Published in final edited form as:

Neurobiol Aging. 2014 July ; 35(7): 1769–1777. doi:10.1016/j.neurobiolaging.2013.12.023.

Severe amygdala dysfunction in a MAPT transgenic mouse model of frontotemporal dementia

Casey Cook^{a,b}, Judy H. Dunmore^{a,b}, Melissa E. Murray^a, Kristyn Scheffel^a, Nawsheen Shukoor^a, Jimei Tong^a, Monica Castanedes-Casey^a, Virginia Phillips^a, Linda Rousseau^a, Michael S. Penuliar^a, Aishe Kurti^a, Dennis W. Dickson^{a,c}, Leonard Petrucelli^{a,c}, and John D. Fryer^{a,c,*}

^aDepartment of Neuroscience, Mayo Clinic Jacksonville, 4500 San Pablo Road, Jacksonville, FL 32224

^cNeurobiology of Disease Program, Mayo Graduate School, 200 First Street SW, Rochester, MN 55905

Abstract

Frontotemporal dementia with Parkinsonism-linked to chromosome 17 (FTDP-17) is a neurodegenerative tauopathy caused by mutations in the tau gene (*MAPT*). Individuals with FTDP-17 have deficits in learning, memory and language, in addition to personality and behavioral changes that are often characterized by a lack of social inhibition. Several transgenic mouse models expressing tau mutations have been tested extensively for memory or motor impairments, though reports of amygdala-dependent behaviors are lacking. To this end, we tested the rTg4510 mouse model on a behavioral battery that included amygdala-dependent tasks of exploration. As expected, rTg4510 mice exhibit profound impairments in hippocampal-dependent learning and memory tests, including contextual fear conditioning. However, rTg4510 mice also display an abnormal hyper-exploratory phenotype in the open field assay, elevated plus maze, light-dark exploration, and cued fear conditioning, indicative of amygdala dysfunction. Furthermore, significant tau burden is detected in the amygdala of both rTg4510 mice and human FTDP-17 patients, suggesting that the rTg4510 mouse model recapitulates the behavioral disturbances and neurodegeneration of the amygdala characteristic of FTDP-17.

Keywords

Frontotemporal dementia; Tau; tauopathy; amygdala; neurodegeneration

© 2013 Elsevier Inc. All rights reserved.

*Corresponding author: Dr. John D. Fryer, PhD, Department of Neuroscience, Mayo Clinic Jacksonville, 4500 San Pablo Road, Jacksonville, FL 32224, Office: 904-953-2317, FAX: 904-953-7370, fryer.john@mayo.

^bThese authors contributed equally to this work.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

CONFLICT OF INTEREST STATEMENT: All authors declare no conflicts of interest. Mayo Clinic has no contracts relating to this research through which it may gain financially.

1. Introduction

In a number of neurodegenerative diseases classified as tauopathies, the microtubule-binding protein tau becomes hyperphosphorylated and aggregates into filaments, losing the ability to bind and stabilize microtubules (Dickson 1999; Buee et al. 2000). These filaments continue to aggregate and form increasingly insoluble deposits referred to as neurofibrillary tangles (NFTs) in diseases such as Progressive supranuclear palsy (PSP), Corticobasal degeneration (CBD), Alzheimer's disease (AD), and Frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) (Dickson 1999; Buee et al. 2000). FTDP-17 is an autosomal-dominant neurodegenerative disease that can be characterized by behavioral disturbances, cognitive impairment, and parkinsonism, though considerable phenotypic variation in patients has been observed (Wszolek et al. 2006). Of note, personality and behavioral changes are frequently the earliest clinical symptoms of FTDP-17 to develop, including a loss of social inhibition, inappropriate emotional responses, and restlessness (Lynch et al. 1994; Wilhelmsen et al. 1994; Yamaoka et al. 1996; Spillantini et al. 1997), suggesting involvement of amygdala dysfunction in the disease.

With the identification of pathogenic mutations in tau associated with FTDP-17, indicating that misregulation of tau function alone is sufficient to cause neurodegeneration (Hutton et al. 1998; Poorkaj et al. 1998; Spillantini et al. 1998), a number of groups generated transgenic mice expressing different variants of tau, including the FTL-associated P301L mutation. In the current study, we utilize the rTg4510 mouse model, which conditionally overexpresses P301L human mutant tau in the forebrain. The rTg4510 mice develop pre-tangles as early as 2.5 months of age, and mature NFTs and neuronal loss is evident by 5.5 months (Santacruz et al. 2005). In addition, due to the localization of tau pathology to forebrain structures, including the hippocampus, rTg4510 mice exhibit severe cognitive deficits in hippocampal-dependent tasks (Santacruz et al. 2005; Berger et al. 2007; O'Leary et al. 2010; Yue et al. 2011). However, the existence of other behavioral abnormalities in rTg4510 mice and their potential relevance to the clinical presentation of FTL-17 has been relatively ignored. Therefore we tested rTg4510 mice and non-transgenic littermates at 2 (early stage), 6 (mid stage) and 10 months of age (end stage) on a behavioral battery that included tasks designed to provide a measure of amygdala function, to determine the extent to which the amygdala was affected in rTg4510 mice. We present evidence to suggest that the rTg4510 mouse model very closely mimics both the behavioral and pathological phenotype of FTL-17.

2. Methods

2.1. Transgenic mice

The rTg4510 model relies on two different transgenes to conditionally express human 4R0N tau containing the P301L familial mutation, which has been linked to frontotemporal dementia. The first transgene is the mutant tau cDNA responder transgene that includes a minimal promoter that is transcriptionally blocked by binding sites for the tetracycline transactivator (tTA). The second transgene is the tTA transgene driven by the CaMKII α promoter, resulting in forebrain-focused neuronal expression of both the tTA and the responding tau transgene. Of critical importance to our current findings, the CaMKII α

promoter does drive transgene expression in the amygdala (Rammes et al., Eur J Neurosci, 2000 and Michalon et al., Genesis, 2005), which we have verified in the rTg4510 model by immunostaining with an antibody specific for human tau (Supplementary Fig. 1). Each of the two transgenic lines (tTA and tau) is maintained independently. The rTg4510 mice used in the current study were produced by an F1 breeding of a tTA parent on a 129S6 background to a tau parent on an FVB/NCrl background. Mouse cohorts were sex and age-matched littermates. The following animal numbers were used for this study: for the 2 month cohort we used n=24 (12 male, 12 female) non-transgenic (NTg) and n=24 (12 male, 12 female) rTg4510 transgenic (Tg) mice; for the 6 month cohort we used n=26 (11 male, 15 female) NTg and n=21 (13 male, 8 female) Tg mice; for the 10 month cohort we used n=14 (6 male, 8 female) and n=16 (8 male, 8 female) Tg mice. All animals were group housed without enrichment structures in a specific pathogen free environment in ventilated cages and tested according to standards established by the Mayo Clinic Institutional Animal Care and Use Committee.

2.2. Behavioral tests

On consecutive days, a behavioral battery was performed consisting of Open Field Assay, Elevated plus maze test, the Light/dark chamber exploration test, and contextual and cued fear conditioning. All mice were acclimated to the room of testing for one to two hours prior to testing and all tests were performed during the first half of the light cycle with the exception of the cued fear conditioning. All behavioral equipment was cleaned with 30% ethanol between each animal. All mice were returned to their home cage and homeroom after each test.

2.3. Open-field assay (OFA)

Mice were placed in the center of an open-field arena ($40 \times 40 \times 30$ cm, W $\times$ L $\times$ H), and allowed to roam freely for 15 minutes. Side-mounted photobeams raised 7.6cm above the floor were used to measure rearing, while an overhead camera was used to track movement with AnyMaze software (Stoelting Co., Wood Dale, IL). Mice were analyzed for multiple measures, including total distance traveled, average speed, time mobile, and distance traveled in an imaginary “center” zone (20×20 cm).

2.4. Elevated plus maze test (EPM)

This is a formal test of anxiety/exploration, which was conducted in a maze consisting of four arms, 50×10 cm, with two of the arms enclosed with roofless gray walls (35×15 cm, L $\times$ H). The entire maze is elevated 50cm from the floor. Mice were tested by placing them in the center of the maze facing an open arm, and their behavior was tracked for five minutes with an overhead camera and AnyMaze software.

2.5. Light/dark chamber exploration test (LDE)

This is another formal test of mouse anxiety/exploration. The light/dark chamber is a square box ($40 \times 40 \times 30$) equally divided into two compartments with a small open door joining the light and dark compartments. The dark compartment was completely covered and mice were tested by placing them at the far end of the lit chamber facing away from the dark

chamber and their activity was tracked for 10 minutes with overhead camera and AnyMaze software.

2.6. Conditioned fear test (CF)

This test was conducted in a sound attenuated chamber with a grid floor capable of delivering an electric shock and freezing was measured with an overhead camera and FreezeFrame software (Actimetrics, Wilmette, IL). Mice were initially placed into the chamber and undisturbed for 2 minutes, during which time baseline freezing behavior was recorded. An 80-dB white noise served as the conditioned stimulus (CS) and was presented for 30 sec. During the final 2 sec of this noise, mice received a mild foot shock (0.5mA), which served as the unconditioned stimulus (US). After 1 minute, another CS-US pair was presented. The mouse was removed 30 sec after the second CS-US pair and returned to its home cage. Twenty-four hours later, each mouse was returned to the test chamber and freezing behavior was recorded for 5 minutes (context test). Mice were returned to their home cage and placed in a different room than previously tested in reduced lighting conditions for a period of no less than one hour. For the auditory CS test, environmental and contextual cues were changed by: wiping testing boxes with 30% isopropyl alcohol instead of 30% ethanol; replacing white house lights with red house lights; placing a colored plastic triangular insert in the chamber to alter its shape and spatial cues; covering the wire grid floor with opaque plastic; and altering the smell in the chamber with vanilla extract. The animals were placed in the apparatus for 3 min and then the auditory CS was presented and freezing was recorded for another 3 min (cued test). Baseline freezing behavior obtained during training was subtracted from the context or cued tests to control for animal variability.

2.7. Histology

Mice were euthanized by cervical dislocation; brains were quickly removed, and subsequently fixed in 10% formalin, embedded in paraffin wax, sectioned coronally at 5 microns, and mounted on glass slides. The tissue sections were deparaffinized in xylene, and rehydrated in a graded series of alcohols. Antigen retrieval was performed by steaming in distilled water for 30 min, and endogenous peroxidase activity was blocked by incubation in 0.03% hydrogen peroxide. Sections were then immunostained with PHF1 (tau phospho-specific antibody detecting Ser396/404; gift from Dr. Peter Davies, Albert Einstein College of Medicine, Bronx, NY) using the DAKO Autostainer (DAKO North America, Carpinteria, CA) and the DAKO EnVision + HRP system. E1 (1:15000; human specific tau antibody) was generated by our group against amino acid residues 19–33 within exon 1 of human tau (Petrucelli et al. 2004; Dickey et al. 2008; Cook et al. 2012). The stained slides were then dehydrated, and cover-slipped for analysis.

Slides were scanned with the Aperio Slide Scanner (Aperio, Vista, CA), and quantitative analyses of tau burden in the amygdala and hippocampus were performed using a custom-designed color deconvolution algorithm and ImageScope software (Aperio, Vista, CA). As previously described, the algorithm was designed to measure the specific optical density of the brown chromagen as a percentage of burden within the annotated region of interest (Janocko et al. 2012).

2.8. Data and statistical analysis

To assess the impact of aging and the tau transgene on experimental results, two-way ANOVAs were performed using GraphPad Prism 5.04. The Bonferroni post-hoc analysis for multiple comparisons was used to evaluate differences between groups at each age. All data is presented as mean $\pm$ SEM.

3. Results

3.1. Progressive development of abnormalities in exploratory behavior in rTg4510 mice

To characterize the functional impact of tau-induced neurodegeneration in a progressive mouse model of tauopathy, we assessed performance in behavioral tasks at 2, 6, and 10 months of age. In the open field assay, rTg4510 mice (in comparison to sex-matched non-transgenic littermates) exhibited hyperactivity as assessed by total distance traveled (Fig. 1A; $F=56.97$, $p<0.0001$), average speed (Fig. 1B), and time spent mobile (Fig. 1C). In addition to hyperactivity, rTg4510 mice also displayed a decreased tendency to explore the center of the open field (Fig. 1D), which is typically characteristic of increased anxiety. Therefore additional behavioral tests were included to evaluate whether rTg4510 mice exhibit a heightened level of anxiety. However, in the elevated plus maze, rTg4510 mice actually spend a greater amount of time in the open arms (Fig. 2A; $F=55.21$, $p<0.0001$), as well as a higher ratio of time spent in open to closed arms (Fig. 2B; $F=19.49$, $p<0.0001$) in comparison to non-transgenic littermates. In addition, both male and female rTg4510 mice displayed hyperactivity in this test, as well as in the open field analysis (Supplementary Fig. 2, 3).

Similarly, in the light/dark exploration test, rTg4510 mice occupy the light chamber for a greater amount of time than non-transgenic littermates (Fig. 3A; $F=12.42$, $p=0.0006$; Supplementary Fig. 4A), and also display a higher ratio of time spent in the light compared to the dark chamber (Fig. 3B; $F=10.06$, $p=0.002$; Supplementary Fig. 4B). These results indicate that rTg4510 mice are more exploratory, and actually appear to be less anxious (or disinhibited) in comparison to non-transgenic littermates. Interestingly, when the rTg4510 mice enter the open arms of the elevated plus maze or the light chamber of the light/dark exploration apparatus, they have an increased tendency to freeze (Supplementary Fig. 5, 6). This could either be interpreted as a flight response, or alternatively the rTg4510 mice may have excessive risk taking behaviors and misinterpret the environment of the open spaces and freeze once they are there. Notably, this phenotype was first observed at 6 months of age, and became more significant with age especially in the elevated plus maze, which is particularly interesting given the progressive nature of this model.

3.2. Severe deficits in fear conditioning

Given the relative disinhibition displayed by the rTg4510 mice in the elevated plus maze and light/dark exploration test, we then wanted to assess amygdala function. Therefore we evaluated performance of non-transgenic and rTg4510 mice in the contextual and cued fear conditioning paradigm, to detect hippocampal or amygdala dysfunction, respectively. Based on the well-documented cognitive deficits observed in the rTg4510 model (Santacruz et al. 2005; Berger et al. 2007; O'Leary et al. 2010; Yue et al. 2011), as well as the development

of mature tau pathology in the hippocampus of rTg4510 mice at 5.5 months of age (Santacruz et al. 2005), deficits in contextual fear conditioning were anticipated. As expected, rTg4510 mice displayed a decrease in the amount of time freezing when returned to the same environment in which they previously received a mild electrical shock (unconditioned stimulus; US), indicative of hippocampal dysfunction (Fig. 4A; $F=67.12$, $p<0.0001$; Supplementary Fig. 7A). Furthermore, the inability of rTg4510 mice to associate the spatial context with the US was observed as early as 2 months of age, and continued to decrease with aging.

In addition, during the cued fear conditioning test, rTg4510 mice exhibited a decrease in the amount of time freezing in response to an auditory tone (conditioned stimulus; CS), which had been previously paired with the US (Fig. 4B; $F=24.35$, $p<0.0001$; Supplementary Fig. 7B). This failure to associate the CS with the US, which is indicative of amygdala dysfunction, was first noted in rTg4510 mice at 6 months. Remarkably, at 10 months of age, rTg4510 mice exhibit a complete lack of freezing during both the contextual and cued fear conditioning paradigm (Fig. 4A, B; Supplementary Fig. 7A, B), reflective of a severe impairment in hippocampal and amygdala function.

3.3. Regional development of tau pathology in rTg4510 mice

To monitor the progression of tau pathology in the hippocampus and amygdala, we immunostained tissue sections from mice at 2, 6, and 10 months of age for PHF1 (an antibody specific for tau phosphorylated on Ser396/404). As expected given the initial characterization of the rTg4510 model (Santacruz et al. 2005), very little PHF1 immunoreactivity is observed in the CA1 region of the hippocampus at 2 months of age (Fig. 5D), but PHF1-positive NFTs are detected by 6 months (Fig. 5E) and is extensive by 11 months of age (Fig. 5F). The development of tau pathology in the amygdala follows a similar time course, with few cells intensely-labeled for PHF1 at 2 months (Fig. 6D), but abundant PHF1-positive NFTs observed at 6 months of age (Fig. 6E) and extensive pathology by 11 months of age (Fig. 6F).

To measure tau burden, we designed the algorithm to quantify the percent area occupied by PHF1-immunopositive pathology in the annotated region of interest. The resulting percentage did not include lightly-stained areas of endogenous mouse tau. In agreement with previous reports, we found an age-dependent accumulation of tau pathology in the CA1 region of the hippocampus (Fig. 7A), but we also observed significant deposition of tau pathology in the amygdala with aging (Fig. 7B). This localization of tau pathology to both the hippocampus and amygdala in rTg4510 mice could account for the observed cognitive deficits in hippocampal and amygdala-dependent tasks described in the current report.

To assess the extent to which rTg4510 mice model FTDP-17, and to further support the relevance of PHF1-positive tau pathology localized in the amygdala, we measured tau burden in two FTDP-17 patients with a P301L mutation (52 and 53 year old males) and a normal control (51 year old male). We chose these P301L cases since this was the same mutation used to create the rTg4510 model. We observed a significant accumulation of tau pathology in the hippocampus and amygdala of both FTDP-17 patients compared to the

normal control (Fig. 8), supporting the pathologic recapitulation of FTDP-17-like neuropathology in rTg4510 mice.

4. Discussion

We performed a thorough behavioral assessment of rTg4510 mice at 3 different time points to evaluate how the development and progression of tau pathology in forebrain structures impacts the behavioral phenotype. Given the well-characterized staging of tau burden in the rTg4510 model, it is known that pre-tangles are observed at 2.5 months of age, while mature NFTs develop at 5.5 months, and extensive neuronal loss is detected at 8.5 months (Santacruz et al. 2005). Therefore we chose to evaluate mice at 2 months of age (prior to the development of mature tau pathology), at 6 months of age (following the development of mature NFTs and significant tau burden), and at 10 months of age (when neuronal loss is evident). Of note, significant abnormalities in amygdala-dependent exploratory behaviors were noted at 6 months of age, which coincides with the development of mature NFTs in the forebrain, as well as significant accumulation of tau specifically in the amygdala. Interestingly, decreased neural activation in the amygdala of rTg4510 mice at 6 months of age has also been observed (Perez et al. 2013), which is most likely due to the extensive tau burden we detected within this region of the brain. In addition, the decrease in neuronal activity in the amygdala also most likely accounts for the abnormal behavioral phenotype rTg4510 mice exhibit in amygdala-dependent tasks.

rTg4510 mice also exhibit significant tau burden in the hippocampus, as well as severe deficits in hippocampal-dependent tasks, in agreement with previous reports (Santacruz et al. 2005; Berger et al. 2007; O'Leary et al. 2010; Yue et al. 2011). It is interesting to note that even at 2 months of age, deficits in the contextual fear conditioning task are observed in the rTg4510 mice, despite relatively low levels of PHF1 staining in the CA1 of the hippocampus (Fig.5). However, as this is only one phosphoepitope, it is possible another tau species negative for phosphorylation on the PHF1 epitope are present in CA1 neurons at 2 months and contributing to neuronal dysfunction. To assess hippocampal function, the reference memory version of the Morris water maze is most typically utilized. However, in the current report, we detected deficits in contextual fear conditioning as early as 2 months of age. Although this may reflect an earlier deficit in the specific type of memory these tasks require (spatial vs contextual), this could also indicate that the fear conditioning test is perhaps more sensitive to detect the cognitive deficits in rTg4510 mice.

Finally, to determine whether the rTg4510 model is reflective of the disease process in FTDP-17, we evaluated regional tau burden in human patients with FTDP-17 with the same P301L tau mutation. Intriguingly, extensive tau burden was observed in both the hippocampus and amygdala of these individuals. The amygdala plays an important role in several aspects of behavior, including fear recognition, emotional learning and memory, attention and perception, social behavior, and emotional inhibition/disinhibition in both rodents and humans (Phelps and LeDoux 2005). In 1939, Kluver and Bucy described a syndrome from temporal lobe lesions to rhesus monkeys resulting in loss of fear and aggressions, hyperorality (excessively placing objects in the mouth), and hypermetamorphosis (the impulse to notice and react to all visual stimuli) (Kluver and Bucy

1997). Several years later this Kluver-Bucy syndrome was described in human patients (Terzian and Ore 1955; Marlowe et al. 1975). While the clinical symptoms reported in frontotemporal dementias can have diverse presentations, as many as 20% may develop Kluver-Bucy syndrome (Mendez and Perryman 2002). Thus, the amygdala is an important brain structure that contributes to several behavioral phenotypes in multiple species. Disinhibition is one of the earliest symptoms to arise in FTDP-17 (Baker et al. 1997; van Swieten et al. 1999; Kobayashi et al. 2002), and our work here demonstrates that this disinhibition is also a prominent and early feature of the rTg4510 mouse model, likely due to severe amygdala pathology. These findings have important implications when assessing the effects of genetic or pharmacologic manipulations of this model.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Dr. Peter Davies kindly provided us with the PHF-1 antibody. This work was supported by Mayo Clinic Foundation (LP and JDF), GHR Foundation (JDF), Alzheimer's Association (JDF), National Institutes of Health/National Institute on Aging [5R01AG026251-04 (LP) and AG17216-10JP3 (LP)] National Institutes of Health/National Institute of Neurological Disorders and Stroke [R01 NS063964-01 (LP), R01 NS077402 (LP), and U01NS065102 (LP)].

References

- Baker M, Kwok JB, Kucera S, Crook R, Farrer M, Houlden H, Isaacs A, Lincoln S, Onstead L, Hardy J, Wittenberg L, Dodd P, Webb S, Hayward N, Tannenberg T, Andreadis A, Hallupp M, Schofield P, Dark F, Hutton M. Localization of frontotemporal dementia with parkinsonism in an Australian kindred to chromosome 17q21-22. *Annals of neurology*. 1997; 42(5):794–798. [PubMed: 9392579]
- Berger Z, Roder H, Hanna A, Carlson A, Rangachari V, Yue M, Wszolek Z, Ashe K, Knight J, Dickson D, Andorfer C, Rosenberry TL, Lewis J, Hutton M, Janus C. Accumulation of pathological tau species and memory loss in a conditional model of tauopathy. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2007; 27(14):3650–3662. [PubMed: 17409229]
- Buee L, Bussiere T, Buee-Scherrer V, Delacourte A, Hof PR. Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. *Brain Res Brain Res Rev*. 2000; 33(1):95–130. [PubMed: 10967355]
- Cook C, Gendron TF, Scheffel K, Carlomagno Y, Dunmore J, DeTure M, Petrucelli L. Loss of HDAC6, a novel CHIP substrate, alleviates abnormal tau accumulation. *Hum Mol Genet*. 2012; 21(13):2936–2945. [PubMed: 22492994]
- Dickey CA, Koren J, Zhang YJ, Xu YF, Jinwal UK, Birnbaum MJ, Monks B, Sun M, Cheng JQ, Patterson C, Bailey RM, Dunmore J, Soresh S, Leon C, Morgan D, Petrucelli L. Akt and CHIP coregulate tau degradation through coordinated interactions. *Proc Natl Acad Sci U S A*. 2008; 105(9):3622–3627. [PubMed: 18292230]
- Dickson DW. Tau and synuclein and their role in neuropathology. *Brain Pathol*. 1999; 9(4):657–661. [PubMed: 10517505]
- Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, Pickering-Brown S, Chakraverty S, Isaacs A, Grover A, Hackett J, Adamson J, Lincoln S, Dickson D, Davies P, Petersen RC, Stevens M, de Graaff E, Wauters E, van Baren J, Hillebrand M, Joosse M, Kwon JM, Nowotny P, Che LK, Norton J, Morris JC, Reed LA, Trojanowski J, Basun H, Lannfelt L, Neystat M, Fahn S, Dark F, Tannenberg T, Dodd PR, Hayward N, Kwok JB, Schofield PR, Andreadis A, Snowden J, Craufurd D, Neary D, Owen F, Oostra BA, Hardy J, Goate A, van Swieten J, Mann D, Lynch T, Heutink P. Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature*. 1998; 393(6686):702–705. [PubMed: 9641683]

- Janocko NJ, Brodersen KA, Soto-Ortolaza AI, Ross OA, Liesinger AM, Duara R, Graff-Radford NR, Dickson DW, Murray ME. Neuropathologically defined subtypes of Alzheimer's disease differ significantly from neurofibrillary tangle-predominant dementia. *Acta neuropathologica*. 2012; 124(5):681–692. [PubMed: 22968369]
- Kluver H, Bucy PC. Preliminary analysis of functions of the temporal lobes in monkeys. 1939. *The Journal of neuropsychiatry and clinical neurosciences*. 1997; 9(4):606–620. [PubMed: 9447506]
- Kobayashi T, Mori H, Okuma Y, Dickson DW, Cookson N, Tsuboi Y, Motoi Y, Tanaka R, Miyashita N, Anno M, Narabayashi H, Mizuno Y. Contrasting genotypes of the tau gene in two phenotypically distinct patients with P301L mutation of frontotemporal dementia and parkinsonism linked to chromosome 17. *Journal of neurology*. 2002; 249(6):669–675. [PubMed: 12111297]
- Lynch T, Sano M, Marder KS, Bell KL, Foster NL, Defendini RF, Sima AA, Keohane C, Nygaard TG, Fahn S, et al. Clinical characteristics of a family with chromosome 17-linked disinhibition-dementia-parkinsonism-amyotrophy complex. *Neurology*. 1994; 44(10):1878–1884. [PubMed: 7936241]
- Marlowe WB, Mancall EL, Thomas JJ. Complete Kluver-Bucy syndrome in man. *Cortex; a journal devoted to the study of the nervous system and behavior*. 1975; 11(1):53–59.
- Mendez MF, Perryman KM. Neuropsychiatric features of frontotemporal dementia: evaluation of consensus criteria and review. *The Journal of neuropsychiatry and clinical neurosciences*. 2002; 14(4):424–429. [PubMed: 12426410]
- Michalon A, Koshibu K, Baumgartel K, Spirig DH, Mansuy IM. Inducible and neuron-specific gene expression in the adult mouse brain with the rTA2S-M2 system. *Genesis*. 2005; 43(4):205–212. [PubMed: 16342161]
- O'Leary JC 3rd, Li Q, Marinec P, Blair LJ, Congdon EE, Johnson AG, Jinwal UK, Koren J 3rd, Jones JR, Kraft C, Peters M, Abisambra JF, Duff KE, Weeber EJ, Gestwicki JE, Dickey CA. Phenothiazine-mediated rescue of cognition in tau transgenic mice requires neuroprotection and reduced soluble tau burden. *Molecular neurodegeneration*. 2010; 5:45. [PubMed: 21040568]
- Perez PD, Hall G, Kimura T, Ren Y, Bailey RM, Lewis J, Febo M, Sahara N. In vivo functional brain mapping in a conditional mouse model of human tauopathy (taup301l) reveals reduced neural activity in memory formation structures. *Molecular neurodegeneration*. 2013; 8:9. [PubMed: 23379588]
- Petrucelli L, Dickson D, Kehoe K, Taylor J, Snyder H, Grover A, De Lucia M, McGowan E, Lewis J, Prihar G, Kim J, Dillmann WH, Browne SE, Hall A, Voellmy R, Tsuboi Y, Dawson TM, Wolozin B, Hardy J, Hutton M. CHIP and Hsp70 regulate tau ubiquitination, degradation and aggregation. *Hum Mol Genet*. 2004; 13(7):703–714. [PubMed: 14962978]
- Phelps EA, LeDoux JE. Contributions of the amygdala to emotion processing: from animal models to human behavior. *Neuron*. 2005; 48(2):175–187. [PubMed: 16242399]
- Poorkaj P, Bird TD, Wijsman E, Nemens E, Garruto RM, Anderson L, Andreadis A, Wiederholt WC, Raskind M, Schellenberg GD. Tau is a candidate gene for chromosome 17 frontotemporal dementia. *Annals of neurology*. 1998; 43(6):815–825. [PubMed: 9629852]
- Rammes G, Steckler T, Kresse A, Schutz G, Zieglgansberger W, Lutz B. Synaptic plasticity in the basolateral amygdala in transgenic mice expressing dominant-negative cAMP response element-binding protein (CREB) in forebrain. *Eur J Neurosci*. 2000; 12(7):2534–2546. [PubMed: 10947828]
- Santacruz K, Lewis J, Spires T, Paulson J, Kotilinek L, Ingelsson M, Guimaraes A, DeTure M, Ramsden M, McGowan E, Forster C, Yue M, Orne J, Janus C, Mariash A, Kuskowski M, Hyman B, Hutton M, Ashe KH. Tau suppression in a neurodegenerative mouse model improves memory function. *Science*. 2005; 309(5733):476–481. [PubMed: 16020737]
- Spillantini MG, Goedert M, Crowther RA, Murrell JR, Farlow MR, Ghetti B. Familial multiple system tauopathy with presenile dementia: a disease with abundant neuronal and glial tau filaments. *Proc Natl Acad Sci U S A*. 1997; 94(8):4113–4118. [PubMed: 9108114]
- Spillantini MG, Murrell JR, Goedert M, Farlow MR, Klug A, Ghetti B. Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. *Proc Natl Acad Sci U S A*. 1998; 95(13):7737–7741. [PubMed: 9636220]

- Terzian H, Ore GD. Syndrome of Kluver and Bucy; reproduced in man by bilateral removal of the temporal lobes. *Neurology*. 1955; 5(6):373–380. [PubMed: 14383941]
- van Swieten JC, Stevens M, Rosso SM, Rizzu P, Joosse M, de Koning I, Kamphorst W, Ravid R, Spillantini MG, Niermeijer, Heutink P. Phenotypic variation in hereditary frontotemporal dementia with tau mutations. *Annals of neurology*. 1999; 46(4):617–626. [PubMed: 10514099]
- Wilhelmsen KC, Lynch T, Pavlou E, Higgins M, Nygaard TG. Localization of disinhibition-dementia-parkinsonism-amyotrophy complex to 17q21-22. *American journal of human genetics*. 1994; 55(6):1159–1165. [PubMed: 7977375]
- Wszolek ZK, Tsuboi Y, Ghetti B, Pickering-Brown S, Baba Y, Cheshire WP. Frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17). *Orphanet journal of rare diseases*. 2006; 1:30. [PubMed: 16899117]
- Yamaoka LH, Welsh-Bohmer KA, Hulette CM, Gaskell PC Jr, Murray M, Rimmner JL, Helms BR, Guerra M, Roses AD, Schmechel DE, Pericak-Vance MA. Linkage of frontotemporal dementia to chromosome 17: clinical and neuropathological characterization of phenotype. *American journal of human genetics*. 1996; 59(6):1306–1312. [PubMed: 8940276]
- Yue M, Hanna A, Wilson J, Roder H, Janus C. Sex difference in pathology and memory decline in rTg4510 mouse model of tauopathy. *Neurobiology of aging*. 2011; 32(4):590–603. [PubMed: 19427061]

Figure 1. rTg4510 mice display increased hyperactivity in the open field

(A) Total distance traveled (effect of transgene $F=56.97$, $p<0.0001$; effect of age $F=26.17$, $p<0.0001$); (B) Average speed (effect of transgene $F=42.99$, $p<0.0001$; effect of age $F=10.64$, $p<0.0001$); (C) total time spent mobile (effect of transgene $F=50.5$, $p<0.0001$; effect of age $F=104.4$, $p<0.0001$); (D) ratio of time spent in the center quadrants to total distance traveled (effect of transgene $F=75.84$, $p<0.0001$; effect of age $F=6.8$, $p=0.002$). Data is presented as mean $\pm$ SEM. *** $p<0.0001$ ** $p<0.01$ * $p<0.05$

Figure 2. Enhanced tendency of rTg4510 mice to explore open arms in the elevated plus maze

(A) Total time spent in open arms (effect of transgene $F=55.21$, $p<0.0001$; effect of age $F=3.5$, $p=0.03$); (B) ratio of time spent in open to closed arms (effect of transgene $F=19.49$, $p<0.0001$; effect of age $F=6.03$, $p=0.003$). Data is presented as mean $\pm$ SEM. *** $p<0.0001$ ** $p<0.001$ * $p<0.01$

Figure 3. Increased exploration of lighted chamber in light/dark exploration

(A) Time spent in light chamber (effect of transgene $F=12.42$, $p=0.0006$; effect of age $F=1.3$, $p=0.2$); (B) ratio of time spent in light to dark chamber (effect of transgene $F=10.1$, $p=0.002$; effect of age $F=1.1$, $p=0.3$). Data is presented as mean $\pm$ SEM.

* $p<0.01$

Figure 4. Severe deficits in contextual and cued fear conditioning

(A) Percent of time freezing in response to placement in environment associated with US, referred to as contextual fear conditioning (effect of transgene $F=67.12$, $p<0.0001$; effect of age $F=2.5$, $p=0.07$); (B) Percent of time freezing in response to CS, referred to as cued fear conditioning (effect of transgene $F=24.35$, $p<0.0001$; effect of age $F=46.8$, $p<0.0001$). Data is presented as mean $\pm$ SEM. *** $p<0.0001$ ** $p<0.001$ * $p<0.01$

Figure 5. Tau pathology develops in the CA1 region of the hippocampus of rTg4510 mice

Immunohistochemical labeling of tissue sections from non-transgenic (A–C) and rTg4510 mice (D–F) with the phospho-specific antibody PHF1 (pSer396/404) reveals age-dependent accumulation of tau pathology in the CA1 of the hippocampus [2 months (A, D, G); 6 months (B, E, H); 10 months (C, F, I)]. H&E staining illustrates age-dependent neuronal loss in the CA1 in rTg4510 mice (G–I). Pictures are representative.

Figure 6. Tau pathology develops in the amygdala of rTg4510 mice

Immunohistochemical labeling of tissue sections from non-transgenic (A–C) and rTg4510 mice (D–F) with the phospho-specific antibody PHF1 (pSer396/404) reveals age-dependent accumulation of tau pathology in the amygdala [2 months (A, D, G); 6 months (B, E, H); 10 months (C, F, I)]. Serial sections stained with H&E to evaluate neuronal morphology in the amygdala of rTg4510 mice (G–I). Pictures are representative.

Figure 7. Hippocampus and amygdala exhibit significant tau burden in rTg4510 mice

(A) Quantification of tau burden assessed by percent of tissue area strongly-positive for PHF1 in the CA1 region of the hippocampus (effect of transgene $F=89$, $p<0.0001$; effect of age $F=20.8$, $p<0.0001$); (B) Percent of tissue strongly-positive for PHF1 in the amygdala (effect of transgene $F=138.3$, $p<0.0001$; effect of age $F=14.10$, $p<0.0001$). Data is presented as mean $\pm$ SEM. ** $p<0.0001$ * $p<0.05$.

Figure 8. Tau pathology in human amygdala and hippocampus

Immunopositive-tau pathology using the PHF-1 antibody in human brain tissue of two P301L mutation carriers mirrors rTg4510 mice, but was not found in age-matched normal human brain tissue sections (A,D,G). The dentate fascia is shown in the top panel (A–C), hippocampal CA1 in the middle panel (D–F), and the amygdala in the bottom panel (G–I). Tau-positive granule cells of the dentate fascia is a characteristic feature of these mutation carriers (B, C). In addition, the insets of both P301L mutation carriers demonstrate pre-tangles in hippocampal CA1 (E,F; 3.7% and 11.5% tau burden, respectively) and amygdala (H, I; 10.4% and 53.7% tau burden, respectively). A–I, 20× with insets shown at 40×. Magnification bar is shown at 100μm, equivalent to 50μm for insets.