A Novel Tau Transgenic Mouse Model Overexpressing Human Tau and an Aggregation-Prone Tau Sequence Shows Tau Hyperphosphorylation and Behavioral Deficits in the Morris Water Maze

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Abstract

Tauopathy is a group of neurodegenerative diseases characterized by hyperphosphorylation, misfolding and aggregation of tau protein, as well as behavioral deficits and neuronal loss. Here, we developed a novel tau Tg mouse model that overexpresses 5-fold all 6 isoforms of the WT human tau (GEM1) and a tetracycline-responsive, Camk2a-activated transgene carrying a human tandem repeat tau (TRT) sequence at lower expression levels (GEM2). The TRT sequence serves as a tau aggregation primer accelerating the tau pathology without having to use known tau mutations such as P301L. Indeed, expression of TRT in HEK293 cells results in formation of soluble, hyperphosphorylated, high-molecular-weight tau oligomers. Brains were collected from WT control, GEM1, and GEM2 mice, as well as from the GEM1 and GEM2 composite mouse (GEM3), at various ages for biochemical and histological evaluation. Despite the high overexpression of WT human tau, GEM1 showed only a modest increase in tau hyperphosphorylation in sagittal sections stained for the tau phospho (Ser202, Thr205)-specific antibody AT8, a marker for pathologically phosphorylated tau. In contrast, the GEM2 and GEM3 transgenic mice showed a dramatic increase in tau hyperphosphorylation. Similar results were obtained with staining of hippocampus and cortex for AT180 antibody, which recognizes hyperphosphorylated Thr231, and for PHF1 antibody, which recognizes tau phosphorylated at Ser396/404. Behaviorally, 20-month-old GEM3 mice showed moderately increased locomotor activity, but normal performance in the rotarod assay, compared to WT controls. In the Morris Water Maze, GEM3 mice showed equivalent performance to age-matched WT mice in a 5-day cued learning task, but impaired learning in a hidden platform task. GEM3 mice also exhibited spatial learning deficits in 3 probe tests (eg, Day 10 probe test: Percent of time spent in island quadrant; WT: 51.5 ± 6.4; GEM3: 31.7 ± 3.8; $P<0.01$), and analysis of swim patterns indicated search strategies reflective of impaired learning/memory. In summary, this novel tau transgenic mouse model recapitulates key aspects of Alzheimer's disease-like symptoms, including tau hyperphosphorylation and learning and memory impairments.

Aim of the study: To develop a novel tau Tg mouse model showing hallmarks of Alzheimer's disease, eg, tau hyperphosphorylation and cognitive deficits, that does not show locomotor hyperactivity like that seen with the commonly used rTg4510 mouse model.

MATERIALS AND METHODS

Animals

GEM1: BAC Tg line overexpressing WT human tau 5-fold

GEM2: TET inducible Tg line overexpressing TRT sequence plus Camk2a-tTA Tg

GEM3: GEM1 plus GEM2 (triple transgenic line)

Locomotor Activity and Rotarod Assessment

Mice were initially examined for general health and for motor performance in a locomotor activity test and rotarod test, to identify any mice that may have motor impairment sufficient to impact water maze performance.

Morris Water Maze

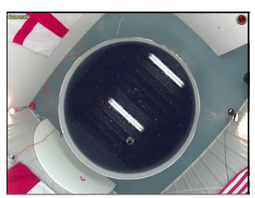
Studies were conducted in a 6-foot-diameter water maze. Flags were used as extra-maze cues. Water was at room temperature.

CUED Learning: For the CUED learning phase, mice were singly placed in the pool and trained to swim to a visible, flagged platform. The mice received 3 trials/day for 3-5 days, depending on performance, and the platform location varied within each block of 3 trials (eg, NE, NW, SE, SW). The maximum duration of each trial was 60 seconds, with approximately 10-min intervals between each trial.

PLACE Learning: In the place phase, the island platform was submerged and hidden from the animal and was at a fixed location for all trials. Mice received 3 trials/day for 10 days. The maximum duration of each trial was 60 seconds, with approximately 10-min intervals between each trial.

PROBE test: On days 5 and 10 of place training, each mouse received a 60-sec probe test, where the island platform is removed and the search pattern of the animal measured over this period. Time spent in each of the 4 quadrants [Adjacent Right (AR), Target quadrant or 'Island' (I), Adjacent Left (AL), Opposite (O)] was recorded. The probe test was conducted approximately 1 hour after the conclusion of the place learning test on each of these days. A third probe test was conducted 7 days post-completion of place training.

All water maze data, such as path length, distance traveled, latency to platform, swim speeds, quadrant preference, and number of platform crossings, was captured by overhead camera and ANYMAZE© software.



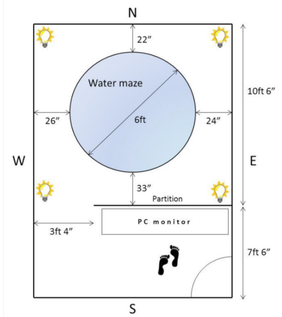
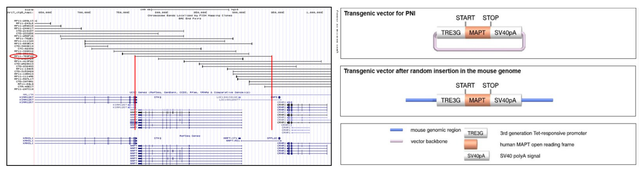
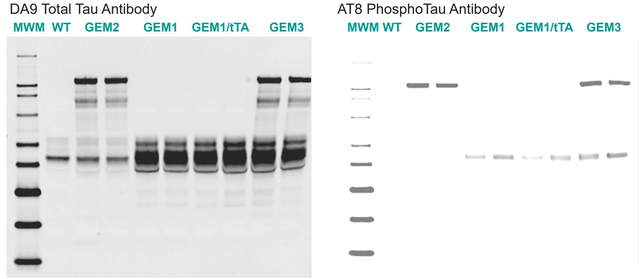


Figure 1. Generation of GEM1, GEM2, and GEM3 Tg Mice



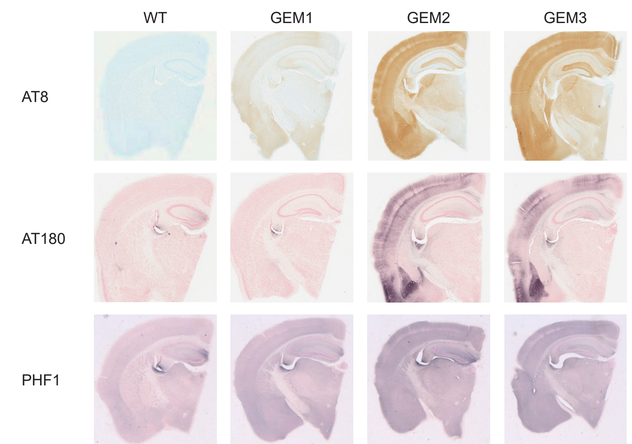
A. "GEM1": BAC RP11-769P22 contains the entire human tau gene and was used for pronuclear injection into fertilized FVB/NTac oocytes at Taconic Biosciences Inc. **B.** "GEM2": A transgenic vector containing the doxycyline-inducible TRE3G promoter, the KOZAK consensus sequence, the human tau open reading frame, and the tandem repeat tau (TRT) construct was used for pronuclear injection into fertilized FVB/NTac oocytes at Taconic Biosciences Inc. GEM1/GEM2 dTg mice in a FVB/NTac genetic background were crossed to Camk2a-tTA Tg mice in a 129 genetic background to generate WT controls, GEM1 single Tg = GEM1, GEM2/tTA double Tg (dTg) = GEM2, and GEM1/GEM2/tTA triple Tg (tTg) = GEM3 in a defined F1 FVB/NTac and 129 genetic background for all experiments.

Figure 2. Western Blot for GEM1, GEM2, and GEM3 Tg Lines



A. Western blot analysis on WT controls, GEM2, hemizygous (Tg) GEM1, GEM1/tTA dTg, and GEM3 Tg mice using DA9 antibody detecting total tau. GEM2 overexpresses the TRT sequence only modestly and a dimer band is visible. In contrast, GEM1 overexpresses 5-fold all 6 human tau isoforms. The GEM3 tTg shows both the expression pattern of the 2 single lines. **B.** Western blot using the AT8 antibody detecting hyperphosphorylated tau. No signal is detected in WT controls, but strong hyperphosphorylated tau is present in the GEM2 dTg line. Again, the GEM3 tTg shows both the expression pattern of the 2 single lines.

Figure 3. pTau in GEM1, GEM2, and GEM3 Mouse Brains



AT8 antibody staining indicates the tau phosphorylation at Ser202 and Thr205 in brain sagittal sections of GEM1 and, to a higher degree, GEM2 and GEM3 Tg mice. Likewise, pathological human tau with phosphorylated Thr231 was identified in hippocampus and cortex of GEM1, GEM2, and GEM3 Tg mice using AT180 and hTau hyperphosphorylated at Ser396/Ser404 using PHF-1 antibody IHC.

Table 1.

	14-Month-Old GEM3	24-Month-Old GEM3
Body weight	Normal	Normal
Rotarod	Normal	Normal
Locomotor activity	Normal	Increased
Cued learning MWM	Normal	Normal
Place learning MWM	Impaired	Impaired
Probe test 2 and 3 MWM	Impaired	Impaired
Swim speed	Normal	Increased
Search strategies	Impaired (increased)	Impaired (increased)

*Comparisons are to the WT control.

Figure 4. Morris WM: Place Learning

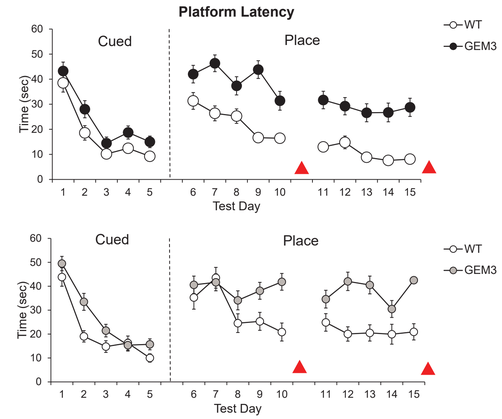


Figure 5. Morris WM: Day 20 Probe Test

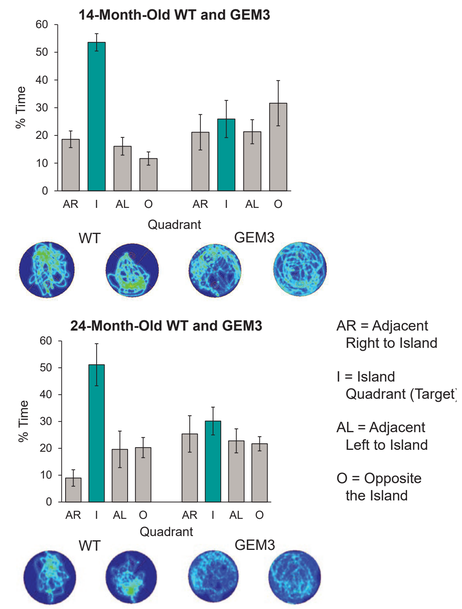
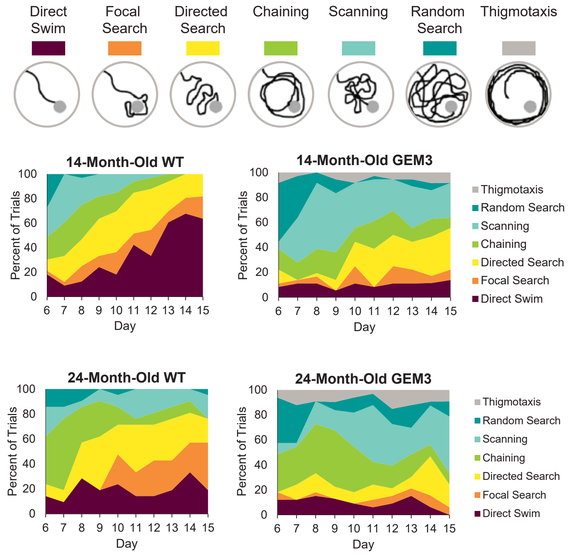


Figure 6. Morris WM: Search Strategies



SUMMARY

- Cellular expression of tandem repeat tau (TRT) resulted in rapid human tau hyperphosphorylation at multiple epitopes. Hyperphosphorylated TRT formed high-molecular-weight oligomeric complexes (shown previously)
- GEM1 Tg mice overexpressed 5-fold all 6 isoforms of the WT human tau, whereas GEM2 Tg mice overexpressed the TRT sequence of human tau at lower levels (**Figure 2**)
- GEM1 Tg mice exhibited only a modest increase, whereas GEM2 Tg mice exhibited a dramatic increase in hyperphosphorylated tau in mouse brains (**Figure 3**)
- Body weight and rotarod performance were normal in 14- and 24-month-old GEM3 Tg mice in comparison to WT controls; locomotor activity was increased in 24-month-old GEM3 Tg mice only (**Table 1**)
- The Cued learning task in the Morris Water Maze was normal in 14- and 24-month-old GEM3 Tg mice, but performance in place learning, probe test trials 2 and 3 was severely impaired to a similar degree in both age groups (**Figure 4 and 5**). Additionally, there were clear differences in the WT and Tg mouse swim strategies (**Figure 6**)
- Preliminary data in 26-month-old GEM2 Tg mice demonstrated that these mice had a similar deficit in the Morris Water Maze to the GEM3 mice, indicating that the behavioral deficits are mainly driven by GEM2, not GEM1. This data needs to be confirmed in a repeat study
- Additionally, young (6-8 months old) GEM3 mice will be tested in future experiments to determine if the behavioral deficit is age dependent
- Future experiments will include Camk2a-tTA single Tg controls, as 5 genes are disrupted at the insertion site of that Tg line (Abbas A, et al. *Nat Commun.* 2019;10(1):2049.)

Acknowledgment

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