Impulsivity, decreased social exploration, and executive dysfunction in a mouse model of frontotemporal dementia

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Abstract

Frontotemporal lobar degeneration (FTLD) is a neurodegenerative disorder, a major subset of which is characterized by the accumulation of abnormal forms of the protein tau, leading to impairments in motor functions as well as language and behavioral alterations. Tau58-2/B mice express human tau with the P301S mutation found in familial forms of FTLD in neurons. By assessing three age cohorts of Tau58-2/B mice in a comprehensive behavioral test battery, we found that the tauopathy animals showed age-dependent signs of impulsivity, decreased social exploration and executive dysfunction. The deficit in executive function was first limited to decreased spatial working memory, but with aging this was extended to impaired instrumental short-term memory. Tau pathology was prominent in brain regions underlying these behaviors. Thus, Tau-58-2/B mice recapitulate neurological deficits of the behavioral variant of frontotemporal dementia (bvFTD), presenting them as a suitable model to test therapeutic interventions for the amelioration of this variant.
Keywords: Behavior; executive function; frontotemporal dementia; frontotemporal lobar degeneration; social exploration; tau

Highlights

• Tau58-2/B mice present with a tau pathology in cortical areas implicated in bvFTD.
• Tau58-2/B mice exhibit motor and sensory gating anomalies at an early age.
• With aging the mice show decreased sociability but no altered exploration.
• The mice further display increased risk-taking behaviors and executive deficits.
• First limited to spatial working memory, it later extends to instrumental memory.

1. Introduction

Frontotemporal lobar degeneration (FTLD) is a form of dementia that is characterized by atrophy of the frontal and temporal lobes and deposition of the microtubule-associated protein tau (Galimberti and Scarpini, 2010; Rademakers et al., 2012; Sieben et al., 2012). Under physiological conditions, tau is mainly localized to the axon of mature neurons where it binds to and stabilizes microtubules; however, tau has also been found in the dendritic compartment where the protein has a role in targeting the kinase Fyn to the dendritic spines (Ittner et al., 2010). Under pathological conditions such as FTLD, tau becomes hyperphosphorylated and forms filaments that eventually form microscopic lesions known as neurofibrillary tangles (NFTs) (Ittner et al., 2015). FTLD has been difficult to diagnose due to the heterogeneity of the associated symptoms, which affect behavior, language and cognition (Bigio, 2013; Mendez, 2004; Seltman and Matthews, 2012).

Frontotemporal dementia (FTD) as the clinical presentation of FTLD is the second most common type of presenile dementia and the fourth most common type of senile dementia, although it is among the more costly due to its symptom characteristics (Neary et al., 2005; Seltman and Matthews, 2012). Clinically, FTD is divided into three subtypes: a behavioral variant (bvFTD) that affects social skills,
emotions, personal conduct, and self-awareness, semantic dementia that compromises language comprehension, and motor variants leading to muscle wasting (Hodges et al., 2004). BvFTD presents with changes in social behavior and conduct, such as loss of social awareness and social withdrawal, restlessness and poor impulse control leading to compulsive behaviors including stereotyped hair-pulling and skin picking (Eslinger, Moore, Anderson, & Grossman, 2011; Lindau et al. 2000; Mendez & Perryman, 2002; Pressman & Miller, 2014; Snowden et al., 2001, 2003). At later stages, FTD patients develop deficits in executive function: they have problems planning, coordinating and executing simple tasks (Harciarek and Cosentino, 2013; Huey et al., 2009; Johns et al., 2009; Moy et al., 2004; Stopford et al., 2012). In addition to the characteristic behavioral changes, the clinical features of FTD can be complicated by neurological signs, such as motor neuron signs, parkinsonism, and gait disturbances, with some patients developing motor problems resulting from motor neuron pathology (Devenney et al., 2015; Merrilees et al., 2010).

To elucidate the contribution of hyperphosphorylated tau pathology to the behavioral manifestations and cognitive deficits observed in FTD, we analyzed the tau expression pattern and studied the effects of tau overexpression by subjecting P301S tau transgenic Tau58-2/B (Tg) mice to a behavioral test battery probing for signs and symptoms relevant for FTD. We analyzed Tau58-2/B mice of three age groups to investigate whether with progressive tau accumulation this would be associated with more pronounced behavioral changes. We found that this murine model is histologically characterized by progressive deposition of hyperphosphorylated forms of tau in neurons. In addition, we found deficits in behaviors modeling the symptoms of bvFTD, including increased impulsivity and risk-taking behaviors, decreased social interest, and executive dysfunction. The distinctive behavioral phenotype was augmented with advanced age, reflecting the age-dependent tauopathy that characterizes the Tau58-2/B mice. Together, this set of FTD-like phenotypic traits and a distinct brain pathology renders the Tau58-2/B strain a suitable model for bvFTD, with the prospect of determining whether therapeutic interventions also ameliorate the phenotype that characterizes the behavioral variant of FTD.

2. Materials and methods
2.1. **Mouse models.** Tau58-2/B mice (Tg) express the human 0N4R tau isoform together with the P301S mutation under control of a murine Thy1.2 promoter on a C75Bl/6 background (Tau58, Novartis Institutes for Biomedical Research, Basel, Switzerland). Mice from the Novartis colony were separately shipped to Australia to establish independent colonies. One colony was mainly analyzed histologically (van Eersel et al., 2015). We established a separate colony by continued breeding of Tau58 onto a C57BL/6 background, generating Tau58-2/B mice. Non-transgenic littermates (wild-type, Wt) were included as controls.

2.2. **Experimental design.** For behavior, mice were divided into the following age groups: 2-3 months, 6-7 months, and 10-11 months. Animals were housed in groups of 2–5 animals per cage. They were kept on a 12 h light/dark cycle (light on at 8:00 AM, with testing during the light period) with free access to food and water unless otherwise stated. For behavioral experiments, the Tau58-2/B mice were directly compared with Wt littermates (5 males and 5 females per genotype per age-group), and tested cross-sectionally using the following experimental sequence: SHIRPA screen, open field, social exploration, light/dark box, Y-maze, and puzzle-box (Supplementary Table 1, Supplementary Table 2). For histological studies (n=3 per age group), the 6-7 month group was used after the behavioral data had been obtained (by then 7-8 months old) and the 10-11 month group after the behavioral data had been obtained (by then 11-12 months old). A separate batch of 16 months old Tau58-2/B mice was included for histology. All animal experiments were approved by the Animal Ethics Committees of the University of Queensland and all procedures complied with the statement on animal experimentation issued by the National Health and Medical Research Council of Australia.

2.3. **Histology.** Animals were anesthetized, perfused, and brains removed and post-fixed in 4% paraformaldehyde at 4 °C overnight, dehydrated, and then embedded in paraffin. Seven µm-thick brain sections were cut with a microtome in the frontal or sagittal plane and mounted on silane-coated slides. Brains of 7-8, 11-12, and 16 month-old Tau58-2/B mice were analyzed for the presence of pathological tau species (Xia et al., 2015) (Fig 1). The following anti-tau antibodies were used: pSer235 (1:500, Thermo Scientific), pSer422 (1:500, Thermo Scientific), AT8
(pSer202/pThr205 Tau, 1:500, Thermo Scientific), AT100 (pThr212/pSer214 Tau, 1:500, Thermo Scientific), AT180 (pThr231/pThr235 Tau, 1:500, Thermo Scientific), and HT7 (pan Tau, 1:500, Thermo Scientific), which recognizes total tau. Bielschowsky silver staining was employed to reveal NFTs (Ke et al., 2009).

2.4. Murine neurobehavioral screen and motor abilities. A comprehensive modified SHIRPA screen (Rafael et al., 2000; Rogers et al., 1997) was performed. Using this test battery, physical characteristics, sensory reactions and reflexes, as well as motor abilities were investigated (for a complete checklist and photos of the setups used see Supplementary Data). The collected physical characteristics included the body weight and general appearance. Next, mouse behavior was observed which included transfer behavior, spontaneous activity, and the occurrence of tremors, palpebral closure and gait (Supplementary Fig 1). Subsequently, reactions to simple stimuli such as touch escape, trunk curl, and the reaching reflex, Preyer reflex, and the toe pinch reflex were assessed. Finally, basic motor abilities were measured using the grip strength, wire hanging and accelerating Rotarod tests.

2.5. Exploratory behavior, sociability and anxiety. General exploration and social exploration (Callaerts-Vegh et al., 2012; Crawley, 1985) were examined using a 30 × 30 cm² square arena of Plexiglas. Animals were placed in the experimental room 30 min before the test started. They were then placed individually in the arena (600 lux) for 10 min, where their movement was recorded using EthoVision video tracking equipment and software (Noldus, Wageningen, The Netherlands). Total distance traveled and velocity were included as measures of locomotor activity. Time spent exploring the center (within an imaginary inner square of 20 x 20 cm) and the corners, and the number of visits to the center and corners were recorded as a measure of anxiety-like behaviors. In addition, heat maps were generated using a custom-made program to examine anxiety and thigmotaxis (wall-hugging behavior) (Van Der Jeugd et al., 2013).

For the assessment of sociability, the open field arena contained a cage with a diameter of 10 cm and a height of 20 cm holding an unfamiliar mouse of the same gender. Again, total distance traveled and velocity were included as measures of locomotor activity. The time exploring the stranger mouse (within an imaginary
annulus of 5 cm around the cage containing the mouse), and the number of visits to the center of the arena was recorded as a measure of social exploration.

2.6. Anxiety, impulsivity and risk-taking behaviors. The light/dark box arena consisted of a Plexiglas box divided by a small underpass into two compartments: a larger brightly lit zone (58 cm long, 28 cm wide, 750 lux) and a smaller covered dark zone (15 cm long, 28 cm wide) (Crawley, 1985; Tasan et al., 2009). Prior to the test, animals were habituated to the dark for 30 min. They were then placed in the dark chamber of the apparatus facing away from the door and tracked for 5 min using EthoVision software (Noldus). The latency to the first entry into the light, the time spent in the illuminated area, and the number of transitions were recorded as indications of impulsivity and anxiety. In order to assess risk-taking behaviors in more detail (Baeta-Corral and Giménez-Llort, 2014; Varty et al., 2002), the frequency of stretch-attendance postures (with the animal stretching forward into the underpass and then retracting to its original position) was also monitored.

2.7. Executive function. Spatial working memory was tested with the spontaneous alternation behavior (SAB) paradigm (Hughes, 2004; Lalonde, 2002; Naert et al., 2013). Testing occurred in a Y-shaped maze with three white, transparent plastic arms arranged at 120° angles (9 cm wide, 40 cm long arms, 30 cm deep). The mouse was placed in the start arm of the Y-maze and allowed to freely explore the three arms in a 5 min trial (extra maze cues were present on the walls). The number of arm entries and the number of triads were recorded in order to calculate the percentage of SAB. An entry occurred when all four limbs were within the arm.

To assess problem solving capacities and short-term memory (STM), the mice were subjected to the puzzle box (Ben Abdallah et al., 2011; Galsworthy et al., 2005). The arena consisted of a Plexiglas box divided into two compartments by a small underpass: a larger brightly lit zone, the start box (58 cm long, 28 cm wide, 750 lux) and a smaller covered dark zone, the goal box (15 cm long, 28 cm wide). Mice underwent a total of six trials over two consecutive days, with three trials per day: the day started with a training session followed by two puzzle sessions (on day 1 these were two dig trials, and on day 2 two plug trials). Mice were deprived of food two days prior to the test (while their body weight was maintained at approximately 85% of its initial weight), and food pellets were presented in the goal box. On training
trials, mice were introduced into the start zone, and taught to move into the goal zone through a narrow underpass (~ 4 cm wide) separating the two compartments. For the burrowing trials, the underpass was filled with sawdust and the mice had to dig their way through. For the plug trials, a square, cotton plug that mice had to pull with their paws to enter the goal zone obstructed the underpass. Time to enter the goal box was measured (with a cut-off of 3 min, thus a 180 s penalty was given when the animal did not solve the puzzle).

2.8. Statistics. ANOVA was used to determine genotype, and gender and interaction effects between the three age groups. All data are represented as means (± SEM), and $p < 0.05$ was considered statistically significant (* for differences between genotypes, # for differences between gender).

3. Results

3.1. Tau transgenic Tau58-2/B mice present with a widespread, progressive tauopathy

In tauopathy, progressive hyperphosphorylation of tau, in particular at pathologic epitopes, occurs as disease progresses, concomitant with NFT formation. To determine the progression of tau pathology in Tau58-2/B mice, the mice were histologically analyzed with a set of phospho-tau-specific antibodies (pS235, pS422, AT8, AT100, and AT180) as well as human tau-specific antibody HT7 (Fig 1A-E). At 8 months of age, Tau58-2/B mice display subtle deposition of hyper-phosphorylated tau mainly in the CA1 region of the hippocampus and the cortex (Table 1). With age, the mice developed a more pronounced tau pathology, with hyperphosphorylated tau being found in the cortex, hippocampus, and cerebellum as shown for 12 months of age. As the mice aged even further, histological analysis revealed NFT deposits; Bielschowsky silver staining of sagittal sections demonstrated stained NFT-like structures and neuronal cell bodies in the hippocampus, throughout the whole frontal cortex including the anterior cingulate cortex andorbitofrontal cortex, and in the cerebellum and brainstem (Fig 1B'-E').

3.2. Higher arousal and reduced neuromotor abilities in Tau58-2/B mice
Assessing the physical characteristics of the mice we found that the Tg mice had a lower body weight than age-matched Wt mice, at all ages tested (three categories: 2-3 months \(F_{1,19}=13.71, p = 0.003\), 6-7 months \(F_{1,19}=39.98, p = 0\), and 10-11 months \(F_{1,19}=21.21, p = 0.001\)) (Table 2). Evaluating the existence of whiskers we did not find a difference between Tg and Wt animals. However, when assessing the constitution of the fur this showed that the 10-11 month-old Tg mice had more bald patches and wounds than their Wt littermates, which was not seen for the two younger age groups (10-11 months: \(F_{1,19}=48.52, p < 0.001\)). When observing the mice for habituation of activity in a new environment, Tg mice were indistinguishable from Wt age-controls. However, we noted some tremors and a less fluid gait in 6-7 and 10-11 month-old Tg mice compared to age-matched Wt mice (tremor: 6-7 months: \(F_{1,19}=7.33, p = 0.02\); 10-11 months: \(F_{1,19}=25.96, p < 0.001\); gait: 10-11 months: \(F_{1,19}=5.15, p = 0.038\)).

In terms of sensorimotor reflexes, we found no difference in visual acuity by reaching reflex, or nociceptive sensation as measured by toe pinch and trunk curl. However, when evaluating the acoustic acuity we found that this elicited a stronger flinch response to the sound in 6-7 and 10-11 month-old Tg mice as compared to their respective age-matched Wt controls (6-7 months: \(F_{1,19}=41.25, p < 0.001\); 10-11 months: \(F_{1,19}=27.40, p < 0.001\)). When evaluating Tg mice for the touch escape response, we also found a higher response in 10-11 month-old Tg mice (10-11 months: \(F_{1,19}=5.15, p = 0.038\)). Finally, assessing the motor abilities of the mice, we found that 10-11 month-old Tg mice were impaired in muscular grip strength (maximal grip: 10-11 months: \(F_{1,19}=31.67, p < 0.001\); mean grip: 10-11 months: \(F_{1,19}=108.07, p < 0.001\)). Also, in the wire hang test, aged Tg mice fell off faster than Wt mice (6-7 months: \(F_{1,19}=6.74, p = 0.025\); 10-11 months: \(F_{1,19}=39.62, p < 0.001\)). Finally, in the accelerating Rotarod test, a similar outcome was observed (maximal latency on the rod: 6-7 months: \(F_{1,19}=21.21, p = 0.001\); 10-11 months: \(F_{1,19}=28.10, p < 0.001\); mean latency on the rod: 6-7 months: \(F_{1,19}=26.1, p < 0.001\); 10-11 months: \(F_{1,19}=37.11, p < 0.001\)). When investigating the main effect of gender (Supplementary Results), differences were observed for weight: at all ages males weighed more than females, irrespective of their genotype. However, in Tg male mice we observed more tremors, bald patches and wounds compared to their female Tg and male Wt controls. Also, in the grip test, both Wt and Tg males outperformed females as measured by
maximum and mean pulling force. Overall, Tau58-2/B mice presented with an overall increase in nervous behavior and arousal at young ages, neuromotor deficits at adult ages, and a lower body weight at all ages.

3.3. Tauopathy mice show no alterations in general exploratory behavior or anxiety

To examine general exploratory behavior and anxiety, mice were observed in the open field test. Total path length and velocity were included as measures of locomotor activity; however, no changes in velocity or overall distance traveled were found between the genotypes at any of the ages tested (Fig 2A). Time spent in the center and corners were recorded as a measure of anxiety, and were found to be unaltered between genotypes at all ages examined (Fig 2B). Numbers of center and corner visits were unaltered; this was also confirmed by displaying the data in heat plots (Fig 2C). No gender differences were found in this test. As indicated above, these findings showed no differences in locomotor activity because the overall spontaneous locomotion and general motor activity was not altered between the genotypes, nor was there any evidence for an anxiolytic or anxiogenic profile in the Tau58-2/B mice.

3.4. Tauopathy mice have deficits in social exploration

The social exploration test is used to study social interactions in mice. No change in total distance travelled or velocity was found in Tg compared to Wt mice at any age tested (Fig 3A). The time spent in the center of the field (within an imaginary annulus of 5 cm around the cage containing the stranger mouse) was recorded as a measure of social exploration. Interestingly, decreased exploration of stranger mice in 6-7 and 10-11 month-old Tg mice was observed compared to Wt littermates (6-7 months: $F_{1,19} = 12.19, p = 0.006$; 10-11 months: $F_{1,19} = 4.70, p = 0.047$) (Fig 3B). This was confirmed by the number of entries into the center of the arena which differed between genotypes: Tg mice visited the center with the stranger mouse less frequently than Wt mice (6-7 months: $F_{1,19} = 14.63, p = 0.03$; 10-11 months: $F_{1,19} = 14.70, p = 0.037$) (data not shown). Heat plots verified that there were fewer activity hot spots near the cage containing the stranger mouse in Tg mice from 6 months onwards (Fig 3C). No gender differences were found in this test. Thus, importantly, although Tg mice did
not show altered overall exploration levels *per se*, they were more reluctant to explore another mouse.

### 3.5. Increased impulsivity and risk-taking behaviors in tauopathy mice

The light/dark box is used in the assessment of anxiety and risk-taking behaviors. The latency to the first entry into the light and the time spent in each of the two compartments was assessed to measure impulsivity and anxiety. We found that Tg mice spent more time in the illuminated area than Wt mice at all ages (2-3 months: $F_{1,19}=7.34; p = 0.019$; 6-7 months: $F_{1,19} = 11.11, p = 0.007$; 10-11 months: $F_{1,19}=16, p = 0.0001$) (Fig 4A). Moreover, we found Tg mice to emerge faster from the illuminated area than Wt mice (2-3 months: $F_{1,19}=14.09, p = 0.003$; 10-11 months: $F_{1,19}=5.06, p = 0.032$) (Fig 4B). We found a gender main effect in the latency to enter the illuminated area at 6-7 months ($F_{1,19} = 5.024, p = 0.041$), with females entering the illuminated area faster than males. There was no difference in the number of transitions between the dark and light compartments between the groups (data not shown). Furthermore, we found fewer stretch-attend postures in Tg mice at 6-7 and 10-11 months compared to their age-matched Wt littermates (6-7 months: $F_{1,19} = 9.73, p = 0.01; 10-11 months: F_{1,19}=17.64, p = 0.001$) (Fig 4C). Hence, one may conclude that the Tg mice act more impulsively and erratically.

### 3.6. Tauopathy mice have deficits in executive function

To assess executive function we first subjected the mice first to the Y-maze that measures spatial working memory. We found that total entries into the Y-maze arms did not differ between Tg and Wt mice, at any of the ages tested (data not shown). However, the percentage of SAB was significantly different between the genotypes, with Tg mice making fewer alternations compared to age-matched Wt mice, with an interaction effect for gender at 6-7 months (main genotype effect: $F_{1,19} = 15.63, p = 0.002$; genotype x gender interaction effect: $F_{1,19} = 6.82, p = 0.024$), and at 10-11 months (main genotype effect: $F_{1,19} = 53.39, p < 0.001$; genotype x gender interaction effect: $F_{1,19} = 5.602, p = 0.032$) (Fig 5A). Heat plots verified this lack of SAB in the Tg mice (Fig 5B). Next, in the puzzle box paradigm, mice were presented with several puzzles of increasing difficulty. In the first phase, a simple emergence test
from the brightly lit arena into the dark goal box, none of the mice were impaired (data not shown). However, in the second phase, that required the mice to dig their way to reach the goal box, Tg mice failed the test from 10 months onwards ($F_{1,19} = 16.446, p = 0.001$) (Fig 5C). Moreover, on the next day thus requiring intact STM, both the 6-7 and 10-11 month-old Tg mice were found to be impaired (6-7 months: $F_{1,19} = 12.46, p = 0.005$; 10-11 months: $F_{1,19} = 30.44, p < 0.01$) (Fig 5D). No gender or genders x genotype interaction effects were found in this test. In conclusion, the Tg mice display specific executive function deficits as shown by working and STM impairments.

4. Discussion

Various aspects of tauopathy have been modeled in mice, but the behavioral aspects that specifically characterize FTD have been less explored. In our study we found that the P301S human tau transgenic Tau58-2/B strain displays a bvFTD-like phenotype. Specifically, we found that this murine model is characterized by the deposition of hyperphosphorylated forms of tau in neurons in frontal and temporal brain areas, and presents with consistent deficits in behaviors resembling the symptoms of bvFTD, including decreased social behavior, impulsivity and increased risk-taking behaviors and impairments in executive function.

Previous studies have shown that motor performance is impaired in Tau58-2/B mice as assessed in the Rotarod, beam and horizontal pole tests (van Eersel et al., 2015). In the present study, we were able to replicate the progressive genotype-linked differences in the Rotarod (however at half the speed in our study), wire hang and grip strength tests, but now add to this a more comprehensive behavioral analysis, addressing aspects of FTD-like behaviors. For instance, we found that Tau58-2/B animals had tremors and displayed a less fluid gait. Indeed, in FTD patients, extrapyramidal symptoms including akinesia, Parkinsonian gait and resting tremor have been reported (Diehl-Schmid et al., 2007). Upon inspection of the cerebellum and brainstem, regions known to contribute to motor problems, we found tau pathology in the older Tg mice.

Moreover, we found evidence for increased arousal and reactivity to sensory stimuli, an aspect of sensory gating that is observed in individuals with FTD and Alzheimer's disease (Gibbons et al., 2008; Grunwald et al., 2003; Knight et al., 1999;
Landqvist Waldö et al., 2014; Perriol et al., 2005; Takeuchi et al., 2011). In our study, we observed that Tau58-2/B mice were more sensitive to an unexpected stimulus, as measured by a higher startle response in the Preyer reflex and touch escape assay. In two related P301S murine models it had been discovered that prepulse inhibition (PPI), another marker of sensorimotor gating, is altered (Koppel et al., 2014; Takeuchi et al., 2011). However, we were prevented from performing a PPI assay with our mice because of the motor weakness that characterizes the aged Tg animals. Sensory gating is highly dependent on the prefrontal cortex (Chao and Knight, 1995; Knight et al., 1999), a brain area for which we found massive immuno-reactivity for hyperphosphorylated tau using a series of epitope-specific antibodies, and NFTs as visualized with Bielschowsky.

Impairment of social interactions, including social withdrawal, loss of empathy and social misconduct, is one of the core characteristics of FTD (Eslinger et al., 2011; Hodges, 2013; Passant et al.; Rankin et al., 2005; Savage et al., 2014). We found that Tau58-2/B animals spend a significantly shorter amount of time with a stranger mouse than Wt mice. This is in accordance with other studies that found deficits in social interactions in mouse models resembling clinical FTD, such as in progranulin-deficient, and senescence-accelerated-prone (SAMP) mice (Filiano et al., 2013; Meeker et al., 2013; Takeuchi et al., 2011; Yin et al., 2010). It has been reported that orbitofrontal dysfunction is related to both apathy and disinhibition in FTD patients (Peters et al., 2006). In accordance with this we noted increased tau immuno-reactivity and NFT formation by silver staining in 12 month-old Tg animals.

It is noteworthy that with the open field test we did not find evidence for an altered general exploration in our tau transgenic model. As the exploration of a stranger mouse is performed in the same arena as the open field test, we conclude that the decrease in sociability we see in the Tg mice is restricted to exploration of a stimulus animal.

Our results revealed that in the light/dark box, the Tg mice spent more time in the illuminated compartment than their age-matched Wt counterparts. This is in contrast to the findings of the open field test where the Tg mice spent an equal amount of time in the center and corners compared to the Wt mice. Moreover, when we assessed marble burying in the mice, we found that Tg mice bury roughly the same number of marbles within a 30 min period as their age-matched Wt littermates (data not shown). The marble burying task can be used as an indicator of both...
obsessive compulsive-like behavior and anxiety-like behavior; however, the neuronal circuitry of this behavior has not been clearly elucidated (Njung’e and Handley, 1991). For some tau transgenic strains, it has been shown that the mice can be anxiolytic and/or thigmotactic correlating with a pathology in the amygdala (Baeta-Corrall and Giménez-Llort, 2014; Egashira et al., 2005; Sterniczuk et al., 2010; Van der Jeugd et al., 2013). Incidentally, obtaining coronal sections, we also found a prominent accumulation of tau in the amygdala of the Tau58-2/B mice (data not shown).

Interestingly, our Tg mice emerged faster into the illuminated area than their Wt littermates after being habituated to the dark half an hour prior to the test. This could indicate increased (motor) impulsivity and behavioral disinhibition in the Tg mice. More evidence for impulsivity comes from the increase in risk-taking behavior that was observed in our Tg animals by decreased stretch-attendance postures in a fearful situation. In individuals with FTD, cases of pathological stealing, an excessive gambling or alcohol consumption have been reported (Cruz et al., 2008; Grochmal-Bach et al., 2009; Manes et al., 2010; Mendez, 2011; Pompanin et al.). All of these violations can be regarded as disinhibitory deficits, which are pathologically related to impairments in specific prefrontal cortex regions. For instance, the ventromedial prefrontal cortex is associated with emotion regulation, the anterior cingulate cortex with emotional empathy, and the orbitofrontal cortex with compulsive behavior (Knutson et al., 2015; Mendez, 2010; Starkstein and Robinson, 1997). Related to this, we observed bald patches and in some cases even skin wounds in 75% of the Tau58-2/B male animals, but only in one Wt mouse. This could be due to excessive barbering, an abnormal repetitive behavior, which is analogous to human compulsive hair pulling and skin picking, as commonly observed in FTD patients (Pompanin et al., 2014). To rule out the possible effect of hetero-barbering or aggression, in a future study repetitive self-grooming and aggression could be examined separately. Increased aggression could also be explained by the higher arousal in male Tg mice as observed by the touch escape and Preyer reflex. In later stages of the disease, a decline in executive function is often seen in FTD patients (Eslinger et al., 2011; Harciarek and Cosentino, 2013; Huey et al., 2009; Johns et al., 2009; Seltman and Matthews, 2012; Shea et al., 2014). To evaluate executive function, the animals were first subjected to the Y-maze test, which depends on their intrinsic motivation to explore a novel environment. Notably, our 6-7 and 10-11
month-old Tg mice showed a working memory deficit in the Y-maze. In rodents, it has been shown that the prefrontal cortex and hippocampus are engaged in this spatial working memory task (Dillon et al., 2008; Naert et al., 2013; Reisel et al., 2002). Moreover, mice were subjected to several problem-solving tasks requiring not only intact spatial STM but also an instrumental response. The sequence over two days (day 1 consisting of two dig trials, day 2 two plug trials) allowed assessing STM for species-specific instrumental responses (Cowan, 2008). We found that only the 10-11 month-old Tg mice performed worse than their Wt counterparts on the dig trials on day 1. However, when tested the next day on the plug trials, now also the 6-7 month-old Tg mice failed, thus suggesting a STM deficit at this age. In support, staining of brain sections with a panel of antibodies revealed hyperphosphorylated tau species and NFTs in the hippocampus and prefrontal cortex in the Tg mice.

In FTLD, tau is abnormally phosphorylated, forming fibrillar aggregates that can be visualized with silver impregnation methods as NFTs. In our Tau58-2/B mice, we found evidence for progressive tau pathology leading to NFTs, including phosphorylation of pathological epitopes in brain areas relevant for FTLD. Bielschowsky silver staining confirmed the presence of NFTs - this was in accordance with another recently published study using Tau58 mice (van Eersel et al., 2015).

In conclusion, we assessed the behavior of three age groups of Tau58-2/B mice with a progressive tau hyperphosphorylation in frontal and temporal regions, focusing on evaluating FTD signs and symptoms. We found that aged Tau58-2/B mice mimic the three core characteristics of FTD: increased impulsivity and risk-taking behavior, impairment of social interactions as shown by decreased social exploration time, and mental rigidity as revealed by poorer working memory and an inability to resolve problem solving tasks. This finding presents Tau58-2/B mice as a model to validate therapeutic interventions to ameliorate the behavioral symptoms of FTD.

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**Appendix A. Supplementary data**

Supplementary data to this article can be found online.
References


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Figure legends

**Figure 1. Tau58-2/B mice present with a widespread, progressive tauopathy.** (A) Drawing of a mouse sagittal section representing the affected brain regions and their underlying functions. ACC, anterior cingulate cortex; HC, hippocampus; OFC, orbitofrontal cortex; PFC, prefrontal cortex; and OFC, orbitofrontal cortex. (B) Representative sagittal section of the brain of a 12 month-old Tau58-2/B mouse stained with HT7 for total tau. (B’) Consecutive section impregnated with Bielschowsky silver staining to visualize NFTs. (C-E, C’-E’) Corresponding higher magnification images derived from hippocampus (C, C’), prefrontal cortex (D, D’), and cerebellum (E, E’). Scale bars: 100µm.

**Figure 2. Tauopathy mice show no alterations in explorative activity or anxiety.** (A) Total distance moved over a 10 min session in the open field test reveals no differences in anxiety between wild-type (Wt) and transgenic (Tg) mice. (B) Tau Tg mice spend the same amount of time as the Wt littermates in the center for all three age groups assessed. (C) Heat maps reveal no thigmotactic or altered anxiety-like behavior in Tg compared to Wt mice at any age tested. Representative traveled paths per genotype are shown.

**Figure 3. From 6 months onwards, tauopathy mice display deficits in social exploration.** (A) In the social exploration test, tau Tg mice are indistinguishable from Wt mice at all age comparisons, when comparing total distance traveled. (B) From 6 months onwards, Tg mice spend less time in the annulus around the cage containing the stranger mouse compared to the age-matched Wt mice, indicative of impaired social interaction. (C) Heat maps confirm the decrease in social exploration in Tg mice. Representative traveled paths per genotype are shown. Brackets indicating differences between genotypes (*), and gender (#); p* < 0.05; p** < 0.01; p*** < 0.001.

**Figure 4. Tauopathy mice show increased impulsivity and risk-taking behaviors.** (A) Tau Tg mice spend more time in the light compartment as compared to Wt mice. (B) As shown by the decreased latency in the light/dark box, dark-habituated tau Tg mice emerge faster from the dark into the light than their age-matched Wt counter-
parts. (C) From 6 months of age onwards, Tau58-2/B mice show an increased risk-taking behaviors compared to Wt mice. Brackets indicating differences between genotypes (*), and gender (#); \( p^* < 0.05; p^{**} < 0.01; p^{***} < 0.001 \).

**Figure 5. Decreased working memory and adaptive problem solving capacities in aged tau Tg mice.** (A) From 6 months onwards, tau Tg mice make fewer alternations in the Y-maze. (B) Heat maps confirm the decrease in alteration in 10-11 months-old Tg mice. Representative traveled paths during the first minute for each genotype are shown. (C) From 10 months onwards, Tg mice take longer to solve the digging problem than the age-matched Wt mice as shown by an increased latency to enter the goal box that contains the food reward. (D) The executive function deficit in aged tau Tg mice is verified in the second puzzle on day 2 when tau Tg mice fail to solve the plug test as compared to age-matched Wt mice. Brackets indicating differences between genotypes (*), and gender (#); \( p^* < 0.05; p^{**} < 0.01; p^{***} < 0.001 \).
Tables

Table 1. Progressive tau hyperphosphorylation and NFT formation in Tau58-2/B mice.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>7-8 mo</th>
<th>11-12 mo</th>
<th>16 mo</th>
<th>7-8 mo</th>
<th>11-12 mo</th>
<th>16 mo</th>
<th>7-8 mo</th>
<th>11-12 mo</th>
<th>16 mo</th>
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<tbody>
<tr>
<td>pSer235</td>
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<td>+++</td>
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<td>+++</td>
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<td>+</td>
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</tbody>
</table>

- No ++, + low ++, ++ moderate ++, +++ high ++ based on positive stained cells and in 7-8, 11-12, and 16 months old tau Tg mice.

Table 2. Differences in general appearance, arousal, and neuromotor performance in Tau58-2/B mice.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>2-3 months</th>
<th>6-7 months</th>
<th>10-11 months</th>
</tr>
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<tbody>
<tr>
<td>Tg</td>
<td>WT</td>
<td>Tg</td>
<td>WT</td>
</tr>
<tr>
<td>Overall health/appearance</td>
<td>Body weight</td>
<td>16.4 ± 0.6**</td>
<td>18.4 ± 1.2</td>
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<tr>
<td></td>
<td>Whiskers</td>
<td>3 ± 0</td>
<td>3 ± 0</td>
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<tr>
<td></td>
<td>Groomed fur</td>
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<tr>
<td></td>
<td>Missing fur</td>
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<td>0.2 ± 0.2</td>
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<tr>
<td></td>
<td>Wounds</td>
<td>0.2 ± 0.1</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>General behavior</td>
<td>Transfer behavior</td>
<td>3.1 ± 0.1</td>
<td>3 ± 0</td>
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<tr>
<td></td>
<td>Spontaneous activity</td>
<td>1.4 ± 0.3</td>
<td>1.3 ± 0.3</td>
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<tr>
<td></td>
<td>Tremor</td>
<td>0.2 ± 0.1</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>Palpebral closure</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td></td>
<td>Gait</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Reflexes and reactions</td>
<td>Touch escape</td>
<td>2.1 ± 0.1</td>
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<td></td>
<td>Trunk curl</td>
<td>1 ± 0</td>
<td>1 ± 0</td>
</tr>
<tr>
<td></td>
<td>Reaching reflex</td>
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<td>4 ± 0</td>
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<tr>
<td></td>
<td>Pupper reflex</td>
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<td>1.2 ± 0.2</td>
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<tr>
<td></td>
<td>Toe pinch</td>
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<td>3 ± 0</td>
</tr>
<tr>
<td>Neuromotor tests</td>
<td>Grip strength max</td>
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<td>1.4 ± 0.2</td>
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<tr>
<td></td>
<td>Grip strength mean</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.2</td>
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<tr>
<td></td>
<td>Wire hang</td>
<td>0.7 ± 0.3</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Rotated max</td>
<td>158.1 ± 7.3</td>
<td>144.8 ± 16.1</td>
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<tr>
<td></td>
<td>Rotated mean</td>
<td>105.5 ± 5.6</td>
<td>104.0 ± 8.0</td>
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</table>

*p < 0.05, **p < 0.01, ***p < 0.001 tau Tg vs. corresponding age-matched WT value.
Figure 2

A. Distance traveled (cm) over time for Wt female and Wt male mice, as well as Tg female and Tg male mice, across three age groups: 2-3 months, 6-7 months, and 10-11 months.

B. Time spent in center (s) over time for Wt female and Wt male mice, as well as Tg female and Tg male mice, across three age groups: 2-3 months, 6-7 months, and 10-11 months.

C. Spatial activity patterns of Wt and Tg mice across three age groups: 2-3 months, 6-7 months, and 10-11 months.
Figure 3

A. Distance traveled (cm) in different age groups (2-3 months, 6-7 months, 10-11 months) for Wt female, Wt male, Tg female, and Tg male genotypes.

B. Time spent in center (s) for different age groups (2-3 months, 6-7 months, 10-11 months) for Wt female, Wt male, Tg female, and Tg male genotypes.

C. Heatmaps showing the distribution of activity for Wt and Tg genotypes at different ages (2-3 months, 6-7 months, 10-11 months).
Figure 4

A

Time spent in light (s)

Wt female

Tg female

Wt male

Tg male

2-3 months 6-7 months 10-11 months

B

Latency to light (s)

2-3 months 6-7 months 10-11 months

C

Stretched postures (#)

2-3 months 6-7 months 10-11 months
Figure 5

A

Alternations (%)

<table>
<thead>
<tr>
<th></th>
<th>2-3 months</th>
<th>6-7 months</th>
<th>10-11 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tg female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wt male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tg male</td>
<td></td>
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</table>

B

Latency to solve dig (s)

C

Latency to solve plug (s)

D

10-11 months
Impulsivity, decreased social exploration, and executive dysfunction in a mouse model of frontotemporal dementia

Ann van der Jeugd¹,², Ben Vermaercke², Glenda M. Halliday³, Matthias Staufenbiel⁴, and Jürgen Götz¹,*

Supplementary Data

Supplementary Results

Gender effects in SHIRPA murine neurobehavioral screen and motor abilities tests. At any age tested, male mice, regardless of their genotype, weighed more than female mice (gender main effect at 2-3 months: $F_{1,19}=36.42, p<0.001$; 6-7 months: $F_{1,19}=34.33, p<0.001$; 10-11 months: $F_{1,19}=31.52, p<0.001$; gender x genotype interaction effects not significant). Moreover, 10-11 month-old Tg male mice had more bald patches and wounds than female mice and their Wt counterparts (missing fur: gender main effect: $F_{1,19}=20.83, p<0.001$; gender x genotype effect: $F_{1,19}=9.75, p=0.007$; wounds: gender main effect: $F_{1,19}=9.83, p=0.007$; gender x genotype interaction effect: $F_{1,19}=9.83, p=0.007$). Also, male Tg mice of 6-7 months of age displayed more tremors than their female and Wt counterparts (gender main effect: $F_{1,19}=7.33, p=0.02$; gender x genotype interaction effect: $F_{1,19}=7.33, p=0.02$).

Finally, we found higher values in the grip strength test for males than for females (grip mean: gender main effect at 2-3 months: $F_{1,19}=4.83, p=0.048$; gender x genotype interaction effects not significant - grip max: gender main effect at 6-7 months: $F_{1,19}=6.12, p=0.031$; gender x genotype interaction effects not significant).
Supplementary Figure 1

Behavioral setups. (A) Open field test, (B) social exploration test, (C) light/dark box, (D) Y-maze, (E) puzzle box dig trial, and (F) puzzle box plug trial.
Supplementary Table 1

Tasks used to compare Tau58-2/B mice to wild-type littermate controls

<table>
<thead>
<tr>
<th>Tasks</th>
<th>Affected regions</th>
<th>Related behavioral abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Touch escape</td>
<td>Sensory cortex</td>
<td>Tactile sensation</td>
</tr>
<tr>
<td>Trunk curl</td>
<td>Sensory cortex</td>
<td>Postural maintenance</td>
</tr>
<tr>
<td>Reaching reflex</td>
<td>Visual cortex</td>
<td>Visual acuity</td>
</tr>
<tr>
<td>Preygor reflex</td>
<td>Auditory cortex</td>
<td>Hearing acuity, Sensory gating</td>
</tr>
<tr>
<td>Toe pinch</td>
<td>Nociceptive system</td>
<td>Pain sensation</td>
</tr>
<tr>
<td>Grip strength test</td>
<td>Cerebellum, Brainstem, Spinal cord</td>
<td>Muscle strength</td>
</tr>
<tr>
<td>Wire hang test</td>
<td>Cerebellum, Brainstem, Spinal cord</td>
<td>Muscle strength</td>
</tr>
<tr>
<td>Rotarod tests</td>
<td>Cerebellum, Brainstem, Spinal cord</td>
<td>Balance, Coordination</td>
</tr>
<tr>
<td>Open field test</td>
<td>Cingulate cortex, Amygdala</td>
<td>Anxiety, Exploratory locomotion</td>
</tr>
<tr>
<td>Social exploration test</td>
<td>Cingulate cortex, Amygdala</td>
<td>Sociability</td>
</tr>
<tr>
<td>Dark light box test</td>
<td>Cingulate cortex, Amygdala</td>
<td>Anxiety, Risk-assessment, Impulsivity</td>
</tr>
<tr>
<td>Y-maze SAB</td>
<td>Hippocampus, Prefrontal cortex</td>
<td>Executive function</td>
</tr>
<tr>
<td>Puzzle box test</td>
<td>Hippocampus, Prefrontal cortex</td>
<td>Executive function, STM</td>
</tr>
</tbody>
</table>

Different tasks used to compare our colony of tauopathy mice to their age-matched W1 littermates; SAB=spontaneous alternation behaviour; STM=short-term memory.
Supplementary Table 2

SHIRPA Murine neurobehavioral screen (based on (Rogers et al., 1997))

Physical factors and gross appearance

- Body weight (g)
- Presence of whiskers
  - 0 = None
  - 1 = A few
  - 2 = Most, but not a full set
  - 3 = A full set
- Appearance of fur (not counting patches of missing fur)
  - 0 = Ungroomed and dishevelled
  - 1 = Somewhat dishevelled
  - 2 = Well-groomed (normal)
- Patches of missing fur on body
  - 0 = None
  - 1 = Some
  - 2 = Extensive
- Wounds
  - 0 = None
  - 1 = Signs of previous wounding
  - 2 = Slight wounds present
  - 3 = Moderate wounds present
  - 4 = Extensive wounds present

Observation of behavior in a novel environment (within a 3 min trial in a tub cage)

- Transfer behavior
  - 0 = Coma
  - 1 = Prolonged freeze (>10 sec.), then slight movement
  - 2 = Extended freeze, then moderate movement
  - 3 = Brief freeze (a few seconds), then active movement
  - 4 = Momentary freeze, then swift movement
  - 5 = No freeze, immediate movement
  - 6 = Extremely excited
- Spontaneous activity
  - 0 = None, resting
  - 1 = Casual scratch, groom, slow movement
  - 2 = Vigorous scratch, groom, moderate movement
  - 3 = Vigorous, rapid/dart movement
  - 4 = Extremely vigorous, rapid/dart movement
• Tremor
  o 0 = None
  o 1 = Mild
  o 2 = Marked

• Palpebral closure
  o 0 = Eyes wide open
  o 1 = Eyes ½ closed
  o 2 = Eyes closed

• Gait
  o 0 = Normal
  o 1 = Fluid but abnormal
  o 2 = Limited movement only
  o 3 = Incapacity

Reactions to simple stimuli and reflexes

• Touch escape (finger stroke from above, starting light and getting firmer)
  o 0 = No response
  o 1 = Mild (escape response to firm stroke)
  o 2 = Moderate (rapid response to light stroke)
  o 3 = Vigorous (escape response to approach)

• Trunk curl (grip tail and lift about 30 cm)
  o 0 = Absent
  o 1 = Present

• Reaching reflex (forelimbs extension when lowered by tail from 15 cm height)
  o 0 = None
  o 1 = Upon nose contact
  o 2 = Upon vibrasse contact
  o 3 = Before vibrasse contact
  o 4 = Early vigorous extension

• Preyer reflex (loud hand clap 30 cm above mouse, watch for pinna reflex)
  o 0 = None
  o 1 = Active retraction, moderate brisk head flick
  o 2 = Hyperactive, repetitive flick

• Toe pinch (apply gentle lateral compression on hind foot of a tail lifted mouse)
  o 0 = None
  o 1 = Slight withdrawal
  o 2 = Moderate withdrawal, not brisk
  o 3 = Brisk, rapid withdrawal
  o 4 = Very brisk repeated extension and flexion

Grip strength and motor coordination
Grip strength was measured using a T-shaped bar connected to a digital dynamometer (Ugo Basile, Comerio, Italy). Mice were placed in such a way that they grabbed the bar spontaneously and were softly pulled backwards by the tail until they released their grip. Ten such readouts were recorded; mean and max pulling force were noted for each mouse.

The wire hang test seeks to evaluate motor function. The animal is placed on a wire cage top, which is then inverted and suspended above the home cage for 30 seconds. Grip is scored as following:

- 0 = Active grip
- 1 = Difficulty to grasp, but hangs on
- 2 = Grasps, but falls of within 10 seconds
- 3 = Unable to grasp, falls within seconds
- 3 = Falls immediately

Motor coordination and equilibrium were tested using an accelerating Rotarod (Ugo Basile, Comerio, Italy). Mice were tested on four trials, during which the rod accelerated from 4 to 20 rpm in 5 minutes. Consecutive trials were separated by a 2-minute intertrial interval. Latency to falling off the rod was recorded up to 5 minutes; mean and max walking latencies were noted for each mouse.