ELSEVIER

Neurobiology of Disease

www.elsevier.com/locate/ynbdi
Neurobiology of Disease 15 (2004) 500–509

Accelerated extinction of conditioned taste aversion in P301L tau transgenic mice

Luis Pennanen,[a] Hans Welzl,[b] Patrizia D'Adamo,[b] Roger M. Nitsch,[a] and Jürgen Götz[a,*]

[a] *Division of Psychiatry Research, University of Zürich, 8008 Zürich, Switzerland*
[b] *Institute of Anatomy, University of Zürich, 8057 Zürich, Switzerland*

Received 1 July 2003; revised 29 September 2003; accepted 18 November 2003

Neurofibrillary tangles, insoluble protein deposits composed of filamentous tau aggregates, are neuropathological hallmarks of Alzheimer's disease and familial frontotemporal dementia (FTDP-17). Transgenic mice expressing the FTDP-17 mutation P301L of tau recapitulate key features of the human pathology, that is, tau proteins aggregate and neurofibrillary tangles begin to appear in the amygdala at 6 months of age. To detect early signs of tau aggregate-associated changes, we investigated behavioral alterations and cognitive deficits in such mice using an amygdala-specific test battery for anxiety-related and cognitive behavior. P301L mice had anxiety levels not different from wild-types, but their exploratory behavior was significantly increased. Acquisition of a fear response to tone and context as well as taste aversion was comparable to wild-types. However, extinction of a conditioned taste aversion was significantly accelerated. We conclude that already aggregation of tau proteins not yet accompanied by massive formation of neurofibrillary tangles causes selective behavioral deficits.

Keywords: Alzheimer's disease; Frontotemporal dementia; Tau; Transgenic mice; Conditioned taste aversion; Amygdala; Neurofibrillary tangles; Extinction

Introduction

Alzheimer's disease (AD) and frontotemporal dementia with Parkinsonism linked to chromosome 17 (FTDP-17) are common forms of age-related dementing diseases. Whereas AD is characterized by extracellular β-amyloid-containing plaques and intracellular neurofibrillary tangles (NFT), in neurodegenerative diseases such as FTDP-17, NFT form in the absence of amyloid plaques (Gotz, 2001; Lee et al., 2001). In cells affected in these tauopathies, the microtubule-associated protein tau is abnormally phosphorylated and relocalized from axonal to somatodendritic compartments where it accumulates in pretangle, filamentous aggregates that eventually assemble into NFT (Buee et al., 2000; Goedert et al., 1995). The discovery of mutations in the tau gene in FTDP-17 established that dysfunction of tau alone can cause neurodegeneration and lead to dementia (Hutton et al., 1998; Poorkaj et al., 1998; Spillantini et al., 1998).

Expression of FTDP-17 mutant tau in transgenic mice caused NFT formation both in neurons (Allen et al., 2002; Gotz et al., 2001a; Lewis et al., 2000; Tanemura et al., 2001; Tatebayashi et al., 2002) and in glial cells (Gotz et al., 2001b; Higuchi et al., 2002; Lin et al., 2003). Whereas extensive behavioral studies have been performed in β-amyloid-forming APP transgenic mice (Chapman et al., 1999; Chen et al., 2000; Dodart et al., 1999; Hsiao et al., 1996; Janus et al., 2000; Morgan et al., 2000; Routtenberg et al., 1997), less information is available for tau mutant mice (Tanemura et al., 2002; Tatebayashi et al., 2002). We investigated our P301L (FTDP-17) mutant mice in several amygdala-dependent tasks because tau aggregates mainly formed in the amygdala (Gotz et al., 2001c). This brain area is involved in mediating effects of emotion and stress on learning and memory as determined in fear conditioning and conditioned taste aversion (CTA) tests (LeDoux, 2000; Welzl et al., 2001). It plays a role in modulating consolidation processes which involve other brain areas (McGaugh et al., 2002). To correlate behavior with tau expression, we determined the distribution of P301L tau in more detail with special emphasis on brain areas shown to be involved in CTA.

We found that the P301L mice showed increased exploratory behavior but normal anxiety levels and no impairment in fear conditioning. CTA is a well-established learning and memory paradigm in which subjects learn to associate a novel taste with nausea and, as a consequence, avoid consumption of this specific taste at the next presentation. Acquisition and consolidation of CTA memory were not significantly affected by the transgene. However, transgenic mice extinguished the CTA more rapidly than wild-type mice. Together, our data show that tau aggregation, as found in particular in the basolateral and basomedial nucleus of the amygdala, has functional consequences for specific forms of learning and memory.

Materials and Methods

Animals

The transgenic mice used in this study express the human pathogenic mutation P301L of tau together with the longest

* Corresponding author. Division of Psychiatry Research, University of Zürich, August Forel Str. 1, 8008, Zürich, Switzerland. Fax: +41-1-634-8874.
E-mail address: goetz@bli.unizh.ch (J. Götz).
Available online on ScienceDirect (www.sciencedirect.com.)

0969-9961/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.nbd.2003.11.020

human brain tau isoform (htau40) under control of the neuron-specific mThy1.2 promoter. Pronuclear injections were done into C57Bl/6 × DBA/2 F2 oocytes to obtain founder animals that were back-crossed with C57Bl/6 mice to establish transgenic lines (Gotz et al., 2001a). Line pR5-183 expressed mutant human tau in many brain areas; however, NFT formation was mainly confined to the amygdala (Gotz et al., 2001c). Here, male wild-type (wt) and P301L mice were sequentially analyzed in two sets, which were balanced for the genotype and subjected to behavioral tests at the age of 6 months when NFT began to form. Data were pooled, as no statistically significant differences were found between sets. Group-housed mice were transferred to individual cages at 6 months of age when testing began. They were kept under an inverted 12 h light/dark cycle with a room temperature of 22°C. Food pellets and water were available ad libitum unless otherwise noted. Thirty minutes before each test session, the mice were transferred to the behavioral room.

Histology

Coronal 4-μm paraffin brain sections were immunohistologically stained as previously described (Gotz and Nitsch, 2001; Gotz et al., 2001a) and brain areas were mapped based on the mouse atlas by Paxinos (1997). Sections were dehydrated in an ascending series of ethanol, stained and flat-embedded between glass slides and coverslips in Eukitt (Kindler, Germany). For signal enhancement, sections were microwave-treated in citrate-buffer pH 5.8 at 70°C for 15 min. They were stained with the human tau-specific antibody HT7 (Innogenetics Inc, diluted 1:200) and the phosphorylation-dependant anti-tau antibody CP13 (Dr. Peter Davies, diluted 1:200) directed against phosphorylated S202/T205. For the peroxidase/DAB stainings, secondary antibodies were obtained from Vector Laboratories (Vectastain ABC kits PK-6101 and PK-6102).

Motor coordination on the Rotarod

The body weight was determined at 6 and again at 8 months of age, when the CTA test was performed. To test locomotor coordination, the accelerating Rotarod (Udo Basile, Milano, Italy) was used which consists of a rotating drum with a diameter of 3 cm covered with knurled Perspex to provide an adequate grip. The mice were first placed on the rod at the lowest speed of 4 rpm for 2 min. Only then the rod was switched to acceleration mode and the time on the rod was recorded for up to 5 min when the speed reached the maximum of 40 rpm. Mice were assessed daily in two trials on three consecutive days, with an intertrial interval of at least 3 h.

Open-field test

The open-field test analyzes spontaneous locomotor activity, exploratory behavior, and anomalies of locomotion patterns. Mice were placed at the border of a dimly lit (50 lx) circular arena (diameter of 150 cm) for 10 min followed by a second session on the following day. The arena was divided into an outer zone (within 7 cm of the wall), an inner zone (inner circle with a diameter of 110 cm), and an intermediate zone. Paths were tracked with an electronic imaging system (EthoVision 1.96, Noldus Information Technology, Wageningen, The Netherlands) at a frequency of 4.2 Hz and a spatial resolution of 256 × 256 pixels. Raw data were analyzed with the Wintrack 2.3 software (Wolfer et al., 2001). Locomotor activity was assessed by measuring the total distance traveled and the total number of zone transitions. Thigmotaxis, that is, moving along the wall, was quantified by measuring the time spent in the outer zone. To determine measures of anxiety and exploratory behavior, the following parameters were assessed: number of visits to the inner zone, average distance to inner zone, and the number of activity state changes. Three activity states were distinguished: progression (periods with a locomotion speed above the progression threshold of 0.085 m/s and a minimal distance moved exceeding 0.05 m), resting (periods lasting at least 2 s with a speed below 0.025 m/s) and scanning (periods meeting neither resting nor progression criteria).

Light–dark (L–D) test

Unconditioned anxiety-like behavior was tested in the light–dark (L–D) test. The mice were placed into the 30 × 20 cm area of a Perspex L–D box that was illuminated with an average of 700 lx. A dark compartment of 15 × 20 cm was attached opposite to the releasing site with an opening facing the center of the illuminated part. Movements were tracked in the illuminated part over a 5-min trial as described above. Anxiety was measured by the latency to enter the dark compartment and the time spent in it. Exploration was defined by the number of rearings in the illuminated part, and the percentage of visited tiles/total of 36 tiles (5 × 3.3 cm) entered with all four paws, defined as exploration index.

Fear conditioning test

The conditioning chamber consisted of a gray opaque box (16.5 × 25 cm) with a grid floor through which shocks could be delivered (0.15 mA) as the unconditioned stimulus (US). The chamber was placed into a dimly lit sound-attenuating box with a speaker on top delivering a 92 dB/2000 Hz tone as the conditioned stimulus (CS). Two measures were used to quantify freezing as the conditioned reaction: a grid of photobeams (1 × 1 cm) detected the inactivity of the mice, which was defined as no photobeam breaks for at least 2 s. Freezing was also detected manually while observing the animal and was defined as no movement except for respiration (as defined and applied by the experimenter). The two measures correlated well with each other. However, we finally used the manually detected measurement due to technical problems with the photobeams during cue testing: sawdust was used in the new environment and unfortunately, from time to time, the photobeams were concealed, which caused a higher, inappropriate measurement of inactivity. During the conditioning session, a 1-min adaptation period in the box was followed by three identical conditioning trials, each trial consisting of 30-s CS presentation, with the US being applied during the last 2 s of the CS, separated by an intertrial interval of 30 s.

Retrieval tests for context conditioning and, 2 h later, for conditioning to tone were carried out 24 h and 15 days after the conditioning session by recording freezing as mentioned above. To evaluate context conditioning, mice were placed for 2 min in the conditioning chamber. To evaluate conditioning to tone, the physical characteristics of the chamber were changed (shape, light, smell, and bedding material). Each mouse was then placed for 2 min into this new chamber, and the CS was presented throughout the second minute.

Conditioned taste aversion test (CTA)

For the first 4 days of the experiment, water-deprived mice were adapted to obtain water only during two daily drinking sessions, a morning session lasting 20 min and an afternoon session lasting 10 min, with a 4-h intertrial interval. Water was presented in two 15-ml bottles that were weighed before and after the test to measure fluid intake. During the morning session on the conditioning day, mice were only allowed to drink a 0.5% saccharin solution (CS) (saccharin sodium salt hydrate, Fluka Chemie, Buchs, Switzerland) from one bottle. 40 min later, the mice were injected intraperitoneally with a 0.14 M LiCl solution (at 2% of body weight) as the nausea-inducing agent (US). Two days after conditioning, during the 20-min morning session, all mice could drink either tap water from one bottle or a 0.5% saccharin solution from another bottle (two-bottle choice test). The percentage of saccharin consumption per total fluid intake was calculated. Extinction was determined by repeating the choice tests 3 days after conditioning and on 3 consecutive days, beginning 1 week after conditioning. The mice of the second set were analyzed in three additional choice tests starting 5 weeks following conditioning.

To determine whether P301L mice were able to discriminate basic taste qualities, a separate group of water-deprived naive mice was adapted to the drinking schedule as described above. Then, they were tested for their natural preference for a 0.5% saccharin solution in a choice test with the second bottle filled with tap water. Finally, to determine the natural aversion towards a bitter taste, a choice test between a 0.02% quinine solution (quinine hydrochloride dihydrate, Fluka, Buchs, Switzerland) and water was carried out.

Data analysis

For statistical analysis, the SPSS software was used. Between-group comparisons were analyzed using a one-way analysis of variance (ANOVA) or a two-way ANOVA with repeated measures. Significant effects were analyzed post hoc using Fisher's PLSD (protected least significant difference) for pairwise comparison. All data are represented as mean ± SEM with a statistical significance given at $P < 0.05$.

Results

Expression pattern of P301L tau

To determine the expression pattern of human P301L tau in more detail, with special emphasis on brain areas involved in the CTA task, we analyzed frontal sections of four P301L tau expressing mice by immunohistochemistry using the human tau-specific antibody HT7 and the phosphorylation-dependant anti-tau antibody CP13 (Fig. 1). We found expression of human tau in the motor, somatosensory and insular cortex (IC) and in the claustrum at position AP +1.1 mm. At AP −0.82, tau was present in cortical motor and somatosensory neurons, and to a variable degree in the posterior part of the agranular insular cortex (AIP), in the anterior cortical amygdaloid nucleus (ACo) and different regions of the thalamus. At position AP −1.34, expression was found in the cortex, in the basolateral (BLA) and the basomedial nucleus of the amygdala (BM), in the dorsal endopiriform nucleus (DEn) and in

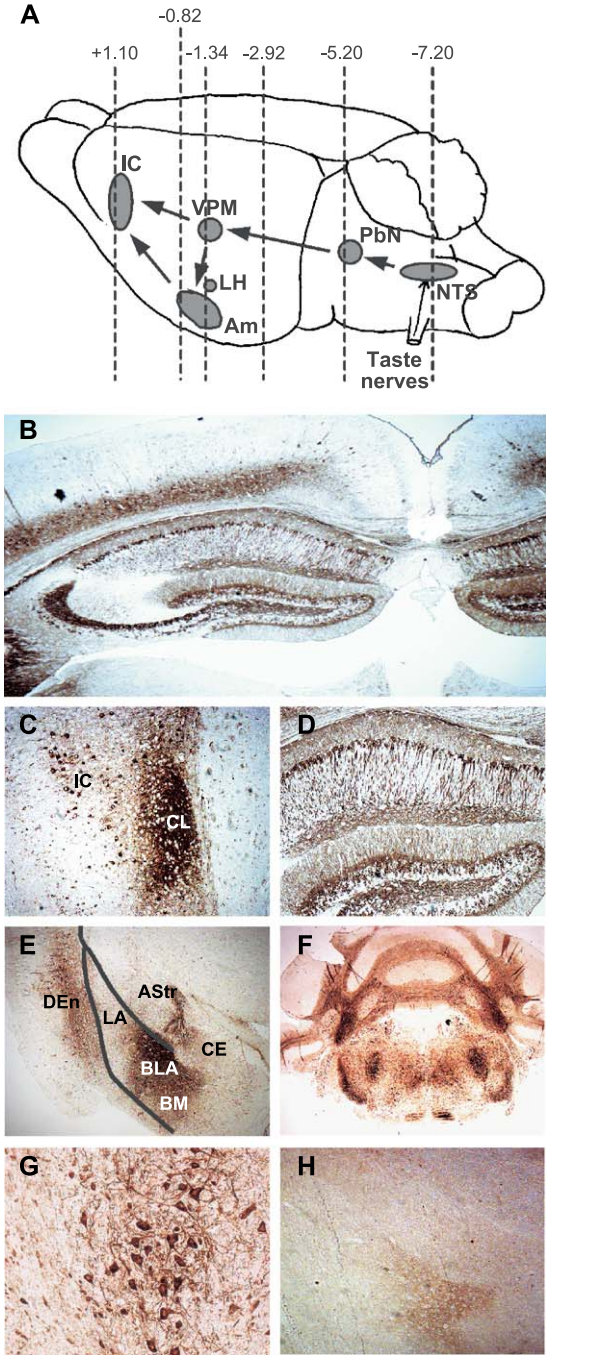

Fig. 1. Expression pattern of tau in P301L tau transgenic mice. (A) Areas involved in CTA are shown in gray: IC, insular cortex; VPM, ventral posteromedial nucleus of the thalamus; LH, lateral hypothalamus; Am, amygdala; PbN, parabrachial nucleus and NTS, nucleus of the solitary tract. (B) Tau was expressed in the cortex, hippocampus and adjacent brain areas. (C) Higher magnification of tau expression in the IC and CL (claustrum), (D) the hippocampus, (E) the DEn (dorsal endopiriform nucleus), the BLA (basolateral nucleus), and BM (basomedial nucleus) of the amygdala, the Astr (amygdalostriatal transition area), but not in the lateral nucleus (LA) and the central nucleus of the amygdala (CE). (F) Tau was expressed in the brain stem. (G) Higher magnification of (F) shows high tau expression in motor neurons of the brain stem. (H) The amygdala of a wild-type mouse is shown as a negative control. The sections were stained with the human tau-specific antibody HT7 (B, D, F, G, H) and the phospho-tau specific antibody CP13 (C, E).

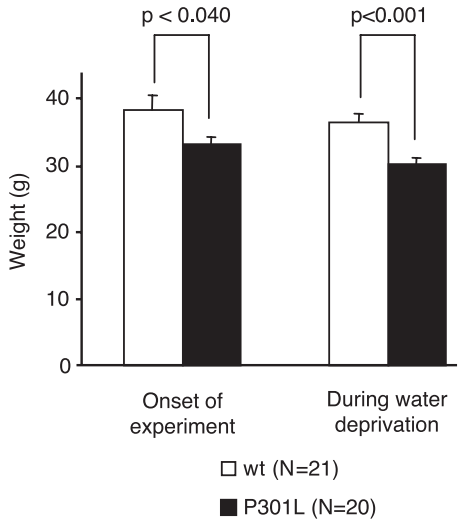

Fig. 2. Weight reduction of P301L mice. The weight of the P301L mice was significantly reduced at the onset of the experiment ($P < 0.04$) and, more pronounced, during the CTA task ($P < 0.001$). The values represent the mean ± SEM.

the ACo. No tau expression was found in the lateral nucleus (LA) and the central nucleus of the amygdala (CE). HT7 staining revealed also a strong expression of human tau in the hippocampus (CA1, CA3, and dentate gyrus), whereas staining of these brain areas with the CP13 antibody was very weak. However, both antibodies strongly stained the posterior part of these hippocampal regions at position AP −2.92 (where human tau was also expressed in cortical neurons), the posterior part of the basolateral amygdala, and to a variable degree, neurons in the red nucleus (parvocellular part, RPC), the ventral tegmental area (VTA) and the posteromedial cortical amygdaloid nucleus (PMCo). At AP −4.60, tau was present in cortical neurons, especially in the lateral entorhinal cortex and the external cortex of the inferior colliculus (ECIC) and to a variable degree in the oral pontine reticular nucleus (PnO), the rostral periolivary region (RPO) and the reticulotegmental nucleus pons (RtTG). Particularly high levels of expression were found in the brain stem (AP −7.20), predominantly in the inferior olive (IO), the ambiguus nucleus (Amb), and in various parts of the reticular formation and medulla. In contrast to the tau expression in the BLA, IC, and some thalamic nuclei, no expression was found in CTA-relevant areas such as the ventral posteromedial nucleus of the thalamus (VPM), the parabrachial nucleus (PBN), and the nucleus of the solitary tract (NTS).

Weight reduction and motor coordination of P301L tau transgenic mice

At the beginning of experiments, body weights of wild-types (38.2 ± 2.2 g) were significantly higher than that of P301L tau transgenic mice (33.0 ± 1.2 g; $P < 0.04$) (wt: $N = 21$; P301L: $N = 20$). This difference was even more pronounced during the CTA task (wt: 36.4 ± 1.2 g; P301L: 30.2 ± 0.8 g; $P < 0.001$) (Fig. 2). To test whether expression of the transgene affected locomotor coordination, the accelerating Rotarod test was performed ($N = 11$/group). P301L mice stayed significantly longer on the Rotarod than wt mice (wt: 124.3 ± 14.2 s; P301L: 178.2 ± 17.5 s; $F(1,20) = 5.316$, $P < 0.032$) and therefore reached a significantly higher average speed (wt: 18.7 ± 2.0 rpm; P301L: 25.3 ± 2.3 rpm; $F(1,20) = 4.647$, $P < 0.043$) before slipping off the rod. However, when the data were normalized for weight (by calculating a weight coefficient), the differences between the two groups disappeared ($F(1,20) = 0.364$, $P > 0.553$), demonstrating a correlation between weight and performance on the Rotarod.

Slightly increased exploration of P301L mice in the open-field and light–dark test

Levels of spontaneous locomotor activity, anxiety-like behavior and exploration were assessed in the open-field and light–dark (L–D) tests. In the open-field test ($N = 21$/group), no genotype

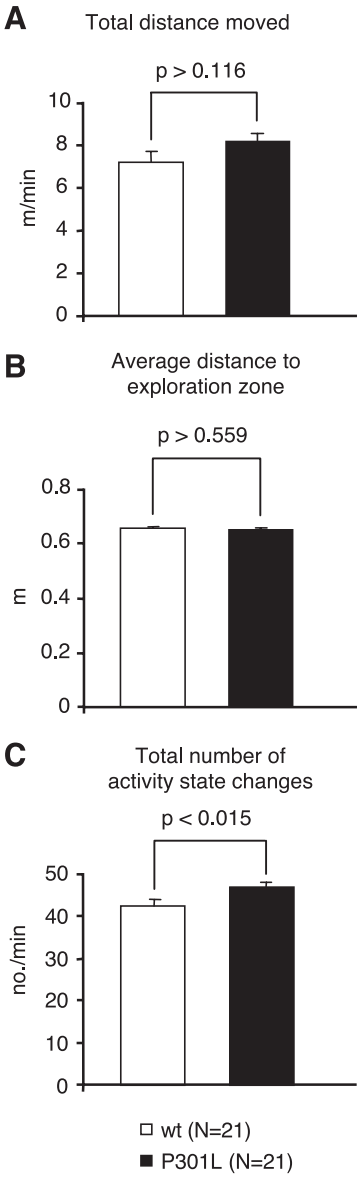

Fig. 3. No altered activity levels of P301L mice in the open-field test but slightly increased exploratory behavior. (A) Total distance moved; (B) average distance to exploration zone; and (C) total number of activity state changes. Values represent the mean ± SEM.

effect was found for measures of locomotor activity [total distance traveled: $F(1,40) = 2.587$, $P > 0.116$; total number of zone transitions: $F(1,40) = 0.003$, $P > 0.956$]. Similarly, no differences were found for measures of anxiety such as thigmotaxis ($F(1,40) = 0.119$, $P > 0.732$), the time spent in the inner zone ($F(1,40) = 0.473$, $P > 0.496$), and the average distance to the inner zone ($F(1,40) = 0.347$, $P > 0.559$). Only for the total number of activity state changes a significant genotype effect was detected ($F(1,40) = 6.469$, $P < 0.015$) (Fig. 3).

No significant differences were found in the L–D test (wt: $N = 20$; P301L: $N = 21$) for measures of anxiety such as the latency to enter the dark compartment ($F(1,39) = 0.082$, $P > 0.776$) and the time spent in the dark compartment ($F(1,39) = 0.040$, $P > 0.843$). In contrast, additional parameters for exploration such as the number of rearings ($F(1,39) = 4.624$, $P < 0.038$), and the exploration index ($F(1,39) = 4.078$, $P < 0.050$) were significantly increased in P301L tau transgenic mice (Fig. 4).

No altered fear conditioning in P301L mice

To test the ability of P301L mice to acquire a conditioned fear response, freezing was recorded during the conditioning session as well as the retrieval sessions for conditioning to context or tone. During conditioning, freezing increased significantly in both groups after the first US has been delivered ($F(7,273) = 38.022$, $P < 0.001$; wt: $N = 21$; P301L: $N = 20$; Fig 5A). Neither genotype nor genotype × interval significantly affected freezing during conditioning (genotype: $F(1,39) = 2.857$, $P > 0.099$; genotype × interval: $F(7,273) = 1.436$, $P > 0.191$), although freezing levels of transgenic mice consistently were below that of wild-type mice (Fig. 5A).

During the retrieval test for context conditioning, no significant differences between transgenic and wild-type mice could be found 24 h ($F(1,39) = 0.344$, $P > 0.561$) (wt: $N = 21$; P301L: $N = 20$) as well as 15 days ($F(1,19) = 0.242$, $P > 0.629$) (wt: $N = 11$; P301L: $N = 10$) post-conditioning (Fig. 5B). No significant genotype

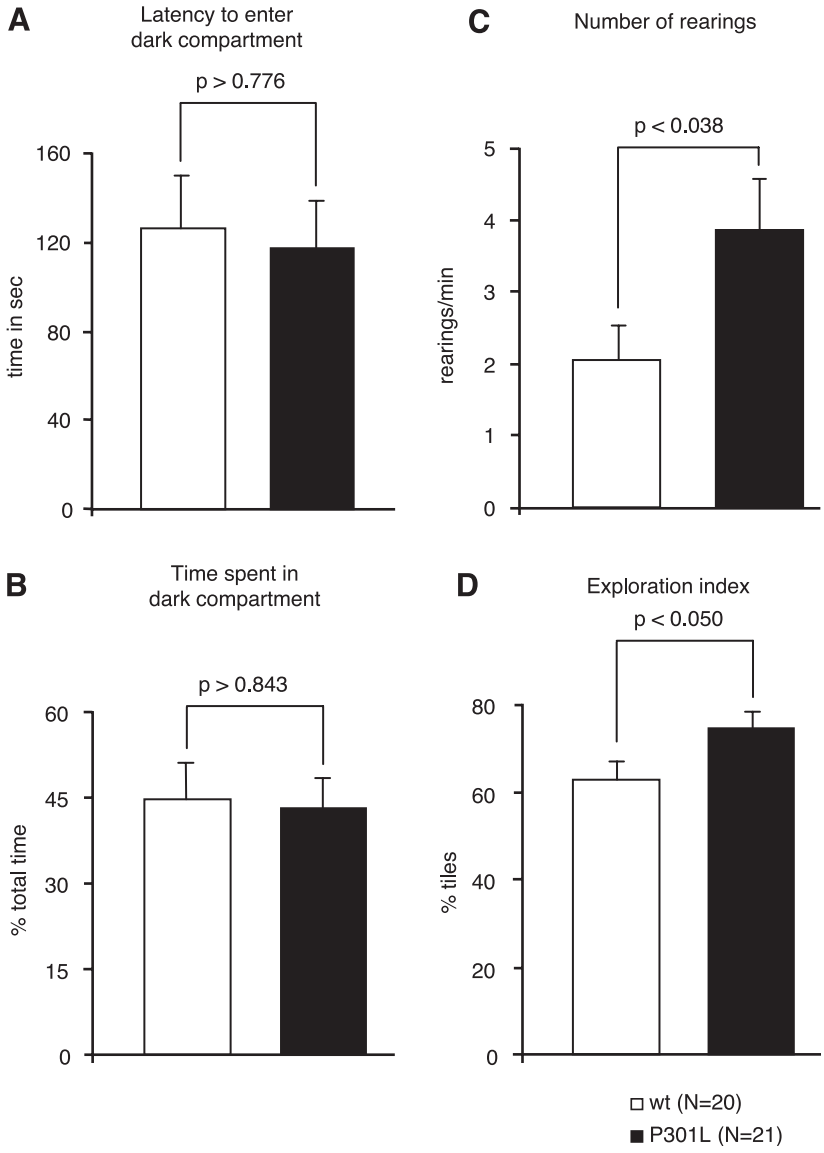

Fig. 4. No altered anxiety levels of P301L mice in the light–dark test but slightly increased exploratory behavior. (A) Latency to enter the dark compartment; (B) time spent in the dark compartment; (C) number of rearings; and (D) exploration index. Values represent the mean ± SEM.

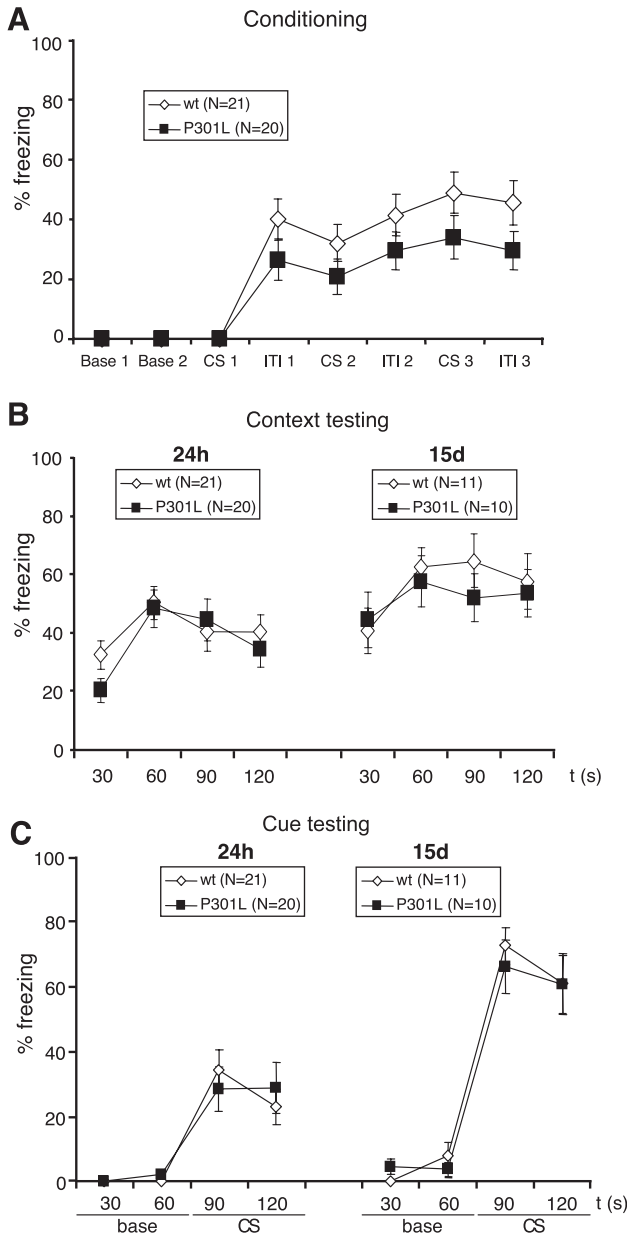

Fig. 5. No altered fear conditioning in P301L mice. (A) Genotype did not significantly affect freezing during conditioning ($P > 0.099$). Base, baseline activity; CS, tone presentation; ITI, inter-trial interval. (B) In the context test, no significant differences between genotypes were detected, neither at 24 h ($P > 0.561$) nor at 15 days ($P > 0.629$) post-conditioning. (C) Conditioning to tone also revealed no significant differences between genotypes, neither at 24 h ($P > 0.911$) nor at 15 days ($P > 0.798$) post-conditioning. The values represent the mean ± SEM.

effect was observed in freezing during the retrieval test for conditioning to tone, neither at 24 h ($F(1,39) = 0.013$, $P > 0.911$) nor at 15 days ($F(1,19) = 0.068$, $P > 0.798$) post-conditioning. Freezing significantly increased in response to tone presentation (second minute of the test) in both groups (24 h: $F(3,117) = 34.287$, $P < 0.001$, 15 days: $F(3,57) = 112.458$, $P < 0.001$) demonstrating that wild-type as well as P301L mice learned to associate the tone with the US. Tone presentation elicited a similar increase of freezing in both groups (genotype × interval interaction: 24 h: $F(3,117) = 0.776$, $P > 0.510$, 15 days: $F(3,57) = 0.512$, $P > 0.676$) (Fig. 5C).

Enhanced extinction of CTA in P301L mice

To test the ability to develop a taste aversion, P301L mice and wild-type littermate controls ($N = 19$/group) were exposed to the novel taste saccharin (CS) followed by a single injection of LiCl (US). A two-way ANOVA with repeated measures over the choice tests conducted 48, 72 h and 1 week after conditioning showed a significant main effect of genotype ($F(1,36) = 8.167$, $P < 0.007$), choice test ($F(4,144) = 8.352$, $P < 0.001$), and genotype × choice test interaction ($F(4,144) = 3.266$, $P < 0.013$) (Fig. 6A). Post hoc

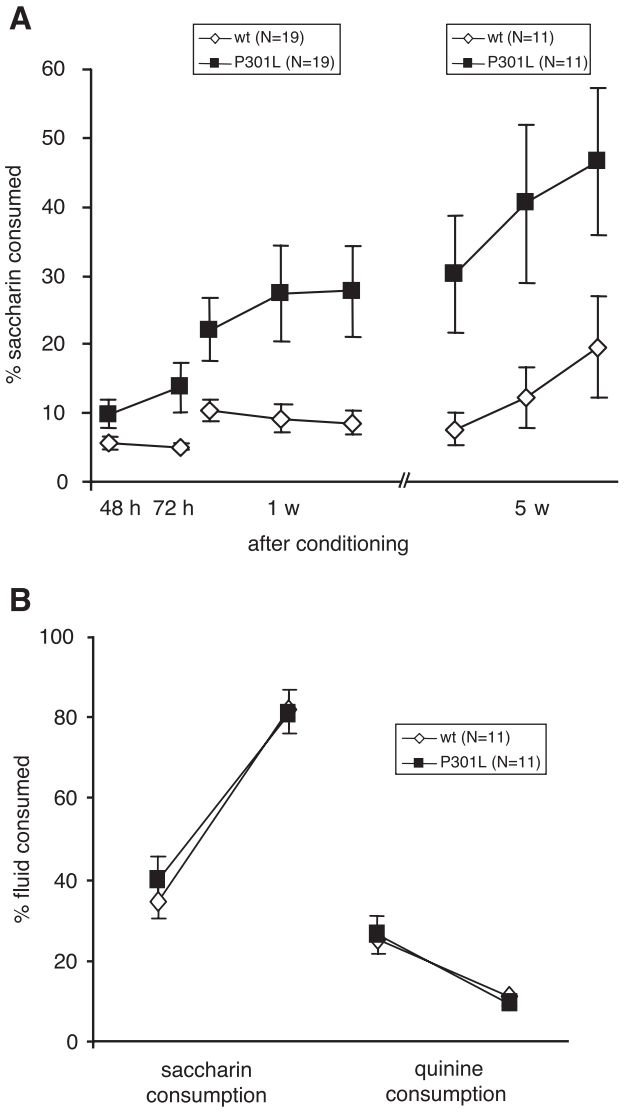

Fig. 6. Accelerated extinction of CTA in P301L mice. (A) During the first choice test 48 h after conditioning no significant genotype effect was observed ($P > 0.094$). However, during all subsequent choice tests, 72 h and 1 week after conditioning, P301L mice consumed significantly more saccharin ($N = 19$/group). Additionally, a subset of mice ($N = 11$/group) was again tested 5 weeks after conditioning. P301L mice continued to show a significantly accelerated extinction. (B) Basic taste qualities in naive mice were not impaired by the tau pathology. Values represent the mean ± SEM.

pairwise comparisons revealed that during the first choice test 48 h after conditioning both groups developed a strong taste aversion for saccharin (percentage of saccharin consumed: wt: 5.6 ± 1.0%; P301L: 9.8 ± 2.1%; $P > 0.094$), indicating that acquisition and consolidation of a taste aversion was not significantly impaired by the tau pathology. However, during the second choice test, P301L mice began to consume significantly more saccharin than wild-types ($P < 0.017$), demonstrating a faster extinction of the taste aversion. Repetition of these choice tests on 3 consecutive days starting 1 week after conditioning showed that CTA memory in wild-type mice extinguished slowly, whereas extinction was significantly faster in transgenic mice. A subset of mice ($N = 11$/group) was again exposed 5 weeks after conditioning to three such choice tests. P301L mice still continued to show a significantly accelerated extinction (main effect of genotype: $F(1,20) = 5.404$, $P < 0.031$) (Fig. 6A). Neither the amount of water intake during the last adaptation day before conditioning (wt: 1.34 ± 0.07 g; P301L: 1.41 ± 0.05 g; $F(1,36) = 0.592$, $P > 0.447$), nor the amount of saccharin consumed on the conditioning day itself (wt: 1.27 ± 0.11 g; P301L: 1.35 ± 0.06 g; $F(1,36) = 0.339$, $P > 0.564$) revealed a significant effect of the transgene. In addition, total liquid intake (water plus saccharin) of P301L mice did not differ significantly from wild-type controls at any time point during the choice tests ($F(1,36) = 0.094$, $P > 0.761$).

Basic taste qualities were assessed in water-deprived naive mice ($N = 11$/group) by presenting saccharin and quinine solutions. This did not reveal any significant differences between transgenic and control mice (for saccharin: $F(1,20) = 0.147$, $P > 0.706$; and for quinine: $F(1,20) = 0.002$, $P > 0.968$), indicating that P301L mice possess a normal taste sensitivity (Fig. 6B).

Discussion

Our immunohistochemical and behavioral analysis of P301L tau transgenic mice revealed a widespread aggregation of tau in the forebrain that is accompanied by selective changes in behavior. Behavioral changes include a small increase in exploratory behavior and an accelerated extinction of an aversion against a taste that has been previously paired with nausea. No changes, with respect to wild-types, were found in locomotor activity, fear conditioning, taste neophobia, and unconditioned natural taste preference for a sweet solution and natural taste aversion against a bitter solution. Extending previously published data, we found tau aggregation in the forebrain in nuclei of the amygdalar complex, the hippocampus and all areas of the neocortex investigated (sensory, motor, and associative areas). Aggregates were also present in several brain stem areas including the red nucleus, ventral tegmental area, and parts of the reticular formation, inferior olive, and ambiguus nucleus. At 6 months of age, NFT began to form in a subset of amygdaloid neurons, possibly reflecting high relative levels of tau expression and/or a selective vulnerability of distinct amygdaloid nuclei (Gotz and Nitsch, 2001; Gotz et al., 2001c).

Our study also revealed that body weights of P301L mice were slightly lower than that of wild-types already at the beginning of behavioral testing. This weight difference was even larger during the final (CTA) task of the test battery. Such a weight loss could be due to disturbances in several different brain sites. In light of the widespread appearance of tau aggregates in the brain, it would be difficult to pinpoint the structure(s) responsible for the weight loss. However, a defect that very unlikely contributes to the observed weight loss is diminished amygdalar function since damage to amygdaloid nuclei has been reported to cause either no change or just the opposite, that is, weight gain in rats (Rollins et al., 2001).

In the open-field, P301L and wild-type mice moved equal distances, but P301L mice changed their activity state somewhat more often from progression to resting to scanning. Furthermore, in the light–dark box they were more active and clearly increased the frequency of rearing, all of which are signs for greater exploratory behavior. In both the open-field and light–dark test, measures of anxiety were unchanged. Several brain structures have been found to be involved in exploratory behavior including the hippocampus and amygdala. That the amygdala might be affected in our transgenic mice is suggested by the observation that amygdala lesions increase exploratory behavior (Kelley et al., 1989). Dysfunction of the amygdala, however, would not affect unconditioned anxiety as measured with an anxiety test (elevated plus-maze) in rats (Treit and Menard, 1997).

Expression of the transgene and formation of tau aggregates in the forebrain did not impair fear conditioning to tone or context. When tested 15 days after conditioning, wild-types as well as P301L mice also showed no signs of extinction and froze even more in response to the conditioned stimuli (context, auditory cue). The non-significant tendency of reduced freezing of P301L mice only during conditioning remains puzzling, and to our knowledge, no treatment with a similar selective effect has been described. Anyhow, it is difficult to imagine how emotional or cognitive changes could affect freezing during conditioning but not during retrieval.

That P301L mice had no deficits in fear conditioning to a tone is in agreement with the expression pattern of tau aggregates in their brains. Conditioning to an auditory CS is dependent on functionally intact lateral and central nuclei of the amygdala (LeDoux, 2000), sites that were free of tau aggregates in our transgenic mice. The involvement of the basolateral amygdala (BLA), a site with tau aggregates in P301L mice, is less well established (but see also Goosens and Maren, 2001). In contrast to fear conditioning to a tone, successful context fear conditioning depends on an intact hippocampus which projects to the BLA (LeDoux, 2000). However, despite a prominent expression of P301L tau in the hippocampus and the BLA of P301L mice, no deficits were found in context fear conditioning compared with control mice. This may be due to the design of the fear conditioning task that was probably not sensitive enough to reveal differences between the two groups. Similar to our P301L mice, aged APP^{Swe} mutant mice also showed no deficits in fear conditioning to a tone or a context when compared to corresponding wild-types (Corcoran et al., 2002). These APP^{Swe} mice had β-amyloid plaques in the hippocampus as well as the amygdala and a reduction in function might have been expected. Only when the salience of the context CS was reduced, an indication of impairment in APP^{Swe} mice appeared. The general lack of massive neurodegeneration in animal models of AD may explain the largely normal performance of both P301L and APP^{Swe} mice in fear conditioning, which is in contrast to the reported impaired fear conditioning in AD patients (Hamann et al., 2002). This demonstrates the importance to design highly sensitive protocols for the measurement of different behavioral tasks.

P301L mice acquired a taste aversion indistinguishable from that of wild-types; that is, when given a choice to drink either a saccharin solution or water 2 days after pairing saccharin drinking with nausea, both genotypes greatly preferred water and avoided

the saccharin solution. However, repeated exposure to such a choice situation attenuated the aversion, and this extinction was drastically accelerated in P301L mice compared to wild-types. The possibility has to be considered that a stronger conditioning in wild-type mice might not have shown due to a flooring effect, that is, wild-type mice performed already close to an optimum with no further capacity of improvement.

The CTA deficits in P301L mice cannot be explained by a reduced neophobia, an increased sweet preference or a reduced aversion for unpleasantly tasting solutions. With respect to these three traits, no genotype differences could be found. P301L, as well as wild-type mice reduced their fluid intake, when first exposed to the saccharin solution (neophobia) developed a strong preference for the sweet solution when drinking was not followed by nausea, and avoided a bitter-tasting quinine solution to the same degree. Further, despite their slightly reduced body weight, P301L mice did not consume less saccharin solution on the conditioning day, that is, exposure to the conditioned stimulus was comparable in both groups.

It has been suggested that lesions of the BLA have a general effect on the response to novel stimuli (such as food) by decreasing neophobia (Dunn and Everitt, 1988). However, in our study, no attenuation of a taste neophobia was found. Thus, the observed formation of tau aggregates in the amygdala in P301L mice very unlikely caused a severe functional impairment of this structure.

Tau was expressed in brain areas which have been shown to be involved in CTA including the BLA, the insular cortex, and some thalamic nuclei (Welzl et al., 2001). No expression was found in other CTA-relevant areas such as the ventral posteromedial nucleus of the thalamus (VPM), the parabrachial nucleus (PBN), and the nucleus of the solitary tract (NTS).

Little is known about what might accelerate extinction of CTA. Several studies implicated hormonal systems, neurotransmitter systems, or specific brain structures in CTA extinction (for review, see Bures et al., 1998); but in general, these studies described retarded, but not accelerated, extinction upon treatments. For example, whereas hippocampal lesions affected acquisition of CTA only mildly or not at all (Best and Orr, 1973; Yamamoto et al., 1995), they slowed extinction of an already conditioned aversion (Kimble et al., 1979). In one study, excitotoxic lesions of the VPM had little effect on the acquisition of CTA but markedly accelerated its extinction similar to what we observed in our transgenic mice (Yamamoto et al., 1995). However, tau is not expressed in the VPM of P301L mice.

Numerous studies support a critical involvement of the amygdala in CTA (Aja et al., 2000; Lamprecht et al., 1997; Yamamoto et al., 1995). Similar to its effect on other aversive memories, the amygdala could modulate consolidation processes (e.g., of CTA) in other brain areas via its projections to these areas (McGaugh et al., 2002). Such a modulation could be achieved by pathways from the amygdala to the insular cortex, a structure critical for storage of a CTA. Stimulation in the BLA induced LTP in the insular cortex which enhanced retention of CTA memory in subsequent extinction trials (Escobar and Bermudez-Rattoni, 2000). Overexpression of P301L tau in the BLA could impair this modulatory effect resulting in accelerated extinction, either directly or by weakening the strength of the memory trace.

Interestingly, a recent study showed that the BLA is essential for extinction of CTA memory whereas acquisition is dependent on an intact central nucleus (Bahar et al., 2003). The differential effect of tau aggregates on fear conditioning and CTA fits well with their distribution pattern in the amygdala. We observed the aggregates in the basolateral but not the lateral and central nucleus. In contrast to CTA, fear conditioning to a tone is not dependent on an intact BLA, but an intact lateral and central nucleus (for review, see Bures et al., 1998; LeDoux, 2000). It also seems noteworthy to mention that, again, APP^{Swe} mice resemble to some degree P301L mice. APP^{Swe} mice expressed amyloid plaques in the amygdala and were drastically impaired in the acquisition of a CTA and, therefore, also in the extinction of this task (Janus et al., 2002).

AD patients exhibit an impairment at all levels of gustatory information processing, in line with the notion of a dissociation between preservation of olfactory and gustatory thresholds and an alteration in odor identification in patients with mild stage AD, suggesting that the alteration is central rather than peripheral (Broggio et al., 2001). Significant losses in the ability to detect the taste of glutamic acid and to recognize odorants were found in demented AD and non-AD patients when compared with age-matched controls (Schiffman et al., 1990). These findings are consistent with our transgenic model as the P301L mice share features of AD and FTD.

A range of behavioral tasks that did not include a test for CTA have been performed with other transgenic mouse strains expressing a mutated form of tau. PrP promoter-driven P301L tau transgenic mice strongly overexpress mutant tau in the brain and in motor neurons of the spinal cord. They develop a progressive motor phenotype commonly not observed in AD (Lewis et al., 2000). V337M tau mutant mice, on the other hand, express mutant tau only in the hippocampus, which is in contrast to our mice that develop a more widespread tau pathology similar to the human tau pathology. V337M mice show an increased locomotor activity and memory deficits in the elevated plus maze, increased spontaneous locomotion in the open-field, but no significant impairments in the Morris water maze (Tanemura et al., 2002). R406W tau mutant mice express tau at highest levels in the hippocampus and to a lesser extent in other cortical and subcortical brain areas. However, in the amygdala, only few cells strongly expressed mutant tau, even in 16- to 23-month-old animals (Tatebayashi et al., 2002). Not unexpectedly, the form of mutant tau and the type of promotor controlling its expression in transgenic mice determines the expression pattern of tau pathology and, as a consequence, results in very different behavioral phenotypes. The distribution of mutant tau in P301L mice investigated in the present study is widespread and comes close to the pattern of tau pathology observed in patients. These mice also lack the motor disturbances observed in other tau mutants, disturbances that are not characteristic of AD. P301L mice share, however, some characteristics with behavioral disturbances observed in APP mutant mice. Furthermore, disturbances can be detected already when mice are 6 months old. Thus, we think that P301L mice are a good model to investigate the contribution of tau pathology, as observed in AD and FTDP-17, to behavioral disturbances.

In summary, although this investigation raised several questions that have to be addressed in future studies, several conclusions on the early effects of tau aggregation in transgenic P301L mutant tau mice can be drawn. Firstly, in the open-field and light–dark box, subtle signs for increased exploratory behavior were manifest in P301L mice. Other behaviors indicative of general activity or anxiety, however, were unaffected by the transgene. Secondly, fear conditioning to tone or to context remained unaffected, probably due to the specific distribution of tau aggregates in the amygdala, and/or the design for this task that was probably not sensitive

enough to detect differences. Thirdly, a selective alteration in the extinction of a taste aversion could be seen in P301L mice. This, again, resembles data collected in APP^{Swe} mice submitted to a similar paradigm. Thus, CTA suggests itself as a sensitive measure of altered brain function in response to the formation of tau aggregates. One possible common factor for all these results could be a dysfunction of specific nuclei of the amygdala.

Acknowledgments

The authors thank Eva Moritz for help with immunohistochemistry, Dr. David Wolfer for data analysis, and Dr. Peter Davies for antibody CP13. This research was supported in parts by grants from the SNF, the ZNZ (Neuroscience Center Zurich), the Hartmann Müller Fund, the Olga Mayenfisch Foundation and by the NCCR "Neuronal plasticity and repair".

References

Aja, S., Sisouvong, S., Barrett, J.A., Gietzen, D.W., 2000. Basolateral and central amygdaloid lesions leave aversion to dietary amino acid imbalance intact. Physiol. Behav. 71, 533–541.

Allen, B., Ingram, E., Takao, M., Smith, M.J., Jakes, R., Virdee, K., Yoshida, H., Holzer, M., Craxton, M., Emson, P.C., Atzori, C., Migheli, A., Crowther, R.A., Ghetti, B., Spillantini, M.G., Goedert, M., 2002. Abundant tau filaments and nonapoptotic neurodegeneration in transgenic mice expressing human P301S tau protein. J. Neurosci. 22, 9340–9351.

Bahar, A., Samuel, A., Hazvi, S., Dudai, Y., 2003. The amygdalar circuit that acquires taste aversion memory differs from the circuit that extinguishes it. Eur. J. Neurosci. 17, 1527–1530.

Best, P.J., Orr Jr., J., 1973. Effects of hippocampal lesions on passive avoidance and taste aversion conditioning. Physiol. Behav. 10, 193–196.

Broggio, E., Pluchon, C., Ingrand, P., Gil, R., 2001. Taste impairment in Alzheimer's disease. Rev. Neurol. (Paris) 157, 409–413.

Buee, L., Bussiere, T., Buee-Scherrer, V., Delacourte, A., Hof, P.R., 2000. Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. Brain Res. Brain Res. Rev. 33, 95–130.

Bures, J., Bermudez-Rattoni, F., Yamamoto, T., 1998. Conditioned Taste Aversion—Memory of a Special Kind. Oxford Univ. Press, New York.

Chapman, P.F., White, G.L., Jones, M.W., Cooper-Blacketer, D., Marshall, V.J., Irizarry, M., Younkin, L., Good, M.A., Bliss, T.V., Hyman, B.T., Younkin, S.G., Hsiao, K.K., 1999. Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. Nat. Neurosci. 2, 271–276.

Chen, G., Chen, K.S., Knox, J., Inglis, J., Bernard, A., Martin, S.J., Justice, A., McConlogue, L., Games, D., Freedman, S.B., Morris, R.G., 2000. A learning deficit related to age and beta-amyloid plaques in a mouse model of Alzheimer's disease. Nature 408, 975–979.

Corcoran, K.A., Lu, Y., Turner, R.S., Maren, S., 2002. Overexpression of hAPPswe impairs rewarded alternation and contextual fear conditioning in a transgenic mouse model of Alzheimer's disease. Learn. Mem. 9, 243–252.

Dodart, J.C., Meziane, H., Mathis, C., Bales, K.R., Paul, S.M., Ungerer, A., 1999. Behavioral disturbances in transgenic mice overexpressing the V717F beta-amyloid precursor protein. Behav. Neurosci. 113, 982–990.

Dunn, L.T., Everitt, B.J., 1988. Double dissociations of the effects of amygdala and insular cortex lesions on conditioned taste aversion, passive avoidance, and neophobia in the rat using the excitotoxin ibotenic acid. Behav. Neurosci. 102, 3–23.

Escobar, M.L., Bermudez-Rattoni, F., 2000. Long-term potentiation in the insular cortex enhances conditioned taste aversion retention. Brain Res. 852, 208–212.

Goedert, M., Spillantini, M.G., Jakes, R., Crowther, R.A., Vanmechelen, E., Probst, A., Gotz, J., Burki, K., Cohen, P., 1995. Molecular dissection of the paired helical filament. Neurobiol. Aging 16, 325–334.

Goosens, K.A., Maren, S., 2001. Contextual and auditory fear conditioning are mediated by the lateral, basal, and central amygdaloid nuclei in rats. Learn Mem. 8, 148–155.

Gotz, J., 2001. Tau and transgenic animal models. Brain Res. Brain Res. Rev. 35, 266–286.

Gotz, J., Nitsch, R.M., 2001. Compartmentalized tau hyperphosphorylation and increased levels of kinases in transgenic mice. NeuroReport 12, 2007–2016.

Gotz, J., Chen, F., Barmettler, R., Nitsch, R.M., 2001a. Tau filament formation in transgenic mice expressing P301L tau. J. Biol. Chem. 276, 529–534.

Gotz, J., Tolnay, M., Barmettler, R., Chen, F., Probst, A., Nitsch, R.M., 2001b. Oligodendroglial tau filament formation in transgenic mice expressing G272V tau. Eur. J. Neurosci. 13, 2131–2140.

Gotz, J., Chen, F., van Dorpe, J., Nitsch, R.M., 2001c. Formation of neurofibrillary tangles in P301L tau transgenic mice induced by Abeta 42 fibrils. Science 293, 1491–1495.

Hamann, S., Monarch, E.S., Goldstein, F.C., 2002. Impaired fear conditioning in Alzheimer's disease. Neuropsychologia 40, 1187–1195.

Higuchi, M., Ishihara, T., Zhang, B., Hong, M., Andreadis, A., Trojanowski, J., Lee, V.M., 2002. Transgenic mouse model of tauopathies with glial pathology and nervous system degeneration. Neuron 35, 433–446.

Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F., Cole, G., 1996. Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. Science 274, 99–102.

Hutton, M., Lendon, C.L., 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, R.C., Stevens, M., de Graaff, E., Wauters, E., van Baren, J., Hillebrand, M., Joosse, M., Kwon, J.M., Nowotny, P., Heutink, P., et al., 1998. Association of missense and 5′-splice-site mutations in tau with the inherited dementia FTDP-17. Nature 393, 702–705.

Janus, C., Pearson, J., McLaurin, J., Mathews, P.M., Jiang, Y., Schmidt, S.D., Chishti, M.A., Horne, P., Heslin, D., French, J., Mount, H.T., Nixon, R.A., Mercken, M., Bergeron, C., Fraser, P.E., St George-Hyslop, P., Westaway, D., 2000. A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. Nature 408, 979–982.

Janus, C., Lovasic, L., Johnson, S.-H., Welzl, H., 2002. Learning and Memory of Transgenic App-Expressing Mice in Conditioned Taste Aversion Paradigm. Program No. 778.2. 2002 Abstract Viewer and Itinerary Planner. Society for Neuroscience, Washington, DC. Online.

Kelley, A.E., Cador, M., Stinus, L., 1989. Exploration and its measurement—A psychopharmacological perspective. In: Boulton, A.A., Baker, G.B., Greenshaw, A.J. (Eds.), Neuromethods. The Humana Press, New Jersey, pp. 95–144.

Kimble, D.P., Bremiller, R., Schroeder, L., Smotherman, W.P., 1979. Hippocampal lesions slow extinction of a conditioned taste aversion in rats. Physiol. Behav. 23, 217–222.

Lamprecht, R., Hazvi, S., Dudai, Y., 1997. cAMP response element-binding protein in the amygdala is required for long- but not short-term conditioned taste aversion memory. J. Neurosci. 17, 8443–8450.

LeDoux, J.E., 2000. Emotion circuits in the brain. Annu. Rev. Neurosci. 23, 155–184.

Lee, V.M., Goedert, M., Trojanowski, J.Q, 2001. Neurodegenerative tauopathies. Annu. Rev. Neurosci. 24, 1121–1159.

Lewis, J., McGowan, E., Rockwood, J., Melrose, H., Nacharaju, P., Van Slegtenhorst, M., Gwinn-Hardy, K., Paul Murphy, M., Baker, M., Yu, X., Duff, K., Hardy, J., Corral, A., Lin, W.L., Yen, S.H., Dickson, D.W., Davies, P., Hutton, M., 2000. Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein. Nat. Genet. 25, 402–405.

Lin, W.L., Lewis, J., Yen, S.H., Hutton, M., Dickson, D.W., 2003. Filamentous tau in oligodendrocytes and astrocytes of transgenic mice

expressing the human tau isoform with the P301L mutation. Am. J. Pathol. 162, 213–218.

McGaugh, J.L., McIntyre, C.K., Power, A.E., 2002. Amygdala modulation of memory consolidation: interaction with other brain systems. Neurobiol. Learn. Mem. 78, 539–552.

Morgan, D., Diamond, D.M., Gottschall, P.E., Ugen, K.E., Dickey, C., Hardy, J., Duff, K., Jantzen, P., DiCarlo, G., Wilcock, D., Connor, K., Hatcher, J., Hope, C., Gordon, M., Arendash, G.W., 2000. A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. Nature 408, 982–985.

Paxinos, K.B.J.F.a.G., 1997. The Mouse Brain in Stereotaxic Coordinates. Academic Press, San Diego.

Poorkaj, P., Bird, T.D., Wijsman, E., Nemens, E., Garruto, R.M., Anderson, L., Andreadis, A., Wiederholt, W.C., Raskind, M., Schellenberg, G.D., 1998. Tau is a candidate gene for chromosome 17 frontotemporal dementia. Ann. Neurol. 43, 815–825.

Rollins, B.L., Stines, S.G., McGuire, H.B., King, B.M., 2001. Effects of amygdala lesions on body weight, conditioned taste aversion, and neophobia. Physiol. Behav. 72, 735–742.

Routtenberg, A., Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F., Cole, G., 1997. Measuring memory in a mouse model of Alzheimer's disease. Science 277, 839–841.

Schiffman, S.S., Clark, C.M., Warwick, Z.S., 1990. Gustatory and olfactory dysfunction in dementia: not specific to Alzheimer's disease. Neurobiol. Aging 11, 597–600.

Spillantini, M.G., Murrell, J.R., Goedert, M., Farlow, M.R., Klug, A., Ghetti, B., 1998. Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. Proc. Natl. Acad. Sci. U. S. A. 95, 7737–7741.

Tanemura, K., Akagi, T., Murayama, M., Kikuchi, N., Murayama, O., Hashikawa, T., Yoshiike, Y., Park, J.M., Matsuda, K., Nakao, S., Sun, X., Sato, S., Yamaguchi, H., Takashima, A., 2001. Formation of filamentous tau aggregations in transgenic mice expressing V337M human tau. Neurobiol. Dis. 8, 1036–1045.

Tanemura, K., Murayama, M., Akagi, T., Hashikawa, T., Tominaga, T., Ichikawa, M., Yamaguchi, H., Takashima, A., 2002. Neurodegeneration with tau accumulation in a transgenic mouse expressing V337M human tau. J. Neurosci. 22, 133–141.

Tatebayashi, Y., Miyasaka, T., Chui, D.H., Akagi, T., Mishima, K., Iwasaki, K., Fujiwara, M., Tanemura, K., Murayama, M., Ishiguro, K., Planel, E., Sato, S., Hashikawa, T., Takashima, A., 2002. Tau filament formation and associative memory deficit in aged mice expressing mutant (R406W) human tau. Proc. Natl. Acad. Sci. U. S. A. 99, 13896–13901.

Treit, D., Menard, J., 1997. Dissociations among the anxiolytic effects of septal, hippocampal, and amygdaloid lesions. Behav. Neurosci. 111, 653–658.

Welzl, H., D'Adamo, P., Lipp, H.P., 2001. Conditioned taste aversion as a learning and memory paradigm. Behav. Brain Res. 125, 205–213.

Wolfer, D.P., Madani, R., Valenti, P., Lipp, H.P., 2001. Extended analysis of path data from mutant mice using the public domain software Wintrack. Physiol. Behav. 73, 745–753.

Yamamoto, T., Fujimoto, Y., Shimura, T., Sakai, N., 1995. Conditioned taste aversion in rats with excitotoxic brain lesions. Neurosci. Res. 22, 31–49.