BEHAVIORAL, ELECTROPHYSIOLOGICAL, AND BIOMARKER LEVEL CHANGES IN THE rTG4510 MOUSE MODEL OF ALZHEIMER'S DISEASE #1259

PsychoGenics
Transforming CNS Discovery

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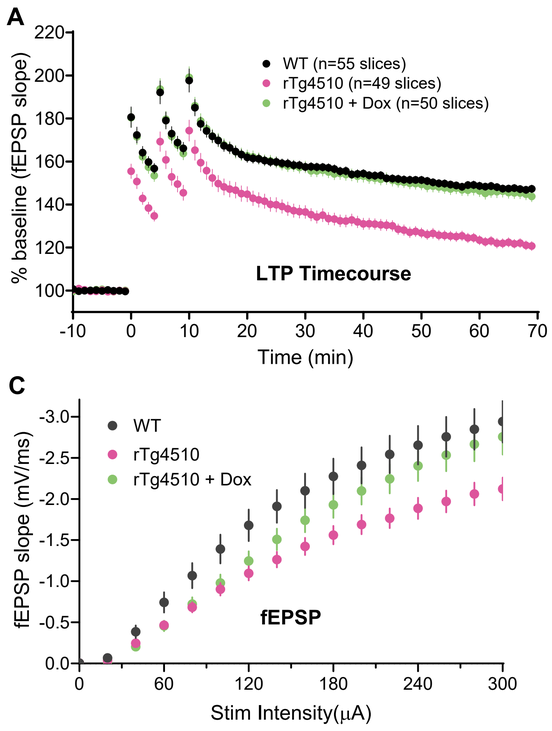
ABSTRACT

Aims: rTg4510 mice conditionally express mutant human Tau in various forebrain regions using Tet-off expression system. This model recapitulates tau pathology observed in AD with age-dependent neuronal loss resulting in cognitive impairment. The objective of the study was to assess age associated behavioral, electrophysiological, and biomarker changes. **Methods**: Female tTA and rTg450 mice were purchased from JAX labs. Locomotor activity was assessed in the open field chambers. Cognitive performance was assessed using the Y maze and Morris water maze tests. Basal synaptic transmission and LTP were assessed by extracellular field potential recording at the Schaffer collateral-CA1 pyramidal. Nf-L levels were measured by Quanterix and inflammatory and Tau by MSD technologies. **Results**: rTg4510 mice showed hyperactivity starting at 4 months of age. Cognitive deficits were seen in the Morris water maze during acquisition and probe tests. Although spontaneous alternations were decreased in rTg4510 mice, number of entries were increased indicating hyperactivity. rTg4510 mice showed elevated tau levels in plasma and CSF at 3 and 6 months of age, with an 11-fold increase in CSF but only two-fold increases in plasma at 6 months. Plasma and CSF Nf-L levels were increased in rTg4510 mice at 6, but not at 3 months. Among the inflammatory markers assessed, only IL-1β was increased in CSF at three months of age. Basal synaptic transmission and LTP were impaired at 6 months of age. Behavioral and electrophysiological effects and levels of all biomarkers were restored by Doxycycline (Dox) treatment. **Conclusions**: Neuropathological changes in rTg4510 mice appear as early as 3 months of age and become widespread by 6 months. Behavioral and biomarker changes can be reversed by Dox treatment, making this model ideal for evaluating Alzheimer's disease therapies.

METHODS

- rTg4510 and tTA mice were purchased from Jackson Laboratory at 6-8 weeks of age and grouped housed at Psychogenics. Animals were maintained on 12/12 light/dark cycles and acclimated to the vivarium for at least 2 weeks prior to any testing
- Animals maintained on dox were provided with dox chow at Psychogenics at the specified ages and maintained as such for the duration of the study For electrophysiological and molecular biological studies, animals were sacrificed at 6-6.5 months of age
- For phospho-Tau quantification: insoluble protein fractions were prepared from hippocampal tissue of 6 months old tTA control, rTg4510, and rTg4510 mice treated with doxycycline from 9 weeks to 6 months of age to stop mutant tau expression.
- GFAP, IL-1b and Tau quantification: Meso Scale Discovery (MSD) electrochemiluminescence detection technology was used to measure GFAP, IL-1B, and Tau levels in CSF and/or Plasma. MSD S-Plex assays were used for the analysis of these analytes, and final concentrations were calculated by extrapolating values from the provided standard curve. Assays were measured using MESO QuickPlex SQ 120MM instrument.
- For quantification of Nf-L in plasma: SIMOA® NF-Light Advantage kit from Quanterix™ was run on the Quanterix™ SR-X Instrument.
- For quantification of Nf-L in CSF: SIMOA® NF-Light Advantage kit from Quanterix™ was used for CSF analysis, using provided NF-L calibrators to generate a standard curve and extrapolate concentrations of NF-L in CSF using the Quanterix™ SR-X Instrument.
- For electrophysiological studies, animals were maintained on regular chow or dox-containing chow as indicated until time of sacrifice. Hippocampi were isolated from freshly dissected brains and acute slices prepared. Recordings were made on interface chambers with temperature maintained at 32°C, continually oxygenated and perfused at 1-2 ml/min. Input-output relationships were established for each slice, with the stimulus intensity then set at 30-50% of maximal to conduct LTP studies. LTP was induced with 3 x 1s trains (100 stimuli @ 100 Hz), with 5 min intertrain intervals

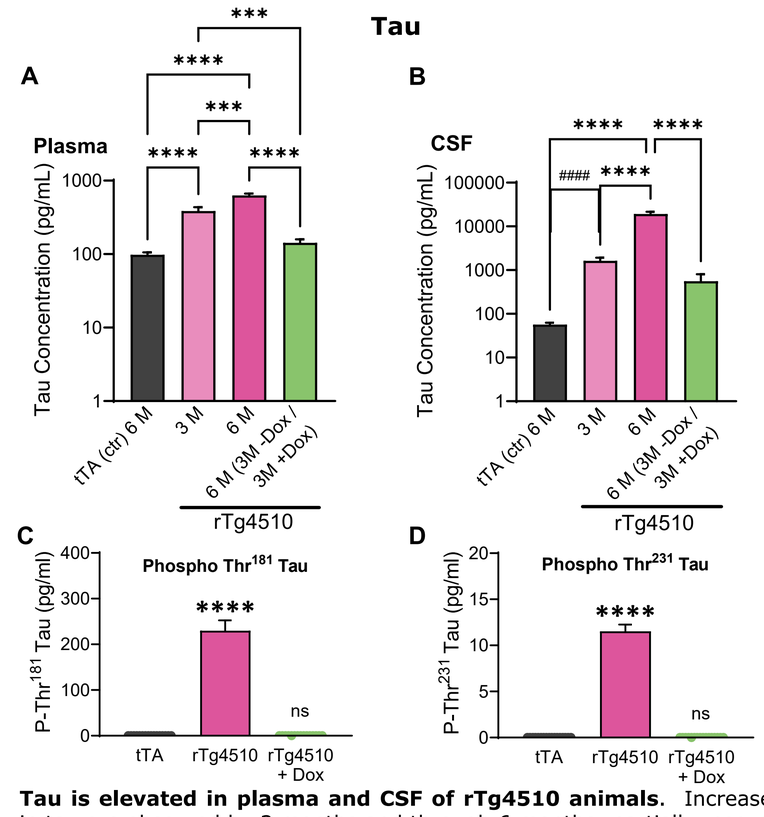
RESULTS



Long-term Potentiation

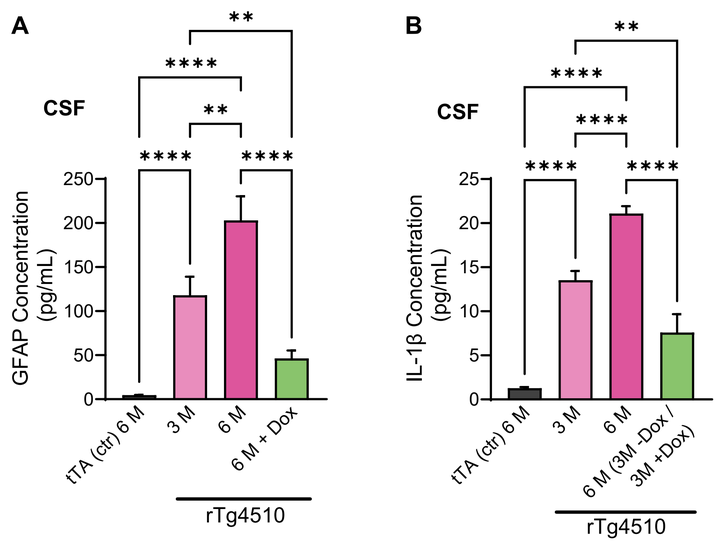
rTg4510 animals show deficits in hippocampal long-term potentiation (LTP) and basal synaptic transmission. (A) LTP is reduced in hippocampal slices from brains of female rTg4510 mice at 6 months of age compared to what is observed in slices obtained from WT brains. LTP was induced by 3 separate stimulus trains, producing post-tetanic potentiation in brain slices from both WT and rTg4510 mice (whether maintained on Dox or not). A significant deficit in LTP (as quantified during minutes 65-70 of the experiment) was observed in brain slices from rTg4510 animals, which was rescued by maintenance of rTg4510 animals on dox **(B)**. **(C)** The input-output relationship of hippocampal fEPSP responses suggests a deficit in basal synaptic transmission; however a concomitant reduction or ceiling effect in presynaptic fiber volley is also observed in rTg4510 animals not maintained on dox **(D),** suggesting that overall neurodegeneration underlies the fundamental decrease in input-output. Doxycycline treatment partially rescues the reduction in fEPSP slope, possibly slowing neurodegeneration.

Tau



Tau is elevated in plasma and CSF of rTg4510 animals. Increases in tau are observed by 3 months and through 6 months; partially rescued by Dox **(A, B)**. Phospho-tau is increased in hippocampal tissue **(C, D)**.

Inflammatory Markers in CSF

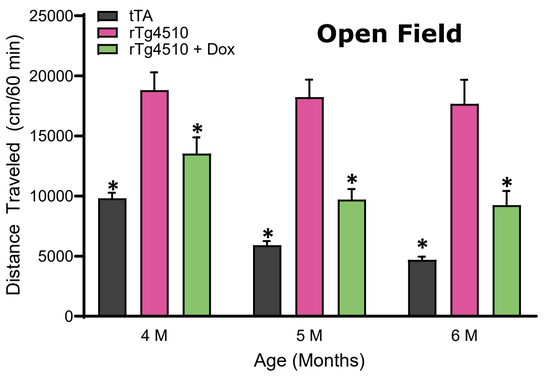


Summary, Inflammatory Markers

Key inflammatory markers are elevated in CSF of rTg4510 animals as early as 3 months of age. (A) GFAP and **(B)** IL-1β is significantly elevated in CSF of rTg4510 animals at 3 and 6 months of age. Maintenance on Dox chow produces significant reductions in both markers through 6 months of age. **Right:** Summary of inflammatory marker protein levels in CSF and plasma.

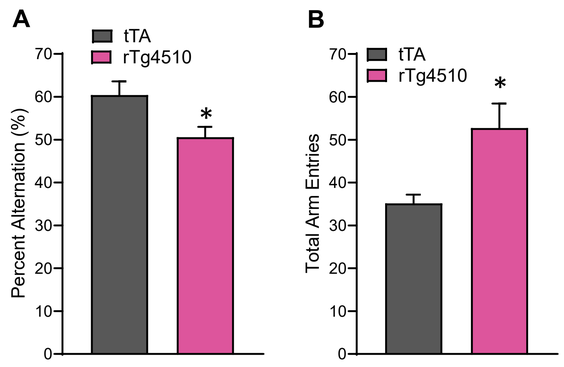
Marker	CSF	Plasma
GFAP	Gene, age, and dox reversal	No differences
INF-γ	No differences	No differences
IL-10	Below detection	Below detection
IL-12p70	Below detection	Below detection
IL-1β	Gene, age, and dox reversal	Below detection
IL-4	Below detection	Below detection
IL-2	Below detection	Below detection
IL-5	Below detection	Below detection
IL-6	No differences	Below detection
KC/GRO	No differences	Below detection
TNF-α	No differences	Below detection

Open Field



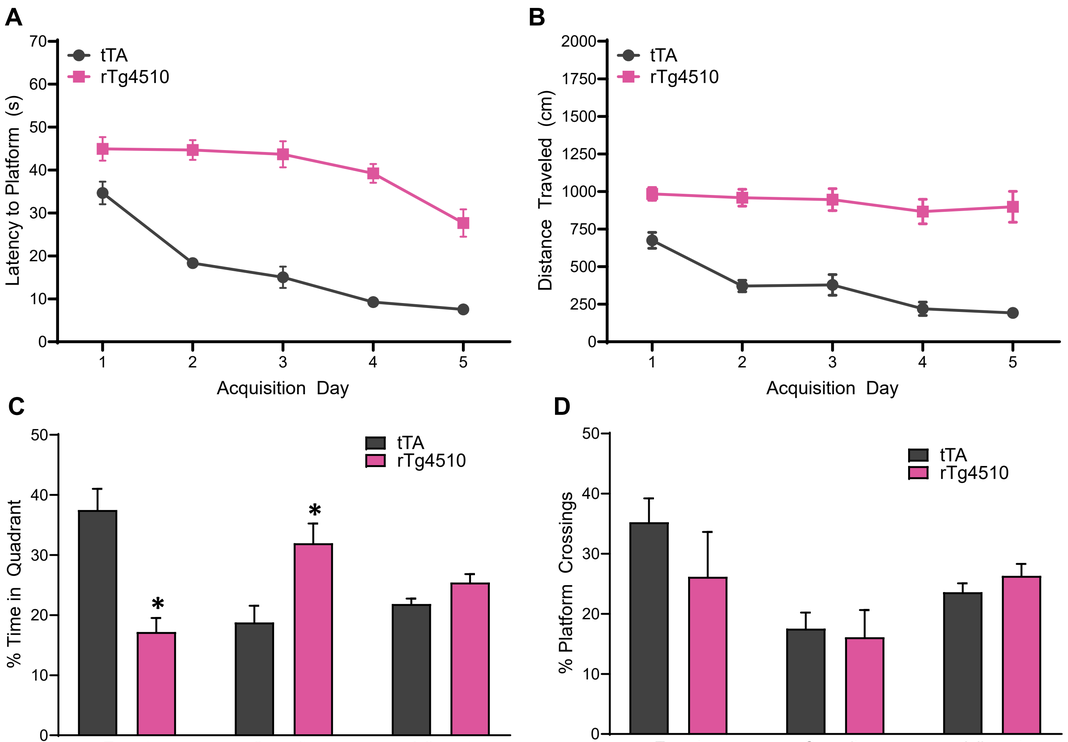
Hyperactive phenotype is apparent as early as 4 months of age in open field. Distance traveled in open field.

Y-Maze

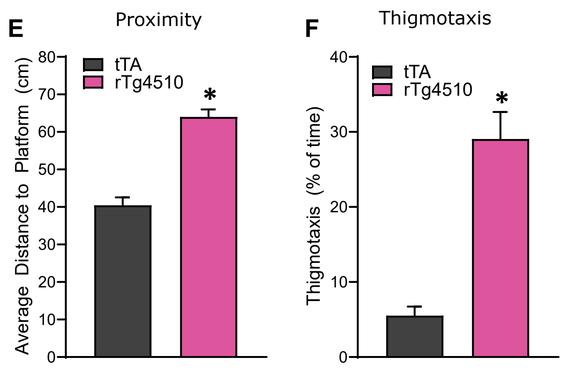


Hyperactive phenotype of rTg4510 animals is revealed by performance in Y-maze. The Y-maze test provides a measure of working memory and exploratory behavior. Alternations between different arms of the maze represent a useful measure of working memory, while global activity is reflected in the total number of arm entries. **(A)** Percent alternations are reduced in rTg4510 mice, consistent with a deficit in working memory. (**B)** Total number of arm entries are increased in rTg4510, indicating an overall increase in activity level.

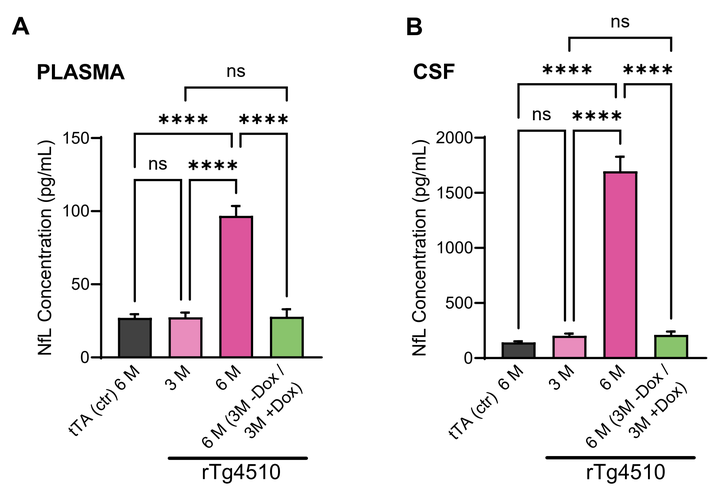
Morris Water Maze



rTg4510 animals show deficits in both acquisition and probe phases of Morris Water Maze, which are prevented by maintenance on Dox. Progress through acquisition phase showing transgenic control tTA mice are faster **(A)** and travel more directly **(B)** to the platform compared to rTg4510 mice without Dox. tTA mice spend significantly more time in the target quadrant and significantly less time in the opposite quadrant than rTg4510 mice **(C)**. While % platform crossings are similar between genotypes **(D)**, tTA mice take a more efficient route to the platform, traveling an overall shorter distance to the target **(E)** and also spend significantly less time overall along the walls of the water maze **(F)**.



Nf-L



Nf-L is elevated in age-dependent manner in plasma and CSF of rTg4510 animals. While no differences in Nf-L levels are observed at 3 months of age, increases in Nf-L are observed by 6 months in both plasma **(A)** and CSF **(B)** of rTg4510 mice, and these elevations can be fully rescued by maintenance on Dox.

SUMMARY

- Here we characterized the behavioral, electrophysiological, and biomarker profile of the rTg4510 mouse model of Alzheimer's disease and other tauopathies to assess its suitability as a preclinical testing model.
- rTg4510 displayed behaviors consistent with a hyperactive phenotype in both open field and Y-maze tests.
- Deficits in hippocampal-dependent learning and memory in rTg4510 mice were evident in the Morris Water Maze, as deficits in performance were observed during both the acquisition and probe phases.
- Hippocampal LTP was impaired in rTg4510 animals, as was basal synaptic transmission. This was largely a product of synaptic dysfunction, likely caused by neurodegeneration as a process of disease pathology. These changes were largely reversed by maintenance on Dox chow.
- Tau and NF-L levels were elevated in an age-dependent manner in rTg4510 plasma and CSF, at least partially reversible by maintenance on Dox chow. Similar elevations of GFAP and IL-1β, regulatable by Dox, were also measurable in CSF.