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PERFORMANCE OF TRANSGENIC TgTau-P301L MICE IN A 5-CHOICE SERIAL REACTION TIME TASK (5-CSRTT) AS A MODEL OF ALZHEIMER'S DISEASE

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SUMMARY

Alzheimer's disease is increasing to epidemic levels with an estimated 36 million people affected worldwide (Wimo 2010). The aetiology of the disease is not known, which is hindering the progression of the treatment. This study is a longitudinal investigation into the performance of TgTauP301L mice as an animal model of Alzheimer's disease on the computer automated touchscreen 5-choice serial reaction time task (5-CSRTT). TgTauP301L mice have a single tau mutation in the P301L gene and develop the tau pathology that represents the observed tauopathy in patients with Alzheimer's disease.

The aim of the investigation is to observe if tau pathology in the TgTauP301L mice causes a cognitive impairment in attention and executive function and at what stage this can be identified by the 5-CSRTT task. This will establish if the animals can be used as a therapeutic model for pre-clinical drug trials and help to identify an early indicator and intervention point in patients with Alzheimer's disease. The animals have previously been studied at 5-months and no differences between performances of the TgTauP301L mice and wild type mice were found (unpublished data). This study measured the performance of the animals at 7-months which is when the tauopathy begins to develop in TgTauP301L mice (Murakami 2005). The results of this study showed that there was no deficit in the performance of the TgTauP301L compared to the wild type mice and there had been no change in the animals' performance compared to at 5-months. The animals will be retested at 12-months once the pathology has extensively spread to see if the tauopathy causes a deficit in performance.

Key words: *Alzheimer's disease - TgTauP301L mice - tau pathology*

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INTRODUCTION

Cognitive deficits of Alzheimer's disease

Alzheimer's disease is often regarded as a memory disorder because patients commonly present with loss of episodic memory (Huff 1987). However, more recent research has shown that when a patient presents with loss of memory there has already been extensive development of pathology. The initial dysfunctions of Alzheimer's disease are thought to be in cognitive deficits specifically in attention and executive control. These have been shown to occur before dysfunction in language, visuospatial and memory domains and may account for impaired performance on other cognitive abilities and difficulties with daily living (Patterson 1996, Buchner & Larson 1987). Within the attentional domain there is a functional and anatomical separability. Patients at the early stages of Alzheimer's disease show a deficit in divided and selective attention e.g. set shifting and response selection, whilst sustained attention is preserved and patients are able to focus their attention on a task over long periods of time (Parasuraman & Haxby 1993, Lezak 1983). Executive functions require cognitive capabilities to plan, initiate and regulate behaviour and actions. Patients with mild Alzheimer's disease show deficits in response control e.g. impaired inhibitory control, which suggests that patients suffer with executive dysfunction. Therefore monitoring attention and executive functions may provide an early diagnostic tool for the disease (Parasuraman & Haxby 1992) that will dissociate Alzheimer's disease from normal ageing.

Tau pathology of Alzheimer's disease

Alzheimer's disease is a neurodegenerative disorder associated with the appearance of beta-amyloid plaques and abnormally hyperphosphorylated tangles of tau protein in the brain (Braak & Braak 1991). Tau is a structural protein that binds and stabilises microtubules and is highly expressed in the axons of neurons and glia. The disruption of the tau protein makes Alzheimer's disease an example of tauopathy. Alzheimer's disease is characterised by the pathological development of intraneuronal fibrous material of hyperphosphorylated tau protein in the form of paired and straight helical filaments threads, predominately of three and four repeat tau isoforms (Greenberg & Davies 1990), as neuritic plaques and neurofibrillary tangles.

The tauopathy spreads via a hierarchical distribution through the medial temporal structures to the association cortices (Braak & Braak 1991) which is consistent with the loss of episodic memory. The early pathological markers are observed in the entorhinal region, specifically layer II (Braak & Braak 1991). The

destruction of this layer affects the transmission to the hippocampus, limbic and association cortices and progressively spreads to these areas. The tau pathology in the limbic cortex may account for the emotional disturbances; and the neurofibrillary tangles in the frontal cortex may contribute to the behaviour and executive dysfunctions (Chu 1997, Milner 1963). It has been proposed that the disease spreads synaptically but the mechanism of propagation is not exactly known (de Calignon 2012).

The development of the tau pathology is not unique to Alzheimer's disease and occurs in other tauopathies, e.g. frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17). FTDP-17 patients have behavioural, cognitive and motor abnormalities (Zbigniew 2006). FTDP-17 is caused by a mutation in the tau gene which leads to the neurodegeneration and pathological development of neurofibrillary lesions and nerve cell degeneration that spread from the temporal and frontal cortex which correlates with Alzheimer's disease (Goedert 2000). A family referred to as Seattle family A who suffered with FTDP-17 and had a mutation in V337M in the third microtubule binding domain of tau developed a tau pathology that was highly representative of Alzheimer's disease with respect to structural and biochemical characteristics; thus emphasising the similarities between FTDP-17 and the tau pathology of Alzheimer's disease (Spillantini 1996). The primary lesion in FTDP-17 is known to be in tau which provides a link between the dementia and tau pathology (Goedert 2000) and suggests that the tau pathology in Alzheimer's disease may be critical, particularly as it is present before significant amyloid beta depositions (Braak & Braak 1991). Given the similarities in the tau pathology of FTDP-17 and Alzheimer's disease, an FTDP-17 animal model is being used to measure the tauopathy in our study.

Animal model of Alzheimer's disease

Animal models provide a simplification of Alzheimer's disease by means of a controlled progression of the neuropathology. The effects of the neuropathology on the animals' cognitive abilities can be measured with behavioural tasks. Studies have shown that transgenic mice that develop both amyloid plaques and tau pathology display deficits in attention and response control when measured with 5-CSRTT (Romberg 2011). To distinguish if tau pathology has a primary causal role in the behavioural deficit in Alzheimer's disease, the mice that we are studying only have the single mutation, P301L.

TgTauP301L mice have a mutation at number 301 in exon 10 with proline changed to leucine amino acid (Murakami 2006). TgTauP301L mice express the FTDP-17 mutation within the longest form of tau (2N, 4R) which represents the biochemical and behavioural pathology of FTDP-17; therefore it is proposed to be a valid model of the tau pathology of Alzheimer's disease. The initial locus of pathology in TgTauP301L is in the hippocampal region which spreads to the amygdala and then the cerebral cortex as the animal ages; this represents the pathology in patients with Alzheimer's disease (Murakami 2006). The TgTauP301L mice develop glial and neurofibrillary tangles in addition to microglial pathology, but do not develop significant tau accumulation in the spinal motor neurons (Murakami 2006). Therefore the animals do not suffer with motor impairment which may disrupt performance on the task. TgTauP301L mice also develop activation of microglia with MHC class II in the: hyperphosphorylated tau regions, fibrillary neuritic plaques and frontal cortex which represents the pathology observed in patients with Alzheimer's disease (Sasakia 2008). Studies have observed that before age 5-months, TgTauP301L mice develop dot-like tau immunoreactivity in the neurons of the pyramidal cell layers of the hippocampus and amygdala, and there is 1.7 times greater net expression of tau-C compared to the controls (Murakami 2006). The pathology continues to progress after 5-months where tufted astrocytes and plaque-like deposits develop in the: cerebral cortex, hippocampus, striatum, brainstem and spinal cord (Murakami 2006). The pathological development has not been observed in the control littermates, which suggests that it is due to the mutation (Murakami 2006).

Our investigation will study the behaviour of the TgTauP301L animals as they age to represent the neurodegenerative nature of the disease. To-date there has been minimal reported behavioural testing before 9-months in TgTauP301L. By 9-months there has been significant development of pathology as there is evidence of the presence of neurofibrillary tangles and glial plaques in the temporal and frontal cortex and behavioural testing has observed a disturbance in spatial working memory (Murakami 2006, Sasakia 2008). By studying the effect of tau pathology on a behavioural task that measures early cognitive deficits of Alzheimer's disease at different stages of the pathology in the TgTauP301L mice it will provide a cognitive profile that can be translated to humans.

Cognitive behavioural task: 5-choice serial reaction time task (5-CSRTT)

Animals with mutations causing the development of amyloid plaques and tau tangles e.g. triple transgenic mice model of Alzheimer's disease (expressing three genetic mutations: APPswe, PS1M145V and tauP301L) show a deficit in performance in the 5-CSRTT compared to the wild-type controls (Romberg 2011). The triple transgenic mice performance were less accurate and showed impaired response control (higher amounts of perseveration) in comparison to the wild-type when the duration of stimulus presentation was shortened (Romberg 2011). The triple transgenic deficit suggest that the

animals had a failure to sustain attention across trials which required greater attentional load, which is comparable to the attentional and vigilance deficits displayed by patients with Alzheimer's disease (Baddeley 1999). Therefore by studying mice with only P301L mutation performing the same task as Romberg (5-CSRTT), we will establish if this decline in performance can be accounted for by the development of tau pathology.

The 5-CSRTT initially designed to study human attentional processes (Leonard 1959) has since been modified for mice and is now a computer automated touchscreen task (Humby 1999). The task measures the ability to selectively detect and appropriately respond to briefly presented visual stimuli that are presented pseudorandomly across 5 horizontal spatial locations. Accurate responding requires attention in visuospatial and temporal components and executive control therefore the task has been proposed to measure: selective, sustained and visuospatial attentional processes as well as response control. By changing the duration of the stimulus, it will manipulate the attentional load required because greater attention and impulse control is required to accurately localize shorter stimulus load (Muir 1996; Bushnell 1998).

The behavioural measures that the 5-CSRTT calculates are: accuracy, omissions, response latency, reward latency, premature responses, perseverative responses and beambreaks front and back (Carli 1983). These measurements are proposed to represent attention, motivation, motor control and response control (impulsivity and compulsivity). Some of the advantages of the 5-CSRTT are: the ease of translation between human touchscreen tasks and their rodent analogues, high degree of automation and standardisation of the experiments and the ability to test numerous subjects simultaneously (Bussey 1994, 2001, 2008, 2012). These increase the reliability when comparing longitudinally throughout this experiment, reduce the effect of extraneous variables and reduce experimental bias. The disadvantage to the 5-CSRTT is that the animals require a long training period to the task, but this is significantly reduced when the animals have follow-up testing. In addition the performance may be impacted by severe motor impairment and there is a low-reward motivation; however, careful monitoring of the dependent variables of the task can demonstrate if these factors may be impinging on performance. Therefore the 5-CSRTT is well suited to assess multiple aspects of attention and has been used to evaluate the neural processes underlying attentional processing in neurodegenerative disorders.

MATERIALS AND METHODS

Subjects

24 male mice were tested: 11 transgenic Tau P301L mice (proline to leucine amino acid mutation at number 301 in exon 10) and 13 wild type non transgenic littermates (as a control group) from University of Toronto, Canada (Murakami 2006). The mice were shipped to the UK for behavioural testing on 06/2012 and throughout the testing the experimenters were blinded as to which were the TgP301L and wild type mice. The animals were housed in groups of 2 or 3 (except mouse 17081 wild type which was housed in its own cage because of the death of his cage mate prior to the experimentation at 7 months). The housing room conditions were a constant temperature (20-24ºC) and humidity (55±10%). The animals were kept under a reversed light cycle (white lights on from 1900–0700 hours and red light from 0700–1900 hours) and all behavioural testing was conducted during the dark phase of the cycle. The mice were at 7-months of age when behavioural testing began but had been previously tested at 5-months of age therefore were already acclimatised to the local animal facility, housing conditions and at being handled. The animals were maintained on a restricted diet (85% of free feeding body weight during experimentation) to increase motivation for the experimental task but water was available ad libitum. All procedures were subject to UK Home office approval (Project license 80/2280) in accordance with UK Animals Scientific procedures Act (1986).

Apparatus

The mice were tested in Campden chambers and each mouse was habituated to their individual operant chambers (see figure 1) (Horner in press; Mar in press). The chambers have a trapezoidal shape (20 h x 18 l x 24 w cm), composed of 3 black plastic walls opening on to the touchscreen which is intended to direct the focus of the animal towards the touchscreen and reward delivery area. The animals are placed into the chamber through the transparent lid which can be secured with latches during the testing to form the roof of the chamber. The floor of the chamber is perforated stainless steel that is raised above a tray lined with filter paper.

Each chamber is equipped with the following: a fan (for ventilation and masking extraneous noise), tone and click generator, LED house light, magazine unit (for the food reward), two infrared photobeams for movement detection in the front (7cm from the screen) and the rear (3.5cm from the magazine) of the chamber, small infrared camera above the chamber to monitor animals' behaviour, touchscreen monitor (30.7cm screen resolution 800 x 600) that does not require the subject to exert any pressure in order for touches to be registered and stimuli (24.3h x 28.0w cm).

All experiments were run using Whisker Server and ABET computer software to control stimulus presentation, reward delivery and to record touchscreen responses and reward collection. The stimulus is pseudorandomly presented by a Latin square design where the stimuli are not presented in the same location more than twice consecutively.

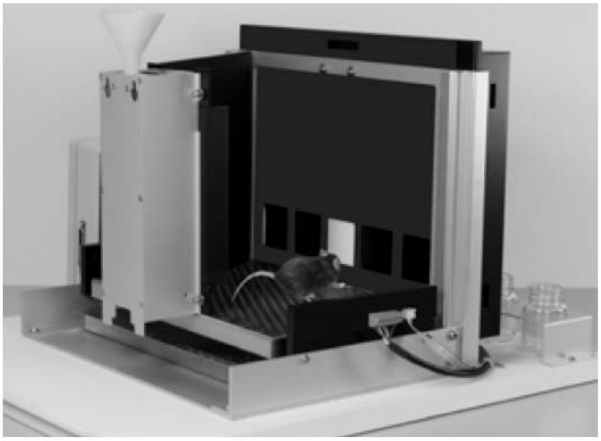

Figure 1. Image of a Campden experimental chamber. Touch-screen stimulus is on the right of the chamber and the magazine (reward collection site) is on the opposite

Reagents

The reagents used were: food reward liquid (20µl Yazoo® strawberry milkshake, Friesland Campina UK Ltd), rodent laboratory food pellets (Rodent Pellets, Special Diets Services, UK), surface disinfectant for cleaning (Trigene or 70% ethanol solution) and animal housing equipment (Horner, in press).

Procedure of the 5-choice serial reaction time task (5-CSRTT)

The animals had all been previously tested 2 months previously on the same experimental task therefore the animals were already habituated to the operant chambers and shaped to the task. The procedure used for the 5-CSRTT was with the same equipment and experimental design that has been previously described in detail by Romberg et al. (2011).

5-CSRTT training

The training requires the animals to detect a brief light stimulus out of the 5 horizontally presented locations. The mouse initiates a trial with a nose poke in the magazine and after a 5 second (s) delay one of the stimulus is pseudorandomly presented by a Latin square design. Before, during and after the presentation of the stimulus the touches to the screen are registered. Responses that occur between initiation of the trial and the stimulus presentation (during the 5s delay) are recorded as premature responses but are not punished. Responses that are correct (touching the stimulus that is lit up) are rewarded which is indicated by the magazine light turning on and the animal has access to the reward. There is a 5s inter-trial interval (ITI) during which no trial can occur to allow for collection and consumption of the reward, the end of the interval is indicated by a click sound. Responses that are incorrect (touching one of the other 4 blank screens) and if no response is made (recorded as an omission) the mouse receives a 5s 'time out' punishment where the chamber light turns and they do not receive access to the reward. The 'time out' punishment signals an incorrect response to introduces selectivity of the response to the stimulus that is lit up. After a correct or an incorrect response the stimulus is removed from the screen immediately.

5-CSRTT probes

When the mouse reaches criteria of greater than 80% accuracy and less than 20% omission for 2 consecutive sessions with 2s baseline stimulus duration, the animal begins on 5-CSRTT probe testing. Probes were tested 7 days a week between 1200 – 1400 hours and the session consisted of 40 trials or a maximum of trials in 60 minutes. Probes are identical to training except that the animals are challenged with reduced stimulus durations. The stimulus durations are reduced from 2s to 1.6s, 0.8s, 0.6s and 0.4s where each stimulus duration is tested for two consecutive days followed by 1 consecutive day of baseline 2s session. The decreasing stimulus duration increases the attentional demand of the task and therefore distinguishes animals that have poorer attentional processing. The intermittent baseline session is a low attentional demand to ensure that the same level of performance occurs for the following probe and that the animals do not disengage with the task.

DATA ANALYSIS AND RESULTS

Throughout the experiment the independent variables are the genotype of the mice (either TgP301L mice or wild type) and the stimulus duration of the probes and baseline trials are the dependent variables for the 5-CSRTT. During the task the following 7 behavioural dependent variable measures for each subject were recorded:

Accuracy: percentage of responses at the correct location divided by the total number of both correct and incorrect trials. This is thought to be the most direct measure of attentional processes.

Omissions: percentage of all the trials which the animal made no response divided by total trials which may indicate basic motivation, motor control, or attention across a delay.

Correct response latency: measures the time delay (ms) between the stimuli appearing on the screen and the animal making a response for correct and incorrect responses. These values may indicate basic motivation or motor control

Reward response latency: measures the time (ms) to collect reward in the magazine after a correct response, which may also represent basic motivation or motor control.

Premature responses: the number of touches to the screen during the 5s delay period after initiation and prior to the stimulus appearing divided by the total trials. This may provide a measure of impaired response control (impulsivity).

Perseverative correct: the number of additional screen touches made after a correct response prior to collecting the reward. This may provide a measure of impaired response control (compulsivity).

Beambreaks front and back: the number of times the mouse crosses the photo-beam at either the front or the back of the chamber. This monitors the motor behaviour of the mice.

Each dependent variable was analysed using SPPS version 21 (IBM) initially with an exploratory data analysis to compare the distribution of the performance of the wild types and TgTauP301L mice at different stimulus duration which revealed no outliers. The graphs produced on Microsoft Excel (2003) display mean scores with standard errors.

Pre-training to reach probes

The wild type and TgTauP301L mice acquired pre-training of the task reaching criteria (accuracy: >80%; omissions: <20%) without any apparent differences. The number of sessions to attain the specified performance to reach criteria were not significantly different between the genotype (see figure 2). The one-way ANOVA with between subject factor as 'genotype' and dependent variable as 'sessions' during pre-training shows no effect of genotype for all the sessions for accuracy and omission in reaching criteria (all sessions $p > 0.05$) (see appendix 1).

Baseline performance

The TgTauP301L and wild type mice both showed normal performance during baseline stimuli for 2s. 2s stimulus duration is a measure of attention at a relatively low demand which is not highly challenging and both groups perform equally well at this stimulus duration. The one-way ANOVA with between subject factor as 'genotype' and dependent variable as 'baseline' sessions reveal no effect of genotype for all intermittent baseline sessions. All variables are $p > 0.05$ for all baseline sessions (see appendix 2).

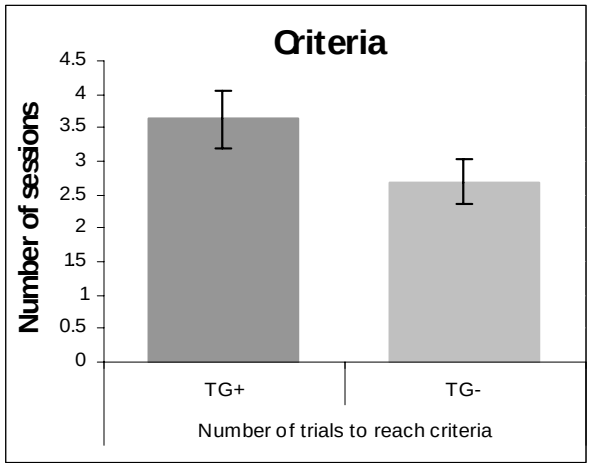

Figure 2. 5-CSRTT training data. The mean number of sessions (40 trials each) required to reach criteria at stimulus duration of 2s for TgTauP301L (n=11) and wild type mice (n=13). Mice were 7-months old at the onset of testing

Probe trials

After pre-training, the mice were challenged with shorter stimulus duration of: 1.6s, 1s, 0.8s, 0.6s and 0.4s. Repeated-measures ANOVA of the results of the dependent measures with within subject factor as 'stimulus duration of probes' and between subject factor as 'genotype' reveal that both TgTauP301L and wild type mice found the shorter duration challenging because there was a significant effect of stimulus duration ($p<0.05$) for both genotypes for all the different variables. But there was no difference between the genotypes ($p>0.05$) and no interaction difference between stimulus duration and genotype ($p>0.05$) (see appendix 3).

The accuracy and omissions data ANOVA showed there was an effect of stimulus duration ($p<0.05$) for both genotypes but there was no significant interaction between genotype and stimulus duration ($p>0.05$) and no effect of genotype ($p>0.05$). Therefore there appears to be no difference in attentional performance with the task between the different genotypes (Figure 3).

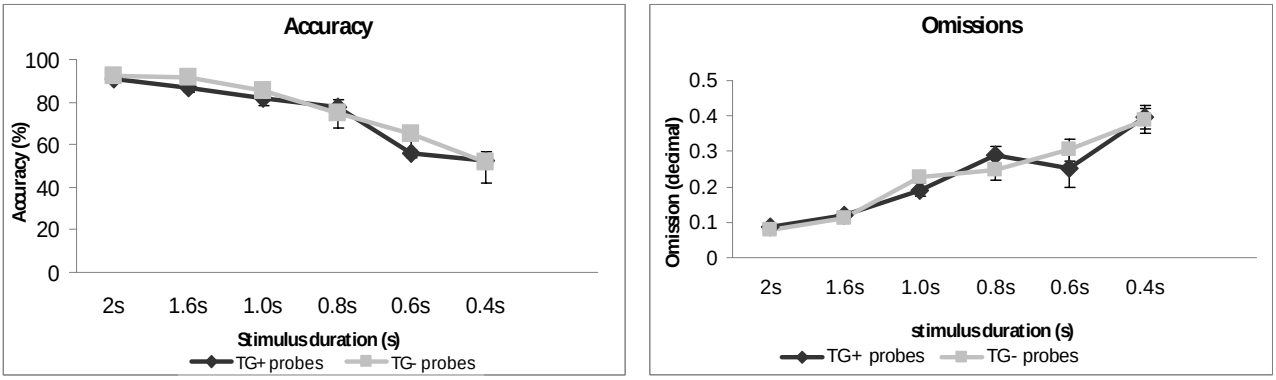

Figure 3. 5-CSRTT performance at reduced stimulus durations for accuracy (left); and omissions (right) for TgTauP301L n = 11 and wild type mice n = 13 at 7-months of age

The premature responses and perseveration between the genotypes was not different. The repeated-measures ANOVA showed that there was an effect of stimulus duration on the task ($p<0.05$) but there was no difference between genotype ($p>0.05$) and there was no interaction effect ($p>0.05$). Therefore there appears to be no difference in impulsive and compulsive behaviour between genotypes (Figure 4).

The response latency to the correct stimulus and reward response latency did not differ between genotypes. There was an effect of stimulus duration ($p<0.05$) but there was no genotype effect ($p>0.05$) and no effect of interaction between genotype and stimulus duration ($p>0.05$). Therefore there was no difference in motivation and engagement with the task between the different genotypes (Figure 5).

The beambreaks front and back measure the number of times the animal crosses the photobeam at the front or back chamber which provides an indication of the movement of the animal. There is an effect of stimulus duration on movement ($p<0.05$) but there is no effect of genotype ($p>0.05$) and no interaction effect ($p>0.05$). Therefore there was no difference in motor abilities between genotypes (Figure 6).

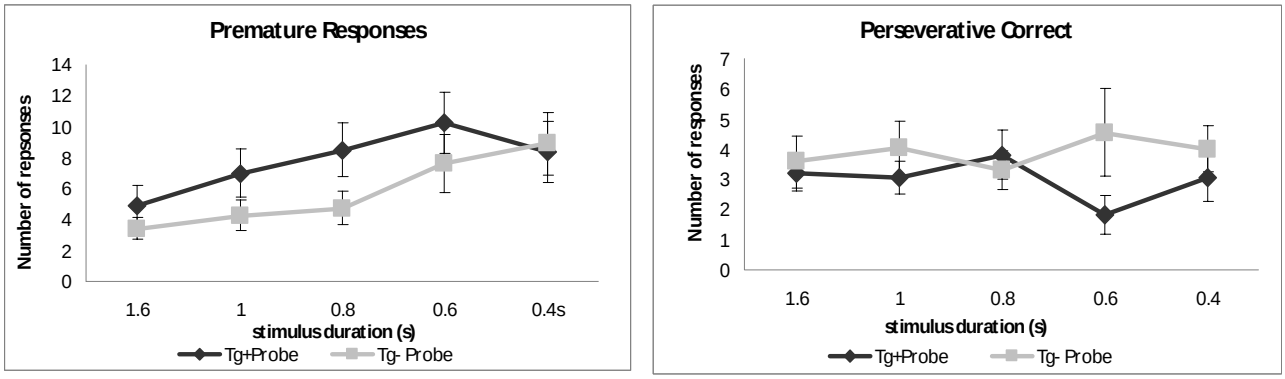

Figure 4: 5-CSRTT performance at reduced stimulus for premature responses (left); and preservative responses (right) for TgTauP301L n = 11 and wild type mice n = 13 at 7-months of age

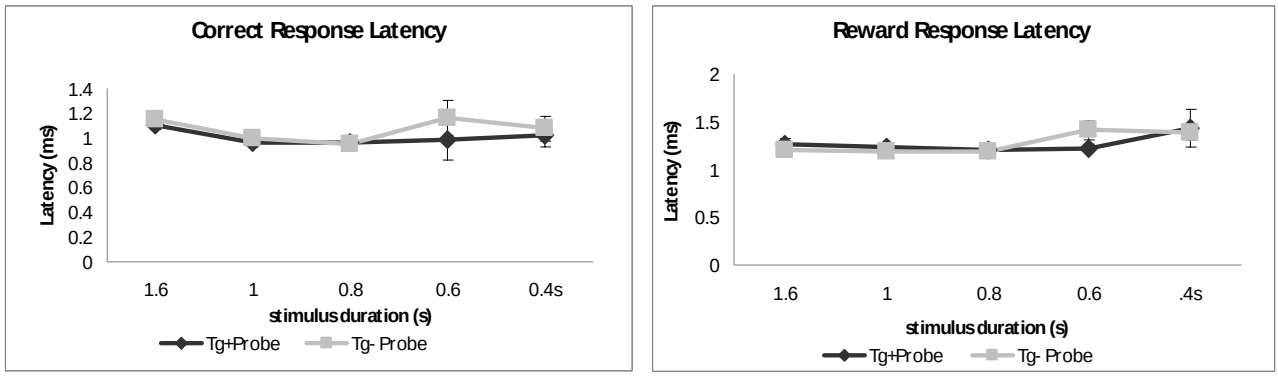

Figure 5. 5-CSRTT performance at reduced stimulus durations for correct response latency (left); and reward response latency (right) for TgTauP301L n = 11 and wild type mice n = 13 at 7-months of age

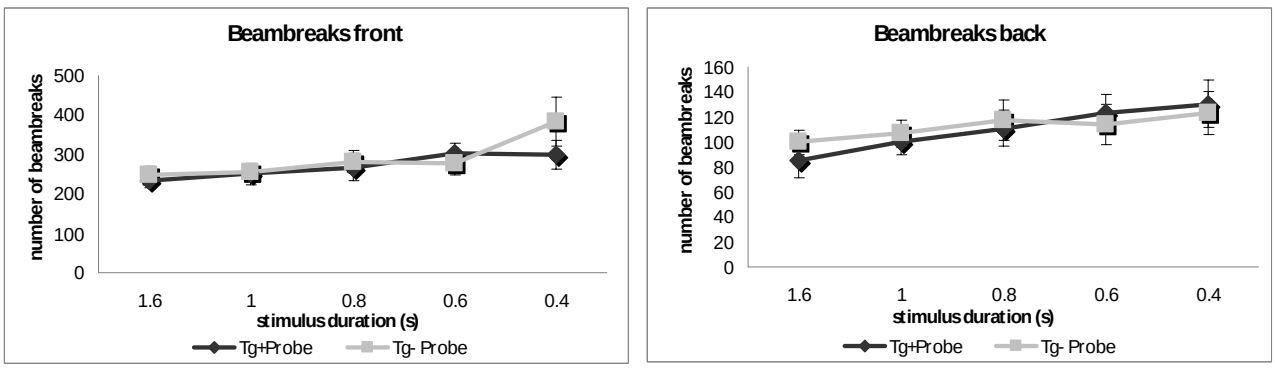

Figure 6. 5-CSRTT performance at reduced stimulus durations for beam breaks front (left); and beam breaks back (right) for TgTauP301L n = 11 and wild type mice n = 13 at 7-months of age

5-months and 7-months comparison

The performance of the mice at 7-months was compared to the performance at 5-months to observe if there had been a change in the ability to perform the task as the animal ages. The results of the repeated measures ANOVA with 'age' as the between subject factor and 'stimulus duration' as the within subject factor reveals that there has been no change in the performance of the task as the animal ages in either genotype groups. Stimulus duration interaction with age shows no significant difference ($p>0.05$) for wild type and transgenics and there is no effect of age ($p>0.05$) for both genotypes (see appendix 4).

DISCUSSION

Tau pathology

The absence of a behavioural impairment in the TgTauP301L mice is likely to be due to insufficient pathology development that has not spread to cause an impingement in the animals' behaviour. From 12-months of age and older the animals show significant tau positive neuronal and glial structures (Murakami 2006, Sasaki 2008). There is evidence of perineural neurofibrillary tangles in the cerebral cortex, hippocampus, amygdala and brainstem and there is a high presence of glial plaques in the cerebral cortex (Murakami 2006). By 18-months the TgTauP301L mice have significant synaptic density reduction and neuronal degeneration in the hippocampus (the pyramidal cell layers in CA1 and CA2 have almost disappeared) (Murakami 2006). There is also evidence of progressive white matter pathology (Lin 2005). Furthermore, behavioural testing with the Morris Water Maze and 8-arm radial task, which evaluate spatial memory, has revealed that the TgTauP301L mice have an impairment at 9-months and at 12-months because they have a longer path length and greater latency on the task performance (Murakami 2006). Therefore when the animals are re-tested on the 5-CSRTT at 12-months when the pathology has spread significantly (particularly within the hippocampus) it is predicted that the TgTauP301L mice will have an impaired performance in the task compared to the wild type.

There has been recent evidence to suggest that neurogenesis occurs at the initial stages of Alzheimer's disease because patients have an elevated expression of immature neuronal marker proteins that signal the birth of new neurons in the dentate gyrus of the hippocampus. There is evidence of an increase in the expression of nerve cell adhesion molecule (doublecortin polysialyated) and an increase in the neurogenic differentiation factor (tuc-4) in neurons in the dentate gyrus which is one of the primary sites of Alzheimer's disease (Jin 2004). It has been proposed that there is an increase in the neurogenesis to replace the degenerating neurons, but as the disease progresses the neurodegeneration exceeds the neurogenic capacity resulting in a net neuronal loss. However, the triple transgenic model of Alzheimer's disease (animals expressing three genetic mutations: APPswe, PS1M145V and tauP301L) do not show an increase in neurogenic capacity; instead an age dependent decrease was observed (Rodriguez 2008). But there has been limited investigation on the effects of only Tau pathology on neurogenesis. Therefore the lack of change in the performance of the TgTauP301L mice from 5-months to 7-months could be due to increase in neurogenic capacity replacing the lost neurons. At the end of this longitudinal study the animals will be sacrificed and their brain tissue will be examined for areas of neurogenesis and neurodegeneration to observe whether Tau pathology has a role in either process.

Although biochemically TgTauP301L mice model have shown to provide an accurate representation for the tauopathy of Alzheimer's disease, it may not be valid because of anatomical differences between FTDP-17 and Alzheimer's disease. TgTauP301L mice are a FTDP-17 model therefore the pathology develops in the frontotemporal regions whereas patients with Alzheimer's disease develop pathology that is in focused in the frontoparietal region. Other animal models for example THY-Tau 22 (express human 4-repeat tau) may provide a better representation of the disease. THY-Tau animals display: hyperphosphorylation, neurofibrillary tangles and astrogliosis on several Alzheimer's disease-relevant anatomical sites (Schindowski 2006). In addition the THY-Tau22 mice display increased anxiety, delayed learning from as early as 3-months and reduced spatial memory at 10-months (Schindowski 2006). Therefore tau pathology in Alzheimer's disease may cause cognitive deficits, but the TgTauP301L animal model may not provide a useful model to represent these deficits that have been observed in patients with Alzheimer's disease.

Other theories of Alzheimer's disease suggest that amyloid-beta peptide overproduction or failure to clear this protein result in amyloid deposition, which results in tau pathology and subsequent neurodegeneration (Hardy 1991). Therefore the attentional deficit observed in triple transgenic Alzheimer's model (Romberg 2011) may be due to the amyloid plaque rather than the tauopathy.

5-CSRTT task

The 5-CSRTT is a behavioural measure of visuospatial attention. The 5-CSRTT may not be a sensitive measure of the animals' cognitive performance because the TgTauP301L mice may be able to use an alternative strategy to complete the task. Human studies suggest that spatial attention is the deficit observed in patients with mild Alzheimer patients and they are not impaired on a visual discrimination touch screen task (Lee 2007). In addition neuroimaging studies have also shown that there is a neuropsychological dissociation between visuospatial attention systems serving to integrate

selective attention, vigilance and dividend attention (Posner & Dehaene 1994, Posner & Peterson 1990). Therefore the TgTauP301L mice may be able to complete the 5-CSRTT task by compensating their deficit in spatial attention with visual attention.

CONCLUSION AND FUTURE DIRECTION

This longitudinal study has found that at 7-months age there are no differences in the performance of TgTauP301L and wild type mice in the 5-CSRTT. Therefore the tau pathology in the TgTauP301L does not appear to be impinging in visuospatial, attentional and executive abilities.

The follow-up study of the TgTauP301L mice performance on the 5-CSRTT at 12-months will help to establish if the tau pathology causes a cognitive and behavioural deficit. If the TgTauP301L mice show a deficit in performance it will provide supporting evidence that the tauopathy aspect of Alzheimer's disease is critical in the cognitive impairment in attention and response control. Therefore this longitudinal study into the performance of TgTauP301L in the 5-CSRTT may provide a cognitive profile of the animals that can be translated to the phenotype of Alzheimer's disease. This will establish if the animals can be used in pre-clinical drug trials to test the drug's feasibility, safety and efficacy before entering clinical trials.

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Conflict of interest: None to declare.

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Appendix 1. One-way ANOVA analysis of pre-training of the mice to reach criteria

	Session 1		Session 2		Session 3		Session 4		Session 5		Session 6	
	F	sig	F	sig	F	sig	F	sig	F	sig	F	sig
Accuracy	1.18	0.289	3.195	0.088	4.861	0.038	1.202	0.285	0.724	0.404	1.009	0.326
Omissions	0.77	0.388	3.539	0.073	0.719	0.406	1.939	0.178	0.646	0.43	0.036	0.852

One-way ANOVA output summary of the pre-training at 2s stimulus duration with subject factor as 'genotype' (wild type (n=13) and TgTauP301L (n=11)) and dependent variable as 'sessions to acquire criteria.' The one-way ANOVA was conducted for accuracy and omissions performance for all pre-training sessions shows no difference between wild type and TgTauP301L mice in acquisition of criteria (criteria: accuracy <80%; omissions >20%). Accuracy: $p>0.05$; Omissions: $p>0.05$ for all the different sessions.

Appendix 2. Baseline performance of the mice at 2s stimulus duration

	TgTauP301L		Wild type	
	Average	SEM	Average	SEM
Accuracy	86.600	1.260	88.470	0.930
Omissions	0.0870	0.010	0.079	0.0077
Premature responses	5.500	0.610	3.820	0.470
Perseverative correct	3.780	0.280	4.590	0.460
Correct response latency	1.139	0.017	1.140	0.018
Reward latency	1.300	0.020	1.280	0.020
Beambreaks front	231.230	9.880	247.320	10.10
Beambreaks back*	80.500	4.550	94.025	4.820

The mean and standard errors (SEM) of all the baseline sessions of wild type (n = 13) and TgTauP301L (n = 11) mice for the different dependent variables.

One-way ANOVA analysis of baseline performance at 2s stimulus duration

	Session 1		Session 2		Session 3		Session 4		Session 5	
	F	Sig	F	sig	F	sig	F	sig	F	Sig
Accuracy	0.136	0.715	0.119	0.733	0.126	0.726	0.645	0.430	0.840	0.369
Omissions	0.135	0.717	0.592	0.450	0.153	0.700	1.186	0.288	0.010	0.971
Premature responses	0.031	0.862	1.201	0.285	0.277	0.604	0.523	0.477	0.122	0.730
Perseverative correct	0.088	0.770	0.033	0.858	1.603	0.219	0.451	0.509	0.446	0.511
Correct response latency	2.537	0.125	0.004	0.952	0.271	0.608	0.480	0.496	0.839	0.370
Reward latency	1.677	0.209	0.008	0.930	0.734	0.401	1.255	0.275	0.753	0.395
Beambreaks front	0.646	0.430	0.010	0.973	0.567	0.459	0.000	0.993	1.155	0.294
Beambreaks back*	0.088	0.770	0.033	0.858	1.603	0.219	0.451	0.509	0.446	0.511

One-way ANOVA output summary of the performance of the mice at the intermittent baseline sessions (2s stimulus duration) for the different variables. For each variable the data was analysed with between subject factor as 'genotype' (wild type (n = 13) and TgTauP301L (n = 11)) and dependent variable as 'intermittent sessions.' The one-way ANOVA shows no difference between wild type and TgTauP301L mice in baseline performance for all the variables.

* The chamber for mice 17004 had faulty equipment for calculating beambreaks front therefore n = 12 for wild type for beambreaks front one-way ANOVA

Appendix 3. Repeated-measures ANOVA analysis of probes performance

	Interaction: stimulus duration*genotype			Tests of within subject effects		Tests of between subject effects	
				Stimulus duration		Genotype	
	F	df	sig	F	sig	F	sig
Accuracy	2.569	5,18	0.064	113.981	0	1.082	0.310
Omissions	1.206	5,18	0.346	26.760	0	0.017	0.898
Premature responses	1.066	5,18	0.412	5.614	0	1.675	0.209
Perseverative correct	1.409	5,18	0.268	5.436	0	1.1019	0.324
Correct response latency	2.327	5,18	0.085	5.140	0	0.750	0.396
Reward latency	2.477	5,18	0.071	5.751	0	0.032	0.860
Beambreaks front	0.684	5,18	0.642	6.023	0	0.592	0.450
Beambreaks back*	0.478	5,17	0.788	5.069	0	0.119	0.733

Repeated-measures ANOVA output summary of the probe trials at shorter stimulus duration (2s, 1.6s, 0.8s, 0.6s and 0.4s). For each variable the data was analysed with between subject factor as 'genotype' (wild type: n = 13; TgTauP301L: n=11) and the within subject factor as 'stimulus duration of probes'. The repeated-measures ANOVA shows that there was an effect of shorter stimulus duration on the performance ($p<0.05$) but there was no effect on genotype ($p>0.05$) and no interaction effect ($p>0.05$) for all the different variables.

* The chamber for mice 17004 had faulty equipment for calculating beambreaks front therefore n = 12 for wild type for beambreaks one-way ANOVA

Appendix 4. Repeated measures ANOVA analysis comparing wild type mice performance at 5 and 7 months

	Interaction: stimulus duration*age			Tests of within subject effects Stimulus duration		Tests of between subject effects Age	
	F	df	sig	F	sig	F	sig
Accuracy	1.864	4,20	0.156	105.607	0	0.06	0.809
Omissions	1.171	4,20	0.353	41.992	0	0.12	0.592
Premature responses	0.871	4,20	0.499	7.7	0	0.138	0.713
Perseverative correct	0.258	4,20	0.901	7.18	0	2.237	0.109
Correct response latency	2.855	4,20	0.051	8.775	0	2.476	0.129
Reward latency	0.585	4,20	0.677	7.026	0	0.095	0.76
Beambreaks front	0.184	4,20	0.944	1.687	0.16	0.015	0.905
Beambreaks back	0.660	4,19	0.627	2.825	0.03	0.11	0.743

Repeated-measures ANOVA output summary of the 5 and 7 months probe trials of the wild type mice. For each variable the data was analysed with between subject factor as 'age' (5-months: n=12*; 7-months: n = 13) and the within subject factor as 'stimulus duration of probes'. The repeated-measures ANOVA shows that there was an effect of shorter stimulus duration on the performance ($p<0.05$) but there was no effect of age ($p>0.05$) and no interaction effect ($p>0.05$) for all the different variables.

* Mouse 17007 did not reach criteria at 5-months of age therefore was excluded in the experiments but did reach criteria at 7-months of age

Repeated measures ANOVA output summary for TgTauP301L mice performance at 5 and 7 months

	Interaction: stimulus duration*age			Tests of within subject effects Stimulus duration		Tests of between subject effects Age	
	F	df	sig	F	sig	F	sig
Accuracy	1.885	4,17	0.159	58.871	0.000	1.019	0.325
Omissions	1.455	4,17	0.259	24.802	0.000	1.377	0.288
Premature responses	2.285	4,17	0.103	1.728	0.152	1.808	0.194
Perseverative correct	2.844	4,17	0.084	3.594	0.010	0.655	0.41
Correct response latency	0.770	4,17	0.559	14.225	0.001	1.790	0.201
Reward latency	3.665	4,17	0.025	9.916	0.005	1.633	0.216
Beambreaks front	0.830	4,17	0.521	2.094	0.089	0.001	0.974
Beambreaks back	0.710	4,17	0.597	3.663	0.009	0.287	0.598

Repeated-measures ANOVA output summary of the 5 and 7 months probe trials of the TgTauP301L mice. For each variable the data was analysed with between subject factor as 'age' (5-months: n=11; 7-months: n=11) and the within subject factor as 'stimulus duration of probes'. The repeated-measures ANOVA shows that there was an effect of shorter stimulus duration on the performance ($p<0.05$) but there was no effect of age ($p>0.05$) and no interaction effect ($p>0.05$) for all the different variables.

*The chamber for mice 17004 had faulty equipment for calculating beambreaks front therefore n=12 for wild type for beambreaks one-way ANOVA

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