

Supplementary methods

Detailed behavioural protocols.

Cued and Reference memory versions of Morris Water maze tests. The water maze apparatus is described in detail elsewhere (Janus, 2004). Briefly, it consisted of a circular pool (1.5 m in diameter and 0.60 m high) made of white plastic, which was elevated 86 cm off the floor level (the pool's rim is 146 cm from the floor level). The pool was filled with water (24 - 25°C), made opaque by the addition of non-toxic white paint, to a depth of 45 cm. During a reference memory training, an escape platform (16cm in diameter) was submerged 0.5 – 1.0 cm under the water level. During a visible platform or cued-response training, the location of a submerged platform was marked with a centrally mounted post (10 cm high, 1 cm in diameter) which was painted with black and white horizontal stripes. During probe trials, evaluating a spatial memory for the platform location, the platform was removed from the pool, and a mouse was allowed to search the pool for the entire allocated time. The search path of a mouse during each swim trial was recorded by a video camera suspended 2.5 m above the centre of the pool and connected to a video tracking system (HVS Image Advanced Tracker VP200, HVS Image, Buckingham, UK) and a PC computer running HVS software.

Cued- (visible platform) response learning. During this test, the water maze tank was surrounded by a curtain which obscures all extra-maze distal spatial cues. The test is carried out for 3 days with 4 swim trials per day. The visible platform location and point of release of a mouse to the pool are changed semi-randomly each trial.

Reference memory (place discrimination) MWM test. Mice were given 9 daily sessions with 4 training trials per session. During training, an escape platform submerged under water was always positioned in the centre of one quadrant (target quadrant, TQ) of the pool (NW in the case of 5.5 month-old cohort and SE in the case of 8-month old cohort). During each 90-second training trial, a mouse was carried to the pool in a holding container and was released into water facing the wall of the pool. During the 9-day learning acquisition training, four 60-second probe trials (as the 1st trial of the day, on D3,

D6, D9 and D10 of training) were administered to evaluate the development of spatial memory for the platform location. The development of spatial memory was analysed in each probe trial separately and as a composite learning index which takes into account learning rate during the whole test (Kotilinek et al., 2002; Westerman et al., 2002).

The spatial memory for the platform location during probe trials was evaluated by the analysis of the dwelling time in each of the pool's quadrant, and the analysis of an annulus crossing index (ACI). Annulus crossing index (referred to as memory index throughout the manuscript), represents the number of crosses over the platform site in the target quadrant, adjusted for crosses over corresponding sites in other quadrants - The ACI represents the number of crosses over the platform site in a quadrant which contained the escape platform (target quadrant, TQ) adjusted for crosses over platform sites in alternative quadrants; i.e. $ACI = \# \text{ of site crosses in TQ} - \text{an average of crosses of sites in other 3 quadrants of the pool}$. ACI represents an unbiased evaluation of spatial memory in the MWM test controlling for non-spatial search strategies like chaining where a mouse learns to search for the platform within the area of the pool at constant distance from the wall (Wehner et al., 1990; Gass et al., 1998; Wolfer and Lipp, 2000; Janus, 2004), or persistent scanning of target and adjacent quadrants, which may yield high platform site crossing scores for scanned quadrants. A strong bias for the spatial location of the platform is expressed by positive ACI values. ACI with low values, close to zero, indicates that platform sites in all 4 quadrants were visited with similar frequency. If mice tend to search quadrants other than the target quadrant, the ACI may have negative values. Since the annulus crossing index transforms the raw data, the total number of platform crosses in all four quadrants of the pool was also analysed. The mean ACI (memory index) represents the average from the last two probe trials at the end of 9 day-long training.

Data analysis. The main analysis of the results was performed by a factorial analysis of variance (ANOVA) with genotype and treatment (where appropriate) as between, and training days as within subject (repeated measure) factors. While performing all repeated measures ANOVAs, departures from the assumption of compound sphericity was evaluated by Mauchly sphericity test with α level set to 0.05. In cases when sphericity

was significantly violated, degrees of freedom were adjusted by Greenhouse-Geisser ϵ -correction. In order to avoid multicollinearity and singularity problems, a correlation matrix of a set of variables characterising a specific behaviour was examined and redundant variables were removed from the final analysis. Global factorial analysis was followed by specific analyses of simple effects (Stevens, 1990) pertaining to each research hypothesis. In analyses requiring multiple comparisons between groups, or analyses of biochemical data referring to hypotheses of tau biochemistry Bonferroni *t* test with alpha level adjusted to minimise Type I (family-wise error rate) error was used.

To investigate possible outliers in the obtained measures we transformed all scores using standard normal *z*-score distribution. The extreme values which occurred with the probability less than 1%, (2.33 standard deviation (SD) from the mean) were treated as outliers.

Preparation of NEM brain extracts

In experiments testing the presence of disulfide bonds, brain was homogenized immediately after scarification of the animal in PBS (pH = 6), 2mM EGTA, 2mM EDTA, protease inhibitor cocktail (Sigma), phosphatase inhibitor cocktail I and II (Sigma), 1 mM PMSF, 25 mM NEM (N-ethylmaleimide, Fluka Biochemika) as previously described (Feng et al., 2006). Briefly, the homogenization buffer was kept under vacuum and repeatedly flushed with argon (5 times for 5 minutes), while stirring. The tube, which the tissue was homogenized in, was also kept under vacuum and flushed with argon 5 times. Both the homogenization buffer and the tube were constantly kept under the atmosphere of argon during the homogenization process.

NEM chemically modifies free SH groups while leaving S-S bonds intact (Feng et al., 2006). Therefore when reducing agents (such as β -mercapthoethanol) are omitted during the sample preparation, tau molecules that have formed S-S bonds *in vivo* will appear as multimers (that can be reduced by β -mercapthoethanol) while tau molecules that contained only SH groups *in vivo* will migrate at the level of monomers. Therefore, this procedure enables to “capture” the tau multimers without artifacts of *in vitro* preparation. Using reducing agents (such as β -mercapthoethanol) enables to test if the existence of at least some tau multimers is dependent on the presence of disulfide bonds.

We used an excess of 10-fold NEM over the expected concentration of SH groups based on protein concentration and average cysteine content. We verified that the reaction of NEM with SH bonds was complete by doing time course and using different concentrations (data not shown).

Gallyas silver staining

For Gallyas silver staining, Sections were deparaffinized and rehydrated to distilled water. Slides were incubated in 5% periodic acid for 5 minutes, and then washed in 2 changes of distilled water for 5 minutes each. Slides were incubated in alkaline silver iodide solution for 1 minute, and then washed in 0.5 % acetic acid for 10 minutes. Slides were immediately placed in freshly made developer solution and developed for 4-6 minutes. Washes were performed in 0.5% acetic acid for 3 minutes. Then, slides were washed in distilled water for 5 minutes. Slides were incubated in 0.1% gold chloride for 5 minutes and rinsed in distilled water. Slides were incubated in 1% sodium thiosulphate solution for 5 minutes, and then washed well in running tap water for approximately 5 minutes. Slides were counterstained in 0.1% nuclear fast red for 2 minutes, and then washed well in tap water, dehydrated and coverslipped.

Supplementary Figure legend

Figure S1. Levels of tau multimers are not affected by reducing conditions.

a, In order to avoid artifacts of *in vitro* preparation in the analysis of disulfide bonds, brain extracts were prepared in the presence of argon (non-oxidizing atmosphere) and NEM (chemical agent irreversibly binding to SH groups and thereby preventing formation of S-S bonds *in vitro*). Tau molecules with already formed S-S bonds *in vivo* are not affected.

b, Total brain extracts were prepared using special procedure (see Material and Methods) to test if the existence of multimers depends on presence disulfide bonds (i.e. on presence of reducing agents) *in vivo*. Tau multimers are not affected by the presence of β -mercaptoethanol in these specially prepared extracts. 1,2 – extracts from two different

rTg4510 (3.5 month-old), tTa1, tTa1 age-matched tTa animals (without mutant P301L tau transgene).

c, The same experiment as in panel *b* but 100mM DTT was used. 1,2 – extracts from two different rTg4510 (3.5 month-old), 3 – extracts from age-matched tTa animal.

Fig. S2. Molecular weight of multimers in human and mouse tissue.

Comparison of molecular weight of multimers present in total lysate. Multimers in transgenic mice appear at approximately 140 kDa and 170 kDa while a smear in this range is visible in AD. Age is indicated in the figure. 10 µg was used in all lanes, except for AD where 5 µg was used. Brain extracts were used, except for JNPL3 mice where spinal cord extracts were used.

Figure S3. Relationships between tau species.

a, Levels of tau140 multimers correlates significantly and positively with levels of tau170 multimers in total extracts at both 5.5 and 8 months.

b, Levels of multimers in total extracts correlates significantly and positively with levels of 64 kDa sarkosyl-insoluble tau in P3 fraction at both 5.5 and 8 months.

c, In sarkosyl-insoluble (P3) fraction, tau 64 kDa and tau multimers exhibit a strong positive correlation at both 5.5 and 8 months.