Research report

Non-cognitive behaviours in an APP/PS1 transgenic model of Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is characterised by progressive cognitive impairment with neuropsychiatric symptoms such as anomalous motor behaviour, depression, anxiety, weight loss, irritability and agitation. The effect of hAPP and PS1 overexpression on cognition has been well characterised in a variety of transgenic mouse models, however, non-cognitive behaviours have not been considered as systematically. The non-cognitive behaviour of the hAPP/PS1 transgenic mouse model (TASTPM) was observed at ages spanning the rapid progression of amyloid neuropathology. TASTPM transgenic mice, of both genders, exhibited decreased spontaneous motor activity, disinhibition, increased frequency and duration of feeding bouts, reduced body weight and, by 10 months, increased activity over a 24 h period. In addition to the aforementioned behaviours, male transgenic mice also displayed enhanced aggression relative to wildtype controls. These data reveal previously unreported disease relevant behavioural changes that demonstrate the value of measuring behaviour in APP/PS1 transgenic models. These behavioural readouts could be useful in screening putative drug treatments for AD.
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1. Introduction

AD is a chronic neurodegenerative disorder that is the most common cause of dementia in the elderly [28]. It is characterised by the accumulation of extracellular beta amyloid into aggregated amyloid plaques, the hyperphosphorylation of tau leading to neurofibrillary tangles (NFTs) and an associated neuroinflammatory state exemplified by activated microglia and astrocytes [46].

Patients initially present mild cognitive impairment that can slowly develop to serious deficits in memory function. At later stages of AD, neuropsychiatric symptoms develop, which range from abnormal motor behaviour, depression or anxiety, and personality alterations such as aggression and irritability, all of which can contribute to patient institutionalisation [2,5,16,34,35,52]. Patients also suffer a fragmented sleep-wake pattern with increased nocturnal activity [54,55,62] that is associated with increased lethargy during daytime hours [44]. Weight loss is an additional common symptom and can contribute to the diagnosis [13]. A recent study [64] reported that over 50% of AD patients developed weight loss despite a majority consuming more calories daily per kilogram body weight than non-demented controls. In determining the relationship between weight loss and behavioural symptoms, a further study [67] revealed a negative correlation between body mass index (BMI) and agitation/aggression, depression, irritability, aberrant motor behaviour and appetite/eating. Additionally, there is conflicting data on the relationship between food intake and weight loss in AD, however, fluctuating feeding patterns positively correlate with increased behavioural problems [57,67–69]. Weight loss is also suggested to be a predictor of mortality in AD with the risk of weight loss increasing with the severity and progression of the disease [66].

In understanding the aetiology of AD, emphasis is placed on the genetic factors that can either predispose or increase the risk of developing the familial early-onset AD. A number

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of mutations have been identified [14,47,48] and subsequently used to develop genetically manipulated animal models that mimic specific facets of the disease, particularly beta amyloid neuropathology.

Transgenic mice with mutated amyloid precursor protein (APP) [12,18,19,21,25,41,42,51], presenilin 1 (PS1), or both [3,15,26,56] can display increased production of beta amyloid protein including increased deposition of insoluble plaque deposits. The Swedish (K670N/M671L) and V717F mutations have been used to develop transgenic mice such as Tg2576 [18,19] and PDAPP [8,12], however, these animals require a period of aging to allow for progressive plaque deposition. The addition of mutant presenilin (a risk factor for familial AD) to create double transgenic models such as PSAPP (Tg2576 × hPS1 (M146L)) [15] results in the acceleration of amyloid plaque deposition in APP mice, although the PS1 transgene alone does not cause deposition [4,6,7,10,20,45]. The PSAPP mouse model displays enhanced plaque deposition at 3 months of age in comparison to plaque onset in Tg2576 at 12 months [36,65]. A majority of literature on double transgenic models covers the assessment of cognitive impairments largely encompassing learning and memory paradigms. Although many studies that primarily focus on the assessment of cognition have included some carefully chosen tests of non-cognitive behaviour, few studies have comprehensively investigated the non-cognitive behavioural consequences of an APP/PS1 genetic manipulation in a single APP or APP + PS1 transgenic model, particularly, those behavioural endpoints pertinent to the psychiatric symptoms associated with AD.

Here we describe the longitudinal behavioural characterisation of a novel double transgenic model, TASTPM. TASTPM mice are derived from the TAS10 APP overexpression model [51] crossed with mutant PS1. Onset of plaque deposition in brain occurs at 4 months of age with heavy plaque deposition exhibited by 10 months [17]. Psychiatric symptoms pertinent to AD may be useful as preclinical markers for the investigation of pharmacological modulation of amyloid deposition in transgenic models and additionally, the symptomatic treatment of non-cognitive behavioural alterations seen in AD. Therefore, the present studies assessed anxiety, aggression, motor behaviour and primary behavioural phenotypic characteristics of TASTPM mice.

2. Methods

2.1. Animals

A breeding program generated heterozygous double transgenic mice (TASTPM) with the Swedish (K670N; M671L) double familial mutation (Thy-1-APP695Sw, Line 10 (TAS10)) [50] and the PS1 (M146V) mutation (Thy-1-PS-1M146V), backcrossed onto a pure C57BL/6 background. As described previously, the insertion of both gene-coding sequences replaced the coding sequence of the murine Thy-1 gene allowing brain-specific expression of the transgene [61,17]. Mice were housed in individual cages and maintained under a standard 12-h light:dark cycle with food and water available *ad libitum*. Testing began when the mice were between 7 and 8 weeks of age.

Ten male and 11 female TASTPM transgenic mice (N12 generation) and 13 male and 12 female wildtype littermates were used in the behavioural assessment. Subsequently, 10 male TASTPM transgenic mice aged 10 months from a

separate cohort and 11 age matched C57BL6/J mice were used to assess food intake. Genotype status was confirmed by analysis of DNA isolated from tail tips removed at termination.

All experiments were conducted according to the requirements of the United Kingdom Animals (Scientific Procedures) Act (1986) and conformed to Glaxo-SmithKline ethical standards.

2.2. Behavioural assessments

Behavioural paradigms took place with at least 24 h between each assessment. More than one paradigm ran within 1 week, however, no two separate tests were run on the same day. Paradigms were assessed in the order described at 2, 5 and 10 months of age. At 2 months, TASTPM transgenic mice display little amyloid plaque load, however, amyloid neuropathology appears at approximately 4 months of age and by 10 months, there is extensive amyloid neuropathology [17]. The behavioural phenotype of the TASTPM mouse was assessed longitudinally including timepoints spanning the course of amyloid neuropathology.

2.2.1. Elevated plus maze (X-maze) test of anxiety

The X-maze is a non-conflict animal model of anxiety, which reflects anxiety through the manipulation of natural fear and exploratory drive upon exposure to novel stimuli. The X-maze (50 cm height from floor) consisted of two enclosed arms and two open arms (all 30 cm long and 5 cm wide, enclosed arm walls 15 cm high in clear perspex), behaviour was determined under red light and the apparatus cleaned thoroughly between each animal (70% ethanol solution). Animals were placed onto the maze in the central (neutral) region facing an open arm. Time spent in and entries into the central region and open or closed arms as a measure of anxiety behaviour were recorded over a period of 5 min using a video tracking system. The percentage of time spent in, and the number of entries into the open arms was analysed as a percentage of total arm (open and closed) time and entry. Total entries into all regions and total entry into arms only was also analysed as a measure of overall maze activity and arm activity, respectively.

2.2.2. Primary behavioural observation screen—SHIRPA

Briefly, SHIRPA is a comprehensive protocol derived from the Irwin Neurologic battery of assessments used to quantify the behavioural and functional characteristics of the mouse phenotype. Here, the primary SHIRPA screen comprising 40 separate observations was carried out to provide a behavioural, neurological and autonomic profile of each mouse [53,30].

2.2.3. LABORAS^TM

Laboratory Animal Behaviour Observation, Registration and Analysis System (Metris b.v. Hoofddorp, The Netherlands) consisted of a triangular shaped sensing platform positioned on two orthogonally placed force transducers and a third fixed point attached to a heavy bottom plate. The whole structure stands on three spikes, which are adjustable in height and absorb external vibrations. Clear polycarbonate/Makrolon type III cages, with a floor covered with sawdust, are positioned directly onto the sensing platform, the upper part of which (including the top, food hopper and drinking bottle) is suspended in a high adjustable frame and was free from the sensing platform. Mechanical vibrations caused by the movement of the animal are transformed into electrical signals, which are modified and stored on a computer. The stored data was processed using several signal analysis techniques to classify the signals into the behavioural categories of feeding, drinking, rearing, climbing, immobility, LMA and grooming. For a more detailed description of this apparatus, refer to [50,60].

2.2.4. Spontaneous locomotor activity (LMA) test

Locomotor activity assessment took place in a bank of activity monitors (42 cm × 21 cm × 20 cm) (AM1052, Benwick Electronics, Essex, UK). A bank consisted of eight clear perspex cages, positioned within a frame equipped with infrared beams. Animal activity was recorded automatically by counting the number of beam breaks in the test period. Each mouse received a single 30-min test session without prior habituation to the apparatus. Data was collected in six blocks of 5-min intervals during the light period.

2.2.5. Reactivity to a novel environment

This assay comprised two tests using the bank of activity monitors described above (Section 2.6). During test 1, animals occupied clear perspex cages that had one wall covered with black and white checked card and the floor covered with lemon-scented sawdust to alter the environment. Activity, during the light period, was recorded by automatic counting of the number of beam breaks during a 30 min period, as described in Section 2.6. Following a 24-h delay, each animal was returned to the cage used in test 1 to determine activity in a familiar environment (test 2).

2.2.6. Accelerating rotarod

The accelerating rotarod (Ugo Basile, Italy) tests balance and co-ordination. It comprised of a rotating drum that accelerated from 4 to 40 rpm over a 5-min period. The latency of each animal to fall from the drum was recorded for three consecutive trials. Latency across trials and best latency were then analysed.

2.2.7. Resident-intruder paradigm

A group housed 'intruder' was placed into the home cage of a singly housed 'resident' and in this study the intruder was always a C57BL6/J mouse whilst the resident was always either a wildtype or transgenic male mouse. Intruder mice were of the same gender as the residents. The behaviour of each mouse was recorded on videotape for a period of 5 min. During this time, offence (frequency, duration and latency to attack) and defence (latency and frequency of defence posture) were recorded. Offensive bouts were defined as individual attack on the opponent separated by non-offensive behaviour.

2.2.8. Food intake during 24 h

Assessment of food intake during a 24-h period occurred in the home cage of each singly housed animal. A preweighed amount of food pellets held in the holder of the cage and weighed every 24 h for 3 days. The average pellet intake during 24 h was calculated for each animal, intake included whole or part pellets dropped within the cage.

2.3. Data analysis

Statistical analyses were conducted using the Proc Mixed procedure in SAS Version 8 (SAS Institute). A general linear mixed model approach was used to assess responses at repeated timepoints within subjects. Planned comparisons on the predicted means from the model assessed individual genotype, gender and age effects over time on each behavioural variable. The non-parametric analysis of SHIRPA measurements was conducted using the Mann–Whitney U-test.

3. Results

3.1. Elevated plus maze

Repeated measures ANOVA indicated an overall effect of genotype $[F_{(1,41)} = 16.14, p < 0.001]$, age $[F_{(2,51)} = 7.54, p < 0.01]$ and a genotype × age interaction $[F_{(2,51)} = 9.86, p < 0.001]$ on percentage time spent in open arms (Fig. 1). A genotype $[F_{(1,43)} = 17.8, p < 0.001]$ and a genotype × age interaction $[F_{(2,77)} = 6.09, p < 0.01]$ were also demonstrated on percentage entries into the open arms (Fig. 1). Total activity, as determined by total overall entries or arm entries alone, did not differ between wildtype and transgenic groups within each timepoint. Male and female TASTPM transgenic mice displayed increased percentage entries and duration in the open arms by 10 months of age ($p < 0.05$) and by 5 months ($p < 0.001$) and 10 months ($p < 0.001$) of age, respectively.
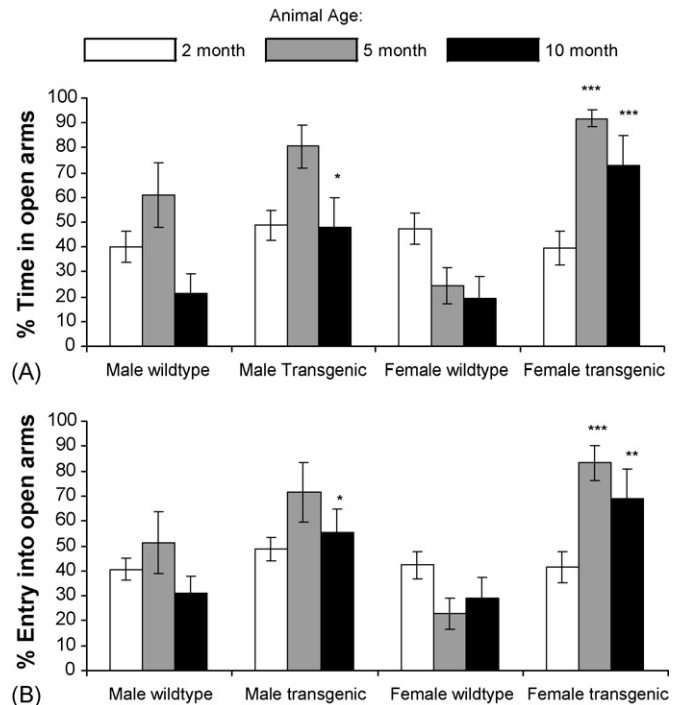

Fig. 1. Percentage time spent in the open arms (A), percentage entry into open arms (B) of aging wildtype and transgenic TASTPM mice. Data represented as observed mean ± S.E.M. ($^*p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$ vs. respective wildtype).

3.2. Primary behavioural observations screen—SHIRPA

At 2 and 5 months, TASTPM mice displayed an enhanced startle response to a 90 dB sound from a click box. TASTPM also showed less resistance to fingertip pressure on the hind paws relative to wildtype mice. TASTPM mice at 5 and 10 months demonstrated a more vigorous escape response to a finger stroke and at 5 months appeared more irritable (Table 1).

Repeated measures analysis of variance indicated an overall effect of genotype $[F_{(1,42)} = 94.51, p < 0.001]$, gender $[F_{(1,42)} = 132.51, p < 0.001]$, age $[F_{(2,76)} = 667.29, p < 0.001]$ and a genotype × age interaction $[F_{(2,76)} = 23.83, p < 0.001]$ on body weight. Post hoc analysis revealed male wildtype mice at 2, 5 and 10 months were heavier than male transgenic ($p < 0.001$, all groups) and female wildtype at 2, 5 and 10 months were significantly heavier than female transgenic mice ($p < 0.001$, all groups). Additionally, male transgenic mice were heavier

Table 1
Primary behavioural observations of the aging TASTPM mouse

Age (months)	Test	Significance, p	Wildtype	Transgenic
2	Startle	0.01	1 (1–2)	2 (1–2)
2	Limb Tone	0.01	2 (2–3)	2 (1–2)
5	Startle	<0.001	1 (1–1)	2 (1–2)
5	Touch Escape	<0.05	2 (2–2)	2 (2–3)
5	Limb Tone	<0.01	2 (2–3)	2 (2–2)
5	Irritability	<0.05	0 (0–0)	0.5 (0–1)
10	Touch Escape	<0.01	2 (2–2)	2 (2–3)

Data are represented as median score with quartile range in parentheses. Non-parametric analysis within each age comparing wildtype and transgenic groups.
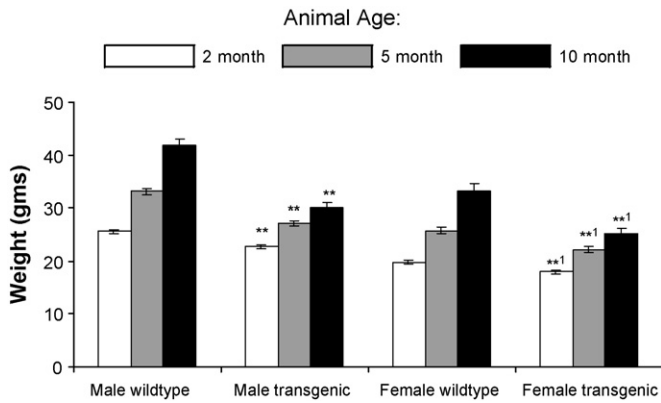
Fig. 2. Comparison of body weight gain in aging male and female wildtype and transgenic TASTPM mice. Data represented as observed mean ± S.E.M. [**]$p < 0.001$ vs. respective MWT and FTG, [**1]$p < 0.001$ vs. respective FWT.

than female transgenic mice at all ages ($p < 0.001$, all groups) (Fig. 2).

3.3. LABORAS[TM]

Statistical analysis revealed no significant effect of genotype, age or gender on measures of frequency and duration of climbing, grooming, drinking or immobility bouts (Table 2).

3.3.1. LMA in 24 h

There were overall genotype [$F_{(1,43)} = 19.94$, $p < 0.001$], age [$F_{(2,35)} = 6.48$, $p < 0.01$], genotype × age [$F_{(2,35)} = 7.00$, $p < 0.01$] and gender × age [$F_{(2,35)} = 4.16$, $p < 0.05$] effects for frequency of locomotor activity over a 24 h duration. Post hoc planned comparisons revealed that male transgenic mice were most active at 5 months ($p < 0.05$) and 10 months ($p < 0.01$) of age (Fig. 3A) whilst female transgenic mice showed greater frequency of activity only at 10 months ($p < 0.01$). There were also significant overall effects of genotype [$F_{(1,41)} = 7.59$, $p < 0.01$] and age [$F_{(2,34)} = 3.16$, $p = 0.05$] with genotype × age [$F_{(2,34)} = 7.85$, $p < 0.01$] and gender × age [$F_{(2,34)} = 3.43$, $p < 0.05$] interactions for overall duration of locomotor activity (Fig. 3B). In a similar pattern to activity frequency, female transgenic mice demonstrated greater duration of activity than female wildtype littermates at 10 months of age ($p < 0.05$) whilst increased activity occurred earlier in the male transgenic mice at 5 months ($p < 0.05$) and 10 months ($p < 0.01$) of age.

3.3.2. Feeding

Repeated measures analysis of variance indicated a significant overall effect of genotype [$F_{(1,32)} = 21.17$, $p < 0.001$] and a genotype × day [$F_{(2,32)} = 5.67$, $p < 0.01$] interaction for frequency of eating. Post hoc planned comparisons revealed that overall, transgenic mice ate more frequently then wildtype mice at 2 months ($p < 0.05$), 5 months ($p = 0.01$) and 10 months ($p < 0.001$) of age. Individual comparisons within genders revealed a non significant trend ($p = 0.07$) at 2 months of age and significantly increased feeding frequency by 5 months ($p < 0.001$) and 10 months ($p < 0.001$) of age in male TASTPM mice versus male wildtype mice (Fig. 4A). Female TASTPM

Table 2
Overview of additional LABORAS results in aging wildtype and transgenic TASTPM mice

	Age (months)					
	2		5		10	
	WT	TG	WT	TG	WT	TG
Climbing (frequency)	177.72 ± 26.33	177.24 ± 31.46	101.00 ± 20.58	91.86 ± 25.88	56.88 ± 13.01	89.80 ± 22.02
Climbing (duration)	4973.18 ± 657.75	4582.16 ± 550.33	2581.38 ± 606.79	3447.70 ± 868.70	1046.09 ± 349.00	1090.71 ± 276.96
Grooming (frequency)	799.94 ± 59.98	862.29 ± 78.68	878.84 ± 66.46	935.29 ± 79.48	1066.60 ± 62.23	1102.27 ± 140.63
Grooming (duration)	6198.42 ± 618.19	6123.46 ± 615.74	6650.62 ± 656.51	6714.63 ± 600.44	9271.85 ± 868.40	7129.20 ± 839.72
Drinking (frequency)	19.67 ± 4.52	22.47 ± 6.14	29.56 ± 5.09	26.33 ± 5.89	30.24 ± 3.62	24.07 ± 5.97
Drinking (duration)	45.55 ± 9.33	63.74 ± 18.08	78.80 ± 15.73	77.64 ± 20.61	75.97 ± 10.16	60.38 ± 14.78
Immobility (frequency)	1828.72 ± 308.93	2134.71 ± 309.48	2085.96 ± 255.63	2206.29 ± 572.88	1734.60 ± 149.61	2061.87 ± 448.97
Immobility (duration)	35516.50 ± 2270.79	31854.02 ± 2648.49	42075.10 ± 1301.72	40157.72 ± 1742.88	39036.67 ± 1493.40	36549.65 ± 3651.49

WT and TG represent genotypes. Data represented as observed mean ± S.E.M.
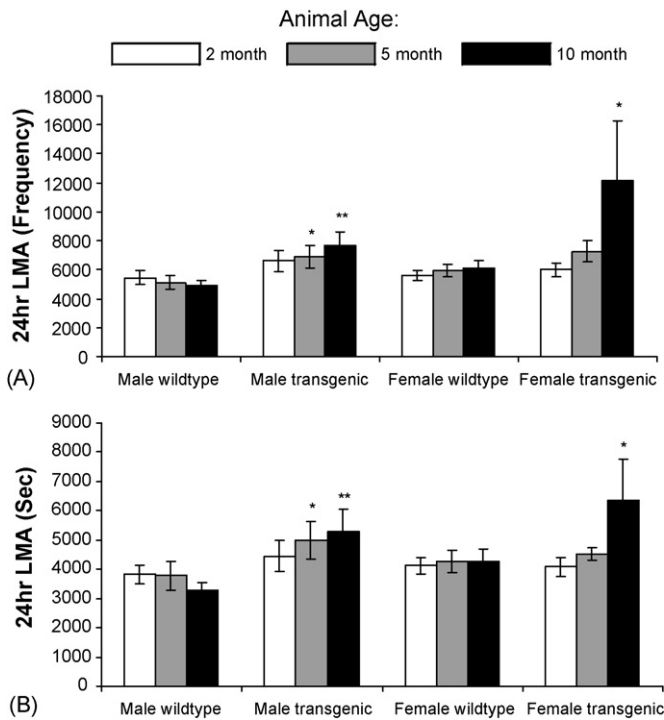
Fig. 3. Frequency (A) and duration (B) of locomotor activity bouts during a 24 h period (17.00–17.00 h) as determined by LABORAS. Data shown as observed mean ± S.E.M. $^*p < 0.05$, $^{**}p < 0.01$ vs. corresponding wildtype group.

mice only exhibited increased frequency of feeding by 10 months of age ($p < 0.01$) (Fig. 4A).

There were significant overall effects of genotype [$F_{(1,38)} = 9.9$, $p < 0.01$] and age [$F_{(2,36)} = 3.91$, $p < 0.05$] on eating duration. Post hoc planned comparisons revealed transgenic mice ate for longer bouts than corresponding wildtype animals at 5 months ($p < 0.05$) and 10 months ($p < 0.001$) (Fig. 4B). Individual comparisons within genders revealed a significant increase in feeding duration by 5 months ($p < 0.05$) and 10 months ($p < 0.01$) of age in male TASTPM mice versus male wildtype mice (Fig. 4B). Female TASTPM mice only exhibited increased duration of feeding by 10 months of age ($p < 0.05$) (Fig. 4B).

3.4. Food intake during 24 h

There were strain [$F_{(1,19)} = 10.90$, $p < 0.01$] and test [$F_{(2,38)} = 6.75$, $p < 0.01$] effects but no strain × test interactions revealed by repeated measures analysis on food consumed across three consecutive 24 h test periods (Fig. 4C). Male TASTPM transgenic mice consumed significantly more food relative to age matched C57BL6/J mice on all three consecutive days ($p < 0.05$, all days).

3.5. LMA in 30 min

3.5.1. LMA in 30 min in aging TASTPM

There were overall genotype [$F_{(1,40)} = 13.93$, $p = 0.001$] and age [$F_{(2,72)} = 3.65$, $p = 0.05$] effects on total locomotor activity calculated from a 30 min period. There were no effects of gender on total activity. Post hoc planned comparisons revealed
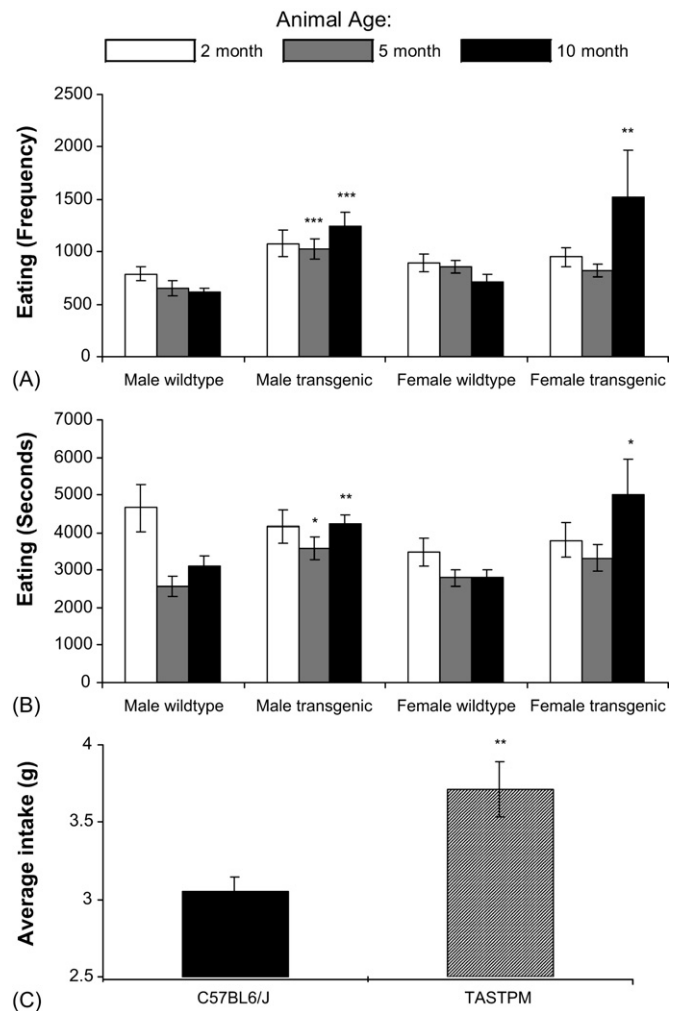

Fig. 4. Frequency (A) and duration (B) of eating bouts during a 24 h period (17.00–17.00 h) as determined by LABORAS at 2, 5 and 10 months of age. Average food intake in home cage (C), data represented as observed mean ± S.E.M. $^*p < 0.05$, $^{**}p < 0.01$ vs. wildtype.

that male and female transgenic mice were significantly less active at 2 months ($p < 0.01$ and $p < 0.05$, respectively), 5 months ($p < 0.05$, both genders) and 10 months ($p < 0.05$, female only) of age (Fig. 5A). Analysis of locomotor activity over time indicated a significant genotype [$F_{(1,80)} = 14.75$, $p < 0.001$] effect with no significant effect of gender or age on locomotor activity during a 30-min period. Both male and female transgenic mice were consistently hypoactive over time, irrespective of age, relative to wildtype animals (Fig. 5B and C).

3.5.2. Reactivity to a novel environment of 10 months TASTPM

Analysis of variance indicated a genotype [$F_{(1,65)} = 22.21$, $p < 0.0001$], test [$F_{(1,44)} = 15.92$, $p < 0.001$] and a significant genotype × gender × time interaction [$F_{(5,163)} = 2.69$, $p < 0.05$]. Transgenic mice were hypoactive during the 30 min period in both a novel (Fig. 6A and B) and familiar environment (Fig. 6C and D), with decreased overall animal activity during test 2 relative to activity in the original novel environment ($p < 0.001$ versus test 1 at 5 min, $p < 0.01$ versus test 1 at 10 min,
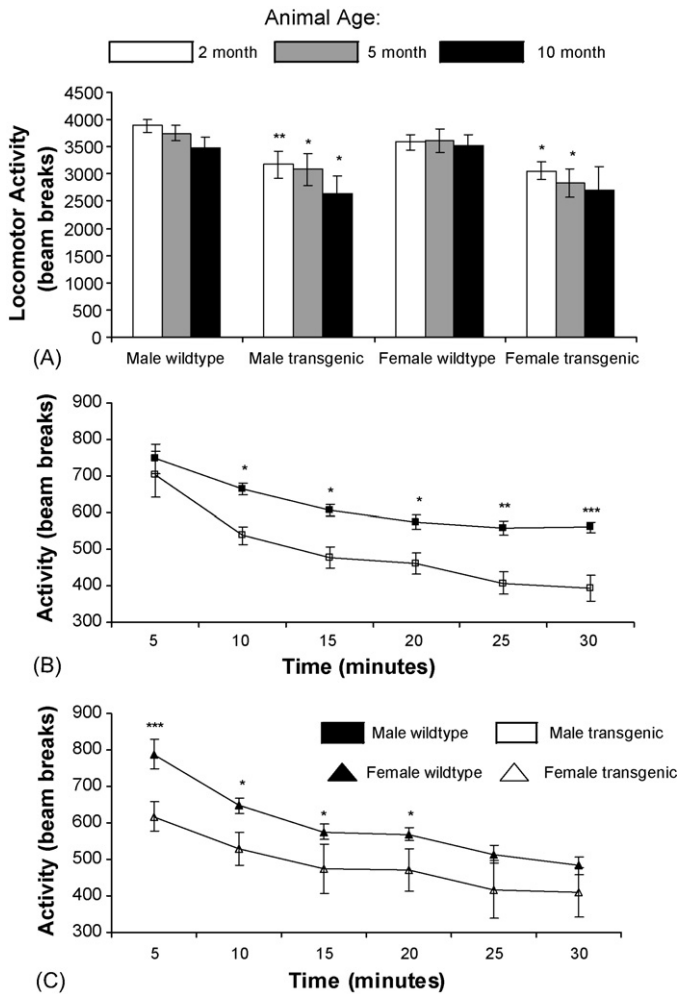
Fig. 5. Total locomotor activity during 30-minute period (A), locomotor activity over time within male (B) and female (C) mice, grouped across age timepoints, data represented as observed mean ± S.E.M. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. respective wildtype.

$p < 0.001$ versus test 1 at 15 and 20 min respective timebins). Analysis of total activity indicated a significant effect of the genotype during test 1 [$F_{(1,35)} = 11.25$, $p < 0.01$] and during test 2 [$F_{(1,35)} = 12.59$, $p = 0.001$]. Male and female transgenics both displayed hypoactivity ($p < 0.01$ and $p = 0.07$, respectively) relative to wildtype animals of the same gender and continued to display hypoactivity in a familiar environment ($p < 0.05$ and $p < 0.01$, respectively).

3.6. Accelerating rotarod

Statistical analysis revealed no significant effect of genotype, age or gender on performance on an accelerating rotarod apparatus (Table 3).

3.7. Resident-intruder

Repeated measures analysis of variance indicated a significant interaction of genotype × age [$F_{(2,29)} = 4.97$, $p < 0.05$] for frequency of offensive attacks. Post hoc analysis revealed transgenic mice attacked more frequently than wildtype mice ($p < 0.01$) at 10 months of age (Fig. 7A). There was also increased duration of offensive behaviour in transgenic mice [$F_{(1,21)} = 5.5$, $p < 0.05$] and an effect of age [$F_{(2,40)} = 4.25$, $p < 0.05$] which was specific to their behaviour at 10 months of age ($p < 0.05$) relative to wildtype mice (Fig. 7B). Typical offensive bouts occurred as neck or a flank attack separated by non-offensive breaks ranging from approximately 10 to 40 s duration.

3.8. Mortality

One hundred percent of wildtype animals, irrespective of gender, survived through to completion of the behavioural testing however only 90% (9/10) of male and 54.5% (6/11) of female TASTPM transgenic mice completed the assessment. Those animals that remained were in good health. Prior to premature death, animals did not exhibit any overt signs of illness or changes in body weight and as such remained in the results analysis. At 5 months of age, behavioural assessments included ten male and nine female transgenic whilst by 10 months group sizes were nine and six, respectively.

4. Discussion

Previous studies have primarily focused on characterising the robust cognitive impairment demonstrated by mice over-expressing APP transgenes, PS1 transgenes, or both, however, non-cognitive behaviours have not been characterised as extensively. The amyloid neuropathology and cognitive impairments exhibited by the TASTPM transgenic mouse were recently described [17]. Amyloid deposits were observed in the brain tissue of all TASTPM mice by 4 months of age. Between 6 and 10 months of age, mice displayed cerebral plaque pathology that

Table 3
Comparison of rotarod performance per trial and best score in aging wildtype and transgenic TASTPM mice

Age (months)	Group	Trial 1	Trial 2	Trial 3	Best score
2	WT	129.32 ± 8.86	152.72 ± 9.15	193.56 ± 11.37	197.08 ± 10.73
	TG	128.55 ± 6.48	157.45 ± 12.35	186.90 ± 10.45	196.90 ± 10.16
5	WT	137.72 ± 9.27	167.52 ± 6.72	181.68 ± 8.51	194.44 ± 8.31
	TG	149.53 ± 9.38	170.63 ± 6.65	188.58 ± 6.72	197.1 ± 65.84
10	WT	132.20 ± 9.35	152.08 ± 9.91	164.76 ± 9.52	179.48 ± 8.54
	TG	138.93 ± 14.03	183.0 ± 13.89	173.80 ± 15.84	208.27 ± 9.86

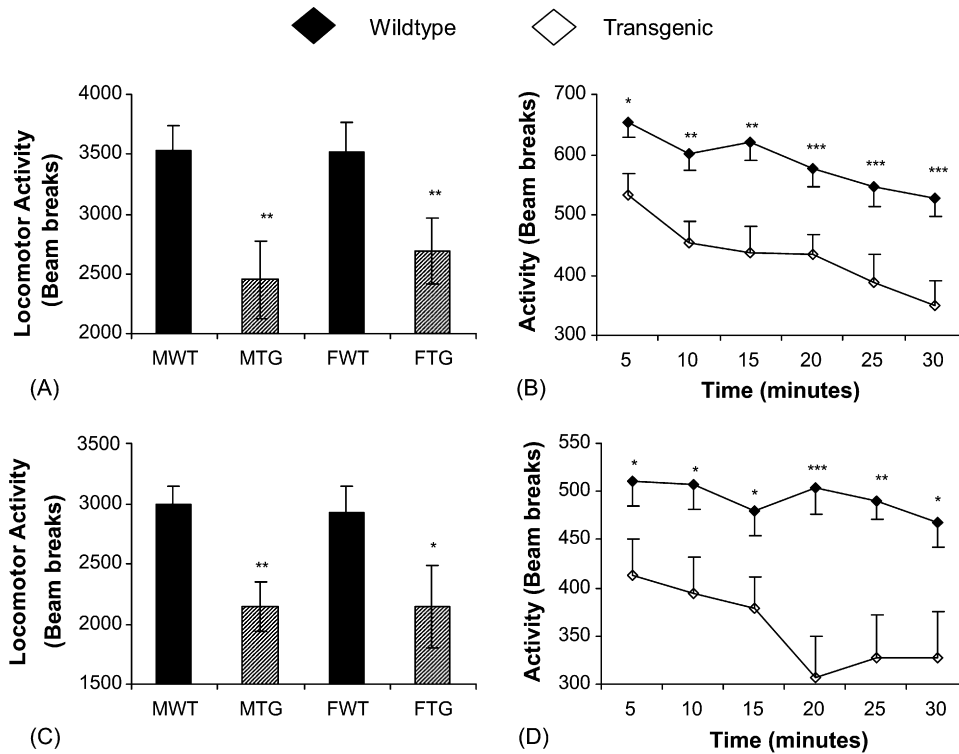Data represented as observed mean ± S.E.M.

Fig. 6. Assessment of reactive behaviour in a novel (A and B) and familiar (C and D) environment in 10-month-old wildtype and transgenic TASTPM mice, data represented as observed mean ± S.E.M. Activity during 30 min (B and D) grouped for gender $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. respective transgenic group.
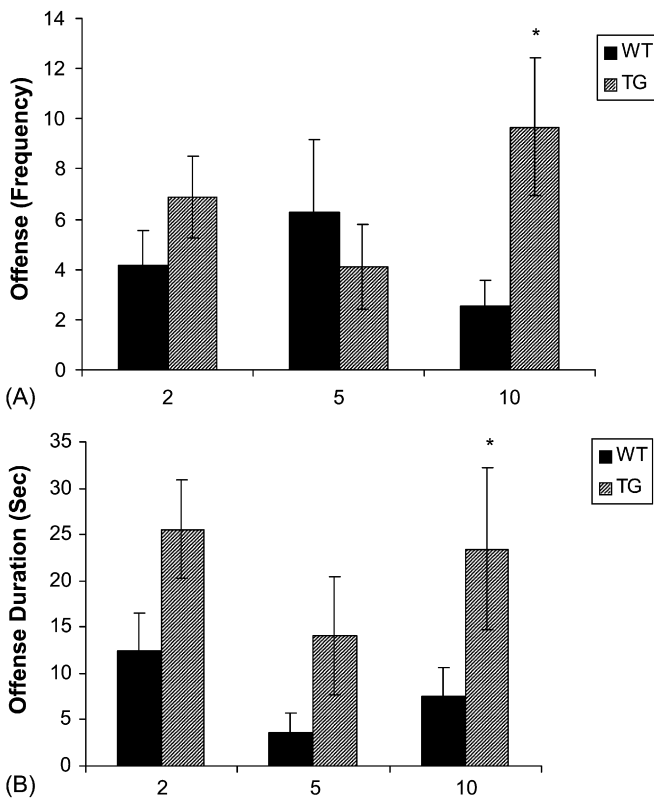

Fig. 7. Resident-intruder aggression frequency (A) and duration (B) in male wildtype and transgenic TASTPM mice at 2, 5 and 10 months of age. Data represented as observed mean ± S.E.M. $^*p < 0.05$ vs. respective wildtype, $^{**}p < 0.01$ vs. respective wildtype.

appeared more extensive in the female TASTPM mice. TASTPM mice, of both genders, exhibited a significantly reduced recognition index, in an object recognition test, by 6 months of age. Here, we provide a comprehensive profile of the non-cognitive behaviours of the APP/PS1 transgenic mouse model, TASTPM. Our studies revealed behavioural impairments in the TASTPM model that may be pertinent to behaviours seen in AD patients and highlight some behavioural alterations not yet reported in APP/PS1 transgenic models.

AD is a form of dementia in which loss of memory is the first and most characteristic symptom, however, as the disease progresses, patients can exhibit depression, irritibility, aggressive outbursts and delusions [34,52]. Late in the disease, patients may also experience social disinhibition and abnormal motor behaviours. A further common occurrence is weight loss that is usually associated with decreased daily activities [13,44,64] and increased nocturnal activity [54,55,62]. Body weight loss is additionally considered to correlate with severity of behavioural disturbances and is suggested to be a useful clinical predictor of mortality in AD [66].

The SHIRPA primary screen, derived from the Irwin test used to assess the neurological side-effects of novel compounds, revealed that on observation, the TASTPM mouse displayed increased irritibility and escape responses, in the absence of any other changes within the battery of tests included. Aggression is a common symptom in dementia, closely linked with agitation and irritability [2,5,16]. The enhanced excitability exhibited by TASTPM mice during SHIRPA may influence the tendency of TASTPM transgenic animals to attack territory intruders. Transgenic mice demonstrated a trend towards elevated duration

of aggressive attacks at all timepoints, which was significant by 10 months of age. Frequency of attacks remained similar to wildtype animals until 10 months of age. The background strain of a transgenic mouse can influence the outcome of a behavioural assessment. The FVB/N strain is used routinely as the background strain in a number of transgenic mouse models, which have subsequently been characterised in tests of aggression [18]. The FVB/N strain possesses a highly aggressive phenotype that makes analysis of territorial aggression difficult [37,39–42,25,49]. More recently, Minkeviciene et al. [38], evaluated aggression in an APP/PS1 overexpressing mouse model on a C57BL6/J background using the resident-intruder test. Our data supports the recent findings that mice with an APP/PS1 genetic manipulation, even on a C57BL6/J background, can exhibit increased aggression.

Numerous studies have also assessed measures of motor activity and postural balance in APP or APP/PS1 overexpressing mouse models [38,43,11,22,58,23,32,24]. It is difficult to determine the precise effect of APP transgenic overexpression on animal activity since there is much conflicting evidence in the literature. Many of the reports suggest that, using the open field test, APP overexpressing mice, namely those with a Swe695 [22,24] or the London mutation (V642I) [23], can exhibit increased activity levels. However, there is evidence for APP transgenic mice to demonstrate minimal locomotor activity changes [58,32]. Many APP transgenic lines can also display impaired balance on rotarod [11,32] or balance beam tasks [22,24]. We assessed the locomotor activity of the TASTPM mouse over 30 min during the daytime period, as measured by number of beam breaks. We also included LABORAS for general activity measures over 24 h and the accelerating rotarod for assessment of balance and co-ordination. Our studies demonstrated task-specific hypoactivity of TASTPM transgenic mice during the light period whilst over a 24-h period (17.00–17.00 h), hyperactivity was observed by 5 and 10 months of age in the male and 10 months in the female transgenic mice. TASTPM mice have been previously reported to demonstrate cognitive deficits in the novel object recognition test [17]. Alterations in locomotor activity may have implications towards the performance of these animals in the novel object recognition test; however, Howlett et al., stated that there was no significant difference in the time spent exploring objects during trial 1 indicating that the cognitive deficit observed was not influenced by hypoactivity. This may further support evidence that alterations in activity levels in TASTPM mice appear task-specific.

It is difficult to determine the exact reasons for the varying activity patterns between tasks. Alterations in circadian rhythm between APP or APP/PS1 overexpressing transgenic and wildtype mice may explain the hyperactivity demonstrated by TASTPM mice over a 24 h period in contrast to the hypoactivity seen using shorter duration tests during the daytime. APP23 mice have displayed nocturnal hyperactivity [59] with decreased motor activity during 2-h recordings taken at dusk. Although we should not extrapolate between rodent and human activity patterns, the disturbance in activity patterns in APP overexpressing mice may indicate some comparison with the increased night-time activity and day-time lethargy exhibited in AD patients

[54,55,62]. Hence, further investigation is required to understand the relative contribution that alterations in circadian rhythm may have on the performance of APP and APP/PS1 overexpressing mice in tests using or assessing locomotor activity.

Further analysis of the locomotor activity of these animals determined their reactivity to a novel environment since a more reactive animal may display enhanced levels of activity when placed into an unfamiliar environment. Since hypoactivity of TASTPM transgenic mice was evident in both familiar and unfamiliar settings this would suggest their activity levels remain lower than control animals regardless of novelty hence familiarity with surroundings has no effect on their behavioural phenotype. Subsequent in-house assessments of TASTPM cohorts have further supported evidence for a robust hypoactivity of these animals using beam break boxes (data not shown).

An assessment of the balance and co-ordination exhibited by TASTPM mice revealed the mice as unimpaired on an accelerating rotarod beam at speeds of 4–40 rpm over 5 min. Mice also appeared normal in tests of co-ordination and balance during the SHIRPA primary screen. The multitude of assays and behavioural protocols used by different authors may explain the lack of abnormalities in postural balance demonstrated by the TASTPM transgenic mice in comparison to other reported transgenic models. Particularly, there have been a number of published protocols for assessment of balance on the rotarod indicating constant beam speeds of approximately 15 rpm may be required to distinguish an impairment of transgenic mice in comparison to wildtype controls [32,11]. A report by Ewers et al. [11] may provide evidence of this since a speed of 15 rpm provided greater separation in the performance of wildtype and transgenic mice than that seen at 25 rpm.

We assessed the activity of TASTPM mice in the elevated plus maze to determine effects of the transgenes on anxiety. TASTPM transgenic mice did not display significantly altered activity levels relative to wildtype controls as measured by either total maze entries or arm entries alone. Transgenic mice, however, exhibited disinhibition as exemplified by increased percentage entry into and percentage time in the open arms of the maze by 5 months of age. Further analysis of TASTPM cohorts between 2 and 5 months of age may clarify more accurately the precise age of onset of disinhibition, particularly how closely the disinhibition correlates with onset of plaque deposition. There are several reports on the performance of APP overexpressing transgenic models in tests of anxiety. Single APP overexpressing animals such as APP23 (APP751) and Tg2576 (APP695) show either no effect or a distinct anxiolytic response on the elevated maze, respectively [29,31,43]. The TgCRND8 transgenic mouse model comprising Swedish and Indiana APP mutations exhibited no difference in anxiety responses to wildtype animals [58] whilst Lee et al. [32] suggested mice with Swe/Indiana APP mutations exhibited increased anxiety and, localised within the amygdala, increased expression of genes implicated in anxiety. There is conflicting literature on the performance of animals overexpressing both APP transgenes and PS1. Arendash et al. [1] did not demonstrate any effect of the transgene on anxiety levels in APP + PS1 mice whilst, more recent data [22], indicates

decreased anxiety in APP + PS1 mice on the elevated plus maze. The variation in APP/PS1 transgenes in mouse models makes it difficult to compare between studies, hence, it is important to include an assessment of anxiety levels in each APP/PS1 mouse model.

Previous reports of mouse models of APP overexpression have shown evidence of a reduction in body weight gain [30]. TASTPM transgenic mice failed to gain body weight at the same rate as wildtype controls of equivalent gender. This weight loss occurred despite TASTPM transgenic mice, of both genders, displaying greater frequency and duration of feeding bouts than wildtype controls, in the absence of any change in drinking patterns, when assessed using LABORAS. TASTPM transgenic mice, in comparison to age-matched C57BL6/J controls, also demonstrated increased food intake during a 24-h period in the home cage which corroborated with data obtained from LABORAS.

Since the reduction in body weight gain was significant prior to onset of amyloid neuropathology, it is likely to be a developmental problem. Lalonde et al. [30] attributed such an effect to reduced milk consumption during weaning and a lack of compensation in feeding post-weaning. Data presented here suggests TASTPM APP/PS1 mice attempt to compensate for reduced body weight post weaning. This is evident as increased frequency and duration of feeding and increased food intake. It would be interesting to investigate the specific reasons for these findings in further studies since it is unclear why compensatory feeding fails to rebalance body weight as the animals' age.

Body weight changes may be indicative of ill health in transgenic mice and a lack of weight gain may predict subsequent mortality. TASTPM transgenic mice used in this study did not appear to demonstrate any overt signs of deterioration in health that correlated with premature mortality, however, predominantly female TASTPM transgenic mice died prematurely. Female TASTPM transgenic mice were reported to have increased amyloid load relative to male TASTPM transgenic mice [17]. Previous literature reports the use of aging transgenic mice that have not demonstrated early mortality despite enhanced amyloid load [63] however numerous reports by Moechars et al., also suggest that early mortality is evident in a majority of APP overexpressing lines [39–42,27,18,19,25,8,9,24]. High levels of mutant APP expression appear to correlate with premature mortality [39] hence; decreasing amyloid load may reduce the risk of premature mortality. Leissring et al. reported that mice with Swe/Indiana APP mutations demonstrated a decline in amyloid brain levels that correlated with a reduction in premature mortality [33]. This may suggest a correlation between premature death and amyloid levels in APP/PS1 overexpressing mouse mice.

Overall, following the longitudinal behavioural assessment of TASTPM mice in a variety of assays, it is clear that decreased weight gain, associated alterations in feeding patterns and changes in activity are most likely a result of developmental problems rather than amyloid neuropathology. In contrast, there were age-dependant changes in anxiety and aggression evident in TASTPM transgenic mice relative to wildtype controls. A longitudinal behavioural assessment requires the repeat testing of all subjects and it is possible that the age-dependant memory deficits exhibited by APP and APP/PS1 transgenic mice may influence the performance of transgenic mice during repeat behavioural testing. Assessing TASTPM mice for reactivity to a novel environment demonstrated that during the subsequent test of locomotor activity in a familiar environment, activity in both TASTPM transgenic and wildtype mice was decreased by approximately 500 beam breaks. This suggests that, at least in short duration behavioural assays, TASTPM transgenic mice can remember the test environment following a 24 h period. In-depth studies would be required to assess the contribution of both short and long term memory alterations demonstrated by transgenic mice to repeat behavioural tests where the delay between testing is longer than 24 h to accurately assess whether genotype differences in behaviour are related to deficits in memory.

In addition, TASTPM APP/PS1 overexpressing mice demonstrate Aβ-induced neuropathology, one aspect of the biochemical alterations that occur in AD. Hence, the non-cognitive behaviours evident in the human AD population may be more heterogenous than that demonstrated by APP/PS1 overexpressing mouse models. Despite this, increased levels of anxiety and aggression in APP/PS1 transgenic mice following onset of amyloid plaque pathology suggests a key involvement of amyloid neuropathology in some of the behavioural symptoms AD patients frequently display. The behavioural phenotype exhibited by TASTPM mice indicates the model could be successfully used to screen putative therapies for neuropsychiatric disorders that occur in AD.

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