prior to PET imaging to stabilize blood glucose levels. P301S and WT mice (n=5 and 7, average BW 40 g and 44 g) were dosed with ca. 15 MBq of ^{18}F-FDG i.v. Static 30 min PET scan (250 – 700 keV) was started 30 min post FDG dosing followed by a CT scan (BioPET/CT, Sedecal). The sinograms were reconstructed with 3D OSEM with attenuation correction and image analysis was performed with PMOD software (v3.7, PMOD Technologies).

Open field: The open field test is performed during the mouse light cycle and under a normal lighting in the sound attenuated cubicles that is divided evenly on to the test cages. The test device (square 27×27×20.3 cm) is constructed of Plexiglas. The paths of the mice were recorded by activity monitor (Med. Associates Inc.) for 30 min.

Fear conditioning: Mice were brought to the experimental room for at least 30 min acclimation to the experimental room conditions prior to testing. The contextual fear conditioning test is modified from Comery et al. (2005) (Comery TA et al., Acute gamma-secretase inhibition improves contextual fear conditioning in the Tg2576 mouse model of Alzheimer's disease. J. Neurosci. 2005 Sep 28;25(39):8898-902). The training and testing were conducted on two consecutive days, using a Coulbourn FreezeFrame system (Coulbourn, Whitehall PA, USA).

Radial arm water maze: Mice are brought to the experimental room for at least 30 min acclimation to the experimental room conditions prior to testing. Two day radial-arm water maze has been described in detail previously (Alamed et al. 2006). Briefly, a six arm maze is submerged in a pool of water, and a platform is placed at the end of one arm. The mouse receives 15 trials per day for 2 d and on each trial is started in a different arm while the arm containing the platform remains the same for each mouse. Using visual cues around the room, the mouse learns the position of the escape platform. The first 10 trials are considered training and alternate between a visible and a hidden platform. The final trials for day 1 and all trials on day 2 use a hidden platform. The number of errors (incorrect arm entries) are counted over a 1 min period. The errors are averaged over three trials resulting in 10 blocks for the 2 d period.

.*All data is mean + SEM and statistics are given as Student's t-test, p-values as indicated in figures.*
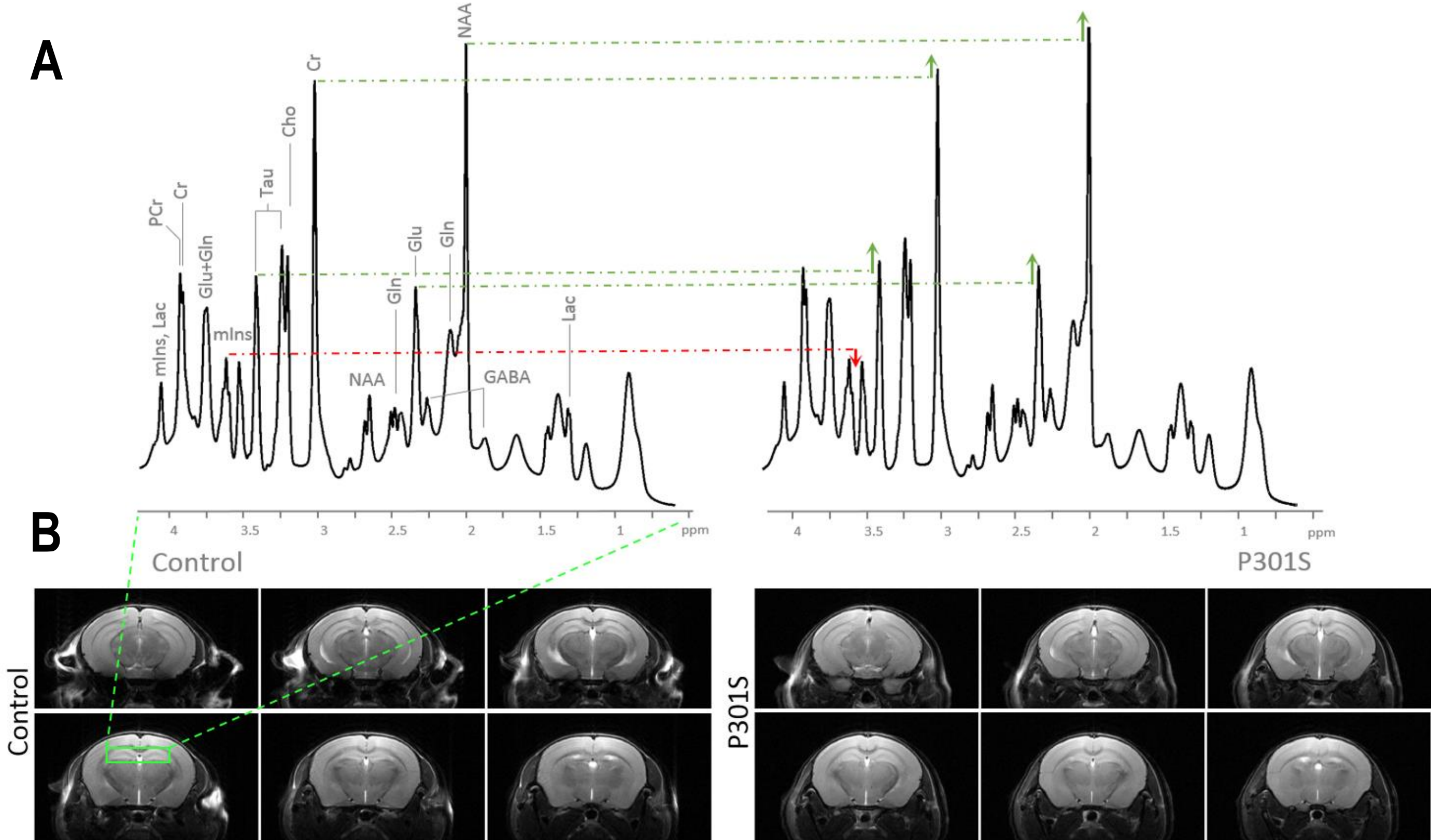
3 RESULTS

Figure 1. Example spectra from P301S and age matched control mice at 2 months of age (A). T2-MRI anatomical images for volumetric evaluation of structures (B) are shown for P301S and aged matched control mouse.
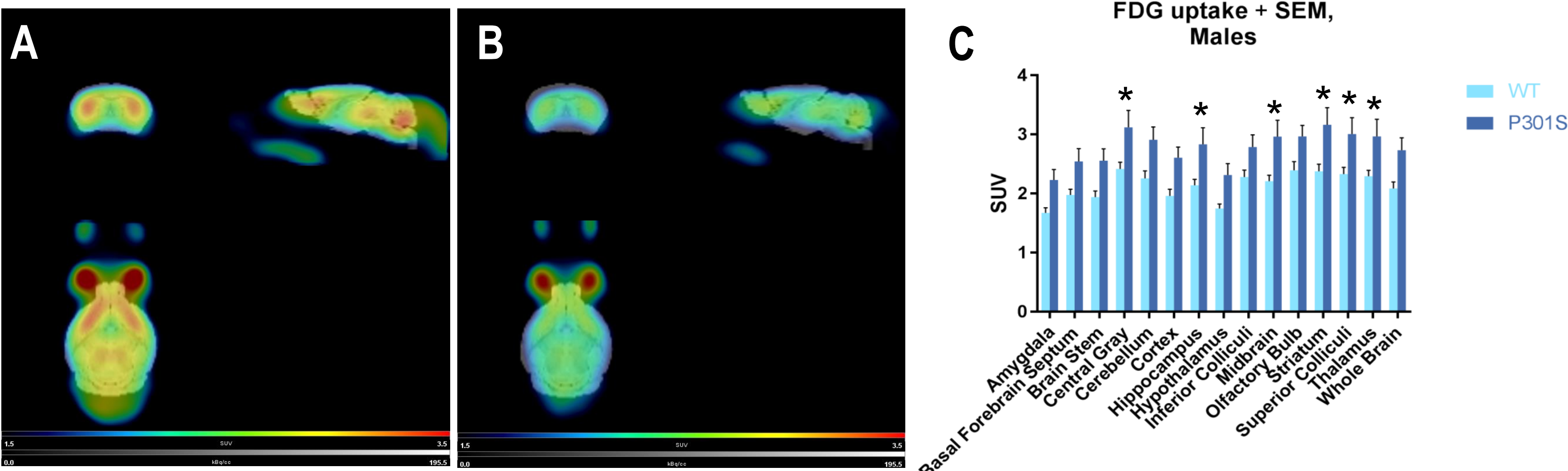
3 RESULTS CONT'D

Figure 2. Example FDG PET imaging data from P301S (A) and age matched control mice (B) at 7 months of age (A). Quantification of FDG consumption was signifcantly higher in multiple brain structures structures (C). Data presented as mean ± SEM, n=5-6 males/genotype. *p<0.05, t-test.
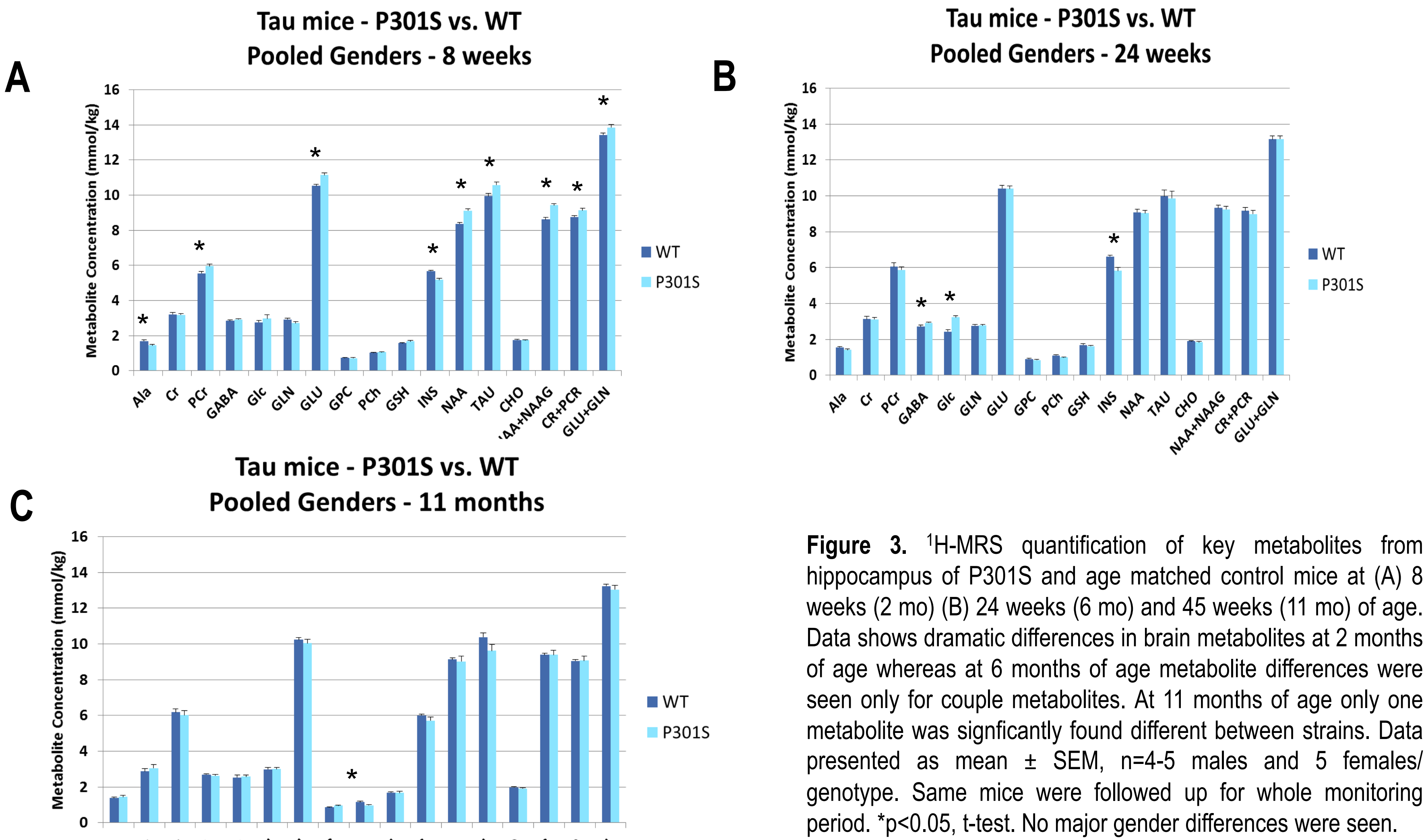

Figure 3. 1H-MRS quantification of key metabolites from hippocampus of P301S and age matched control mice at (A) 8 weeks (2 mo) (B) 24 weeks (6 mo) and 45 weeks (11 mo) of age. Data shows dramatic differences in brain metabolites at 2 months of age whereas at 6 months of age metabolite differences were seen only for couple metabolites. At 11 months of age only one metabolite was signifcantly found different between strains. Data presented as mean ± SEM, n=4-5 males and 5 females/genotype. Same mice were followed up for whole monitoring period. *p<0.05, t-test. No major gender differences were seen.
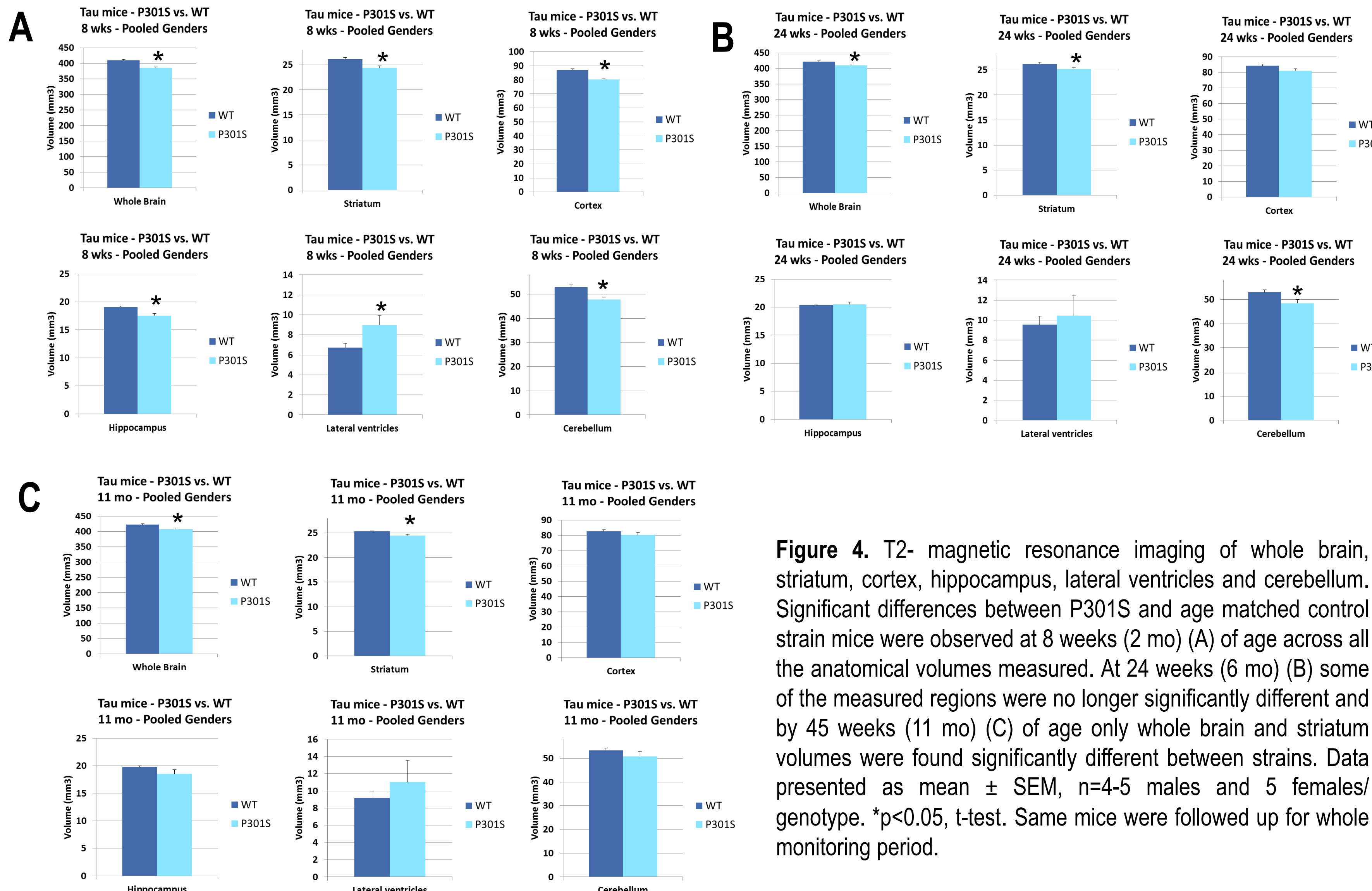

Figure 4. T2- magnetic resonance imaging of whole brain, striatum, cortex, hippocampus, lateral ventricles and cerebellum. Significant differences between P301S and age matched control strain mice were observed at 8 weeks (2 mo) (A) of age across all the anatomical volumes measured. At 24 weeks (6 mo) (B) some of the measured regions were no longer signifcantly different and by 45 weeks (11 mo) (C) of age only whole brain and striatum volumes were found significantly larger than P301S mice. Data presented as mean ± SEM, n=4-5 males and 5 females/genotype. *p<0.05, t-test. Same mice were followed up for whole monitoring period.
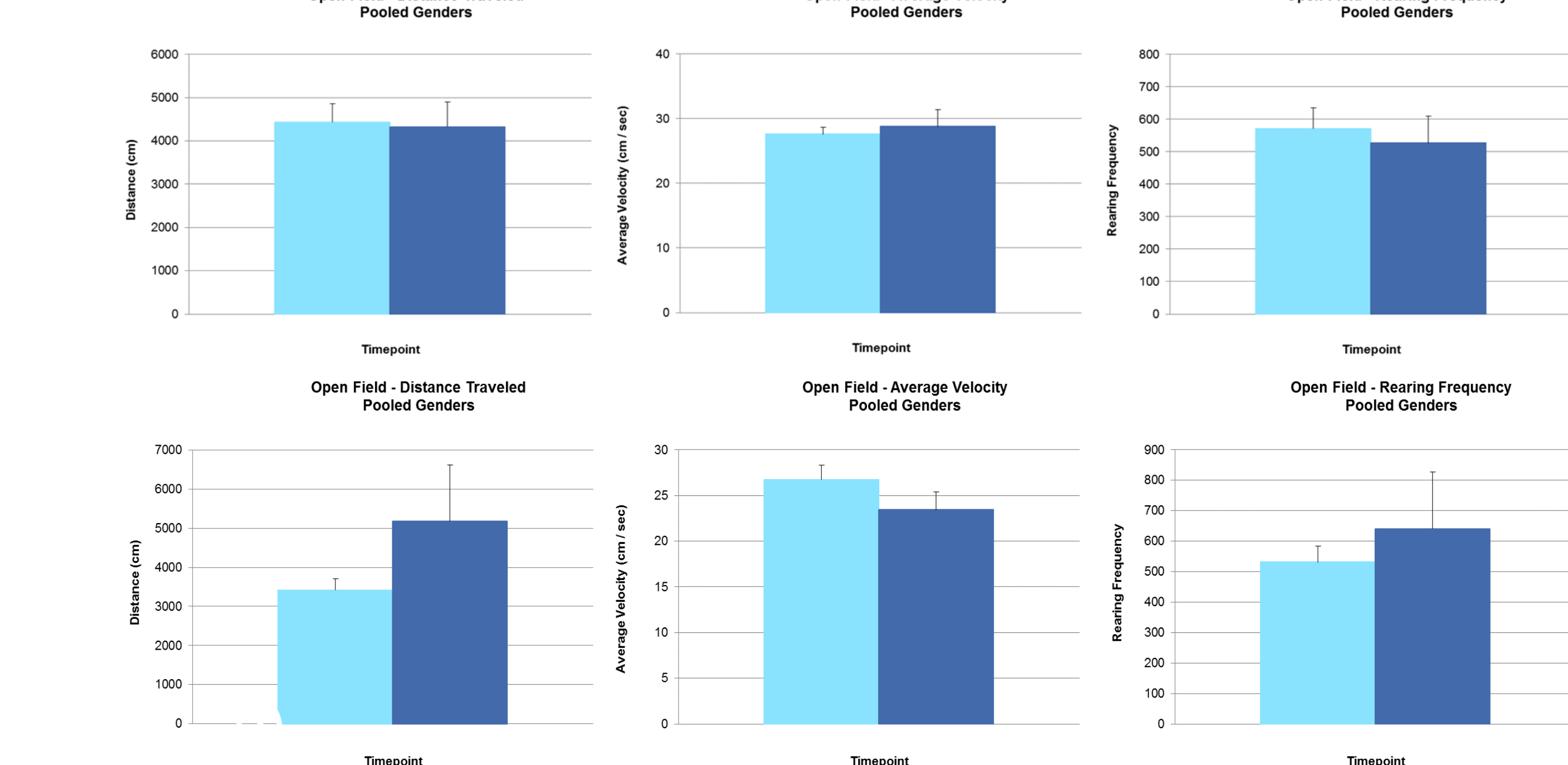
3 RESULTS CONT'D

Figure 5. Open field spontaneous locomotor analysis at 8 weeks (2 mo) or 24 weeks (6 mo) between P301S and age matched control strain mice. No significant differences were seen. Data presented as mean ± SEM , n=4-5 males and 5 females/ genotype. Same mice were followed up for whole monitoring period. No significant gender differences were observed (data not shown).
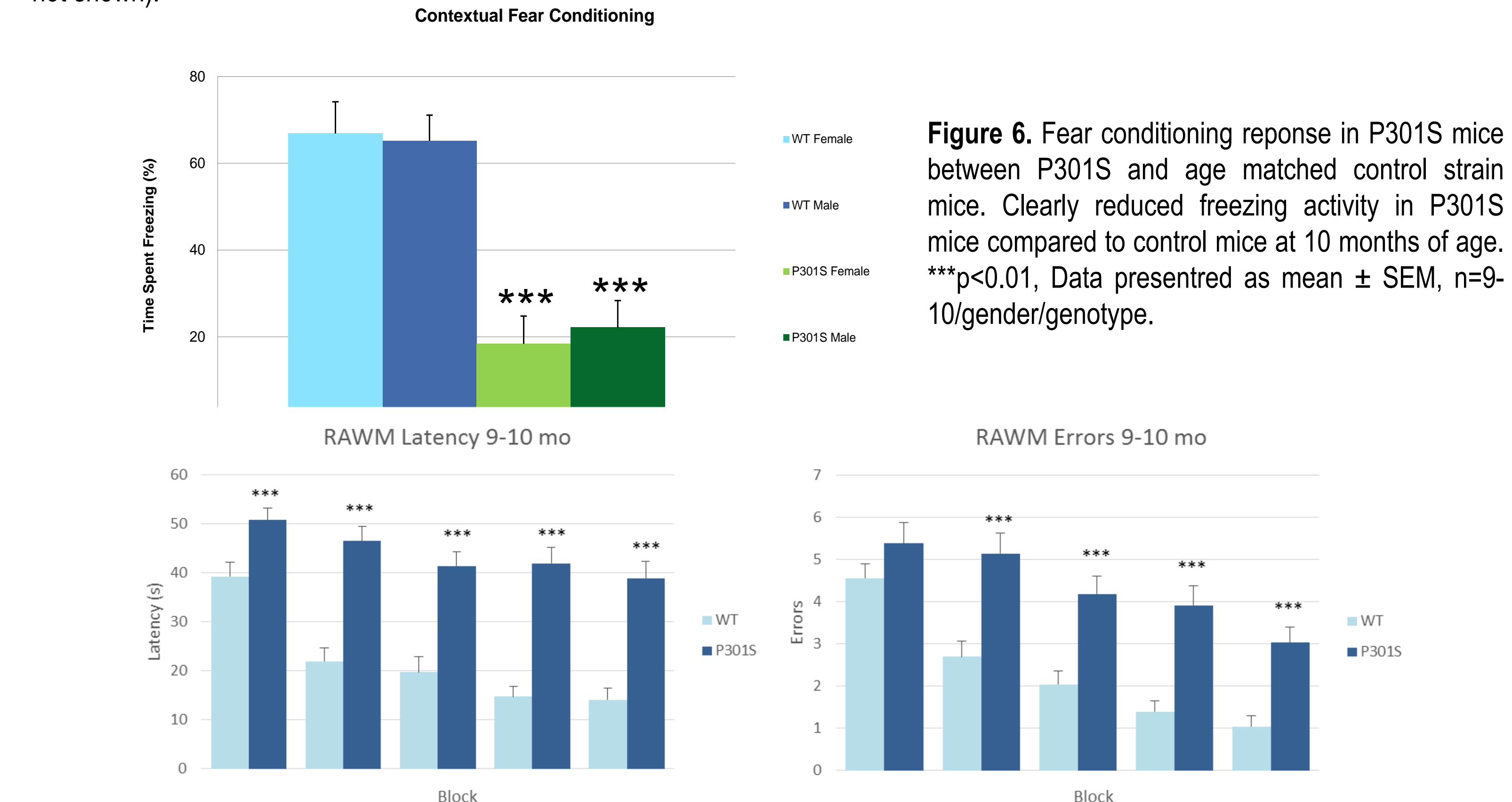

Figure 6. Fear conditioning reponse in P301S mice between P301S and age matched control strain mice. Clearly reduced freezing activity in P301S mice compared to control mice at 10 months of age. ***p<0.01, Data presrented as mean ± SEM, n=9-10/gender/genotype.

Figure 7. Radial arm water maze (RAWM) performance in P301S mice was found to be clearly impaired when compared to age matched control strain mice as seen as increased latency to find the platform or increased number of errors. ***p<0.01, Data presrented as mean ± SEM, n=19-20/genotype. No differences between genders were observed.

4 CONCLUSIONS

In this study we looked at known tau expression mouse model longitudinally for possible changes and differences over time when compared to control strain mice. We specifically looked at brain anatomical and metabolic differences and temporal changes during aging and found:

1) 1H-MRS and T2-MRI showed signficant differences between P301S and control strain mice, especially at early time-points, but later found these differences to level off. Metabolic differences were found to be present in both genders (data not shown) with some differences in metabolites with significant differences. Previously reported atrophy in the P301S mice between 6 months of age to 11 months were not confirmed in this study, although control strain whole brain and striatal volumes were found slightly but significantly larger than P301S mice.

2) FDG consumption was found to be signifcantly higher in multiple brain structures in P301S when compare to control mice

3) Open field spontaneous locomotor activity was not found to be different between P301S and control strain mice at 2 or 6 months of age.

4) Fear conditioning responses and radial arm water maze performance were found signficantly different between P301S and age matched control strain of mice at 9-10 months of age.

Taken together, data presented here suggests there are major differences in brain anatomical volumes and key metabolites in P301S mice compared to aged matched control strain (B6CH3F1) mice. Differences were more pronounced at young age rather than older age. Spontaneous gross motor activity was not found different between strains duirng the study. Cognition readouts were found to be highly signficantly different in P301S mice compared to control strain mice. Ongoing tau analysis in the brain tissues is ongoing to evaluate key protein expression levels in this known mouse model for tau expression and cognitive deficits. Different options for using aged matched littermate mice as controls are currently being investigated.

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