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Author/s:

Murphy, M;Wilson, YM;Vargas, E;Munro, KM;Smith, B;Huang, A;Li, QX;Xiao, J;Masters, CL;Reid, CA;Barrett, GL

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Reduction of p75 neurotrophin receptor ameliorates the cognitive deficits in a model of Alzheimer's disease

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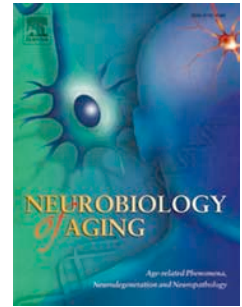
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p75 reduction ameliorates the cognitive deficits in a model of Alzheimer's disease

Authors: Mark Murphy^{1*}, Yvette M. Wilson^{1*}, Ernesto Vargas², Kathryn M. Munro¹, Belinda Smith³, Amy Huang³, Qiao-Xin Li², Junhua Xiao¹, Colin L. Masters², Christopher A. Reid² and Graham L. Barrett³.

Departments of ¹Anatomy and Neuroscience, ²The Florey Institute of Neuroscience and Mental Health, and ³Physiology, University of Melbourne, Melbourne, Victoria 3010, Australia. *These authors contributed equally to this work.

Corresponding author: Mark Murphy

Department of Anatomy and Neuroscience

University of Melbourne

Grattan St

Melbourne

Victoria 3010

Australia

Phone 613 8344 5785

Email: m.murphy@unimelb.edu.au

ABSTRACT

Alzheimer's disease (AD) is an extremely prevalent cause of dementia. It is characterized by progressive memory loss, confusion and other behavioural and physiological problems. The Amyloid- β ($A\beta$) protein is thought to be involved in the pathogenesis of AD and there is evidence that $A\beta$ may act through the p75 neurotrophin receptor (p75) to mediate its pathogenic effects. This raises the possibility that reducing levels of p75 could be a treatment for AD by preventing the effects of $A\beta$. In this study we have crossed the transgenic AD model mice, Tg2576, with p75^{-/-} mice to generate Tg2576/p75^{+/-} mice with reduced levels of p75. These mice are rescued from the deficits in learning and memory and hippocampal function which were found in the Tg2576 mice. These findings suggest that reduction of p75 can ameliorate some of the primary symptoms of AD.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder which often initially manifests as short term memory loss and over a period of years progresses to more severe long term memory loss, confusion, changes in mood and ultimately loss of bodily functions leading to death. It is the most common form of dementia and usually occurs over the age of 65. It is accompanied by characteristic brain pathology, the hallmark of which is the appearance of amyloid plaques and neurofibrillary tangles in the brain. The cholinergic neurons in the basal forebrain are also lost from early times in the progression of AD.

A prominent theory for the development of AD is that a 42 amino acid peptide, termed amyloid β ($A\beta$) is involved, at least in some forms of AD (Benilova et al., 2012; Crouch et al., 2008; Hardy and Selkoe, 2002). In this theory, people who develop AD have high levels of $A\beta$ due to either overproduction or poor degradation. As a result, $A\beta$ aggregates into oligomers which act on neurons to inhibit synaptic activity and are directly neurotoxic (Benilova et al., 2012; Crouch et al., 2008; Hardy and Selkoe, 2002). Continued aggregation of the $A\beta$ oligomers results in the formation of the amyloid plaques.

Alternatively, the early loss of the cholinergic neurons in AD forms the basis of the cholinergic theory of AD. An important role of the cholinergic input to the hippocampus is the regulation of memory processing (Bartus et al., 1982; Bartus et al., 1981). The cholinergic input to the cerebral cortex is thought to be important in the processes of selective attention and arousal (Richardson and DeLong, 1988). Memory impairment and attentional deficits are two of the hallmarks of AD, and are consistent with the importance of the cholinergic deficit. The drugs that are presently used to treat dementia in AD are cholinesterase inhibitors that work by increasing the lifetime and concentration of acetylcholine in the brain.

The p75 neurotrophin receptor (p75) is involved in signaling of the four "classical" neurotrophins and their pro-form precursors (e.g. Pro Nerve Growth Factor (NGF)). In the

mature brain, p75 is only expressed on the cholinergic neurons of the basal forebrain (Schatteman et al., 1988). However studies of mice deleted for the p75 gene (p75^{-/-} mice) indicate that p75's predominant action on these cholinergic neurons is negative regulation of growth and neurotransmitter synthesis (Barrett et al., 2010; Greferath et al., 2000; Yeo et al., 1997; Zagrebelsky et al., 2005). Our studies showed p75^{-/-} mice displayed marked improvement in spatial memory on the Barnes maze and enhanced hippocampal LTP compared to control mice (Barrett et al., 2010; Greferath et al., 2000), albeit others find subtle spatial memory impairment of p75^{-/-} mice in the Morris water maze (Catts et al., 2008; Dokter et al., 2014). p75 can also trigger apoptosis in developing neurons in the peripheral nervous system (Barrett and Bartlett, 1994; Rabizadeh et al., 1993).

The fact that p75 negatively regulates growth and function of the forebrain cholinergic neurons, and that cholinergic degeneration is prominent in AD is intriguing. It is possible that p75 is involved in cholinergic degeneration in AD. Indeed there is accumulating evidence that p75 binds A β directly and this binding may be responsible for some of the neurotoxic actions of A β (Coulson et al., 2009; Dechant and Barde, 2002; Fombonne et al., 2009; Knowles et al., 2009; Yaar et al., 2002). In a mouse model of AD (Thy1-hAPP^{Lond/Swe}) with genetic ablation of p75, there was reversal of forebrain cholinergic neurite degeneration and decreased hippocampal neuritic dystrophy compared with Thy1-hAPP^{Lond/Swe} mice expressing normal levels of p75 (Knowles et al., 2009). These findings thus indicate that p75 is involved in cholinergic degeneration in AD.

What is unclear is if reducing levels of p75 in the brain can result in a rescue in the primary symptoms of AD, the memory deficits. In order to investigate this possibility we have studied a mouse model of AD with genetically reduced levels of p75. We have used the Tg2576 mouse, which expresses the human APP gene, containing the Familial AD Swedish mutation K670N/M671L, throughout the brain. The Tg2576 mouse was crossed with p75^{-/-}

mice to generate Tg2576/p75^{+/-} mice. Our findings suggest that reduction of p75 can attenuate the behavioural deficits in this model of AD.

METHODS

Animals and genotyping

Male and female p75^{-/-} mice (Lee et al., 1992), were bred and maintained on a 129T2/Sv genetic background at the Biological Research Facility, University of Melbourne (Melbourne, Australia). Tg2576 mice were imported from Jackson Laboratories (Maine, USA) and were backcrossed 10 times with 129T2/Sv mice to generate Tg2576 mice on an essentially pure 129T2/Sv genetic background. All mice were subsequently housed in the Biological Research Facility, University of Melbourne. These Tg2576 mice were then crossed with p75^{-/-} mice to generate Tg2576/p75^{+/-} mice. Littermates were used for all behavioural experiments. Mice were kept on a 12h light: 12h dark cycle schedule in groups of one to five per cage. The mice were given food and water *ad libitum* during housing and treated humanely throughout. All procedures were approved by the animal ethics committees of The University of Melbourne and the Howard Florey Institute.

Polymerase chain reaction (PCR) was used to determine Tg2576 and p75 genotypes. The following alleles were tested for each sample: p75 wild type, p75 mutant and Tg2576. The primers sets used for the p75 genotype were as follows: p75 common forward primer GCTCAGGACTCGTGTCTCC; p75 wild type reverse primer CCAAAGAAGGAATTG GTGGA; p75 mutant reverse primer TGGATGTGGAATGTGTGCGAG. The p75 common forward and wild type reverse primers yielded a 386 bp product and the p75 common forward and p75 mutant reverse primers yielded a 193 bp product. Primers for the Tg2576 transgene were: forward primer GGTGAGTTTGTAAGTGATGCC; reverse primer

TCTTCTTCTTCCACCTC, yielding a 360 bp product. The PCR cycle for all alleles comprised incubation for 10 min at 96°C, 30 cycles each of 45 s at 95°C, 60°C, and 72°C, then seven min at 72°C.

Western blot analysis

Brains of wt, p75^{+/-}, Tg2576 and Tg2576/p75^{+/-} mice were isolated and lysed as previously described (Wong et al., 2013; Xiao et al., 2010). Total protein was separated by SDS-PAGE, transferred to PVDF membrane, and probed with antibodies against p75 intracellular domain (#G712A, Promega & # 9992, a gift from Prof. Moses Chao), full-length p75 (#9561, a gift from Prof. Moses Chao) or β -actin (#5541, Sigma) as a loading control. The two antibodies against p75 intracellular domain have been previously shown to detect both full-length p75 protein and its cleaved intracellular fragment in either tissues or cell lines (Matusica et al., 2013; Xiao et al., 2010; Zampieri et al., 2005). They are also sufficient to detect the short form p75 that incorporates the intracellular domain of p75 (von Schack et al., 2001). All blots were imaged and visualised using a Chemiluminescence imaging machine (BioRad ChemiDoc MP) and only unsaturated blots were used for densitometric analysis. All blots shown are representative of at least 3 independent experiments. The optical density value for each band was determined using NIH ImageJ, corrected to the actin loading control, then normalised against the wild type control animals.

Fear conditioning

Fear conditioning was undertaken on 4 – 7 month old male mice as a modification of that previously described (Wilson et al.; Wilson et al., 2011), in a plexiglass chamber equipped with a shock grid floor made of stainless steel rods. On training day, mice were placed in the shock chamber and allowed to explore for 180 s before receiving a 1.0 mA

shock for 3 s duration. After the shock, the mice remained in the chamber for an additional 60 s before being returned to their home cage. Testing occurred 24 h later, when the mice were returned to the chamber for 180 s and no shock was administered. All training and testing sessions were recorded by a miniature colour camera (GeoVision, Clayton, Victoria, Australia) located directly above the chamber. Scoring occurred either live during training and testing or later using the recorded videos. One video rater did all the scoring, and was not aware of the genotype of the mice. Freezing time (Blanchard and Blanchard, 1988) was scored with the criterion for freezing behaviour being the absence of movement except breathing. Freezing time was measured continuously during two periods: 1. During the 60 s immediately post shock, and 2. during the 3 min period of testing; and was expressed as percent of time each mouse froze during this period. The minimum time the mouse must be still to be scored as freezing is approximately 2 seconds.

Y Maze

A modified Y maze with visual cues was used as a test of spatial memory. The exploratory Y maze consisted of three equally spaced arms with the dimensions: 30 cm (length) x 11 cm (width) x 18 cm (height). Images were placed along and at the end of the arms as visual cues. In the training trial, one arm of the maze was blocked by a removable divider. The mouse was placed in the home arm and was free to explore the two available arms for ten min. After a two h interval, each mouse performed a test trial whereby all arms were available to explore including the third 'novel' arm. During testing, the mouse was placed in the home arm to start and was then free to explore all three arms for five min. The amount of time spent in the novel arm was compared to the average time spent in each of the two 'familiar' arms. The maze was thoroughly cleaned between each trial. An entry was scored when the mouse put all four paws in an arm. Between 23 and 33 mice per genotype

were included in the final analysis. Mice were excluded from analysis if they did not leave the home arm within the first min of training or testing, or if they did not investigate all three arms during testing (mice excluded: wild type = 5; Tg2576 = 7; p75^{+/-} = 2; Tg2576/p75^{+/-} = 2). Male and female mice between 4 – 6.5 months old of each genotype were included as there was no significant effect of gender.

Barnes Spatial Maze

Male and female mice between 5 – 8 months and 12 – 14 months old were subjected to a modification of the Barnes spatial maze (Bach et al., 1995). The maze consisted of a white circular platform (1.2 m in diameter and 0.9 m above floor level) containing 40 holes of 5 cm diameter spaced evenly around the periphery. An escape tunnel was positioned underneath one of the holes. The spatial version of the task requires that the location of the escape tunnel remain fixed for each mouse for all trials. Visual cues were placed around the platform at a distance of at least 50 cm from the platform. On the first day of behavioral training, trained mice were first adapted to the maze by being placed in the escape tunnel for 2 min. The first training trial commenced immediately following the adaptation trial. At the beginning of each trial, the mouse was placed under a cylindrical chamber in the middle of the platform. Lights (500 W) were turned on and after 20 s had elapsed, the chamber was lifted and the trial commenced with the mouse free to explore the maze. Following a further 20 s, the second aversion stimuli, a buzzer (80 dB), was turned on. The trial ended when the mouse entered the escape tunnel or after 4 min had elapsed. When the mouse entered the escape tunnel, the buzzer was turned off and the mouse was allowed to remain in the dark for 1 min. Following each trial, the platform was rotated and the surface wiped down with 70% ethanol to ensure randomization of the particular hole by which the mouse accessed the escape tunnel and prevent the use of olfactory cues.

Mice were given three trials a day until they reached the spatial learning criterion (3 or less errors on 3 consecutive trials). A record of the number of errors, as well as the type of search strategy employed by the mice based on the order of holes searched was recorded for each trial according to that described by Bach et al., (1995). A random search strategy entails exploring many holes in a haphazard, unsystematic fashion with many centre crossings. The serial search strategy is characterized by the animal exploring consecutive holes in a clockwise or counter-clockwise fashion. The most efficient spatial search was when the position of the escape tunnel was located in 3 or less errors by the mouse moving directly from the centre of the maze in the direction of the escape tunnel.

Electrophysiology

Hippocampal slices were cut from 4 month old mice (Reid and Clements, 1999). Briefly, mice were anesthetized with isoflurane, decapitated and the brain removed and placed immediately into ice-cold *cutting* artificial cerebro-spinal fluid (aCSF), containing 125 mM NaCl, 3 mM KCl, 1.25 mM KH_2PO_4 , 25 mM NaHCO_3 , 7 mM MgCl_2 , 1 mM CaCl_2 , and 10 mM glucose, saturated with 95% O_2 and 5% CO_2 . 300 micron thick horizontal sections were cut with a vibratome and transferred to normal aCSF containing 1.8 mM MgCl_2 . Slices were held at room temperature for at least 1 h prior to recording.

Synaptic potentials were evoked in the CA1 by stimulating the Schaffer collateral pathway with a 0.1 ms pulse through a bi-polar stimulating electrode (FHC, Bowdoin, ME, USA). Field excitatory postsynaptic potentials (fEPSP) were recorded with an aCSF filled glass microelectrode (2-4 μm) placed in the stratum radiatum ~300–400 micron from the stimulating electrode. Potentials were amplified using an AM Systems 1800 AC differential amplifier and digitized through a Digidata 1322A (Molecular Devices, Sunnyvale, CA, USA). Data were acquired and analyzed using Axograph software (Axograph X, Australia). fEPSPs

were evoked every 30s. Synaptic strength was determined by measuring the slope of the rising phase of the fEPSP between 20 and 80%. Slopes of fEPSPs were normalized to a 15 min pre-tetanic baseline. Paired pulse facilitation was determined by giving two consecutive stimulation pulses separated by 70 ms. For LTP experiments the stimulation intensity was set so that the fEPSP amplitude was 1/3 of maximum amplitude. A high frequency tetanic stimulation was used to induce LTP (3 trains of 100 stimuli at 100 Hz with a 30 s interval between trains). For statistical analysis the average fEPSP amplitude was measured over a 45 to 60 minute epoch following tetanic stimulation. Recordings were excluded if they displayed an unstable baseline (> 10% change over the first 15 min of recording). All experiments were undertaken blind to mouse genotype.

Enzyme-linked immunosorbent assay (ELISA)

ELISA was used to compare total human A β levels in the brains regions of wild type, Tg2576, p75^{+/-} and Tg2576/ p75^{+/-} mice, using a procedure modified from a previous study (Fodero-Tavoletti et al., 2007). Mice were culled via cervical dislocation; the brains removed and snap frozen in liquid nitrogen. Subsequently, brains were homogenised in buffer containing 50mM Tris-HCl, pH 7.5, 150mM NaCl, and protease inhibitor cocktail (Sigma; 1:10 w/v). To measure total A β , the homogenates were mixed with 70% formic acid, neutralised, and then diluted into 50% phosphate buffered saline (PBS) containing 0.05% (v/v) Tween 20 (PBST)/50% casein before adding to the plate. To measure soluble A β , the homogenates were centrifuged at 100,000 g for 60 min at 4°C, and the supernatants were used for ELISA. A MaxiSorp 384 well plate was coated with 0.5 μ g/well WO2 monoclonal antibody, which specifically binds to the N-terminal region of human A β , in carbonate-bicarbonate coating buffer, pH 9.6, and incubated overnight at 4°C. The wells were then washed with PBST. Blocking buffer (0.5% (w/v) hydrolysed casein in PBS, pH 7.4) was

added, followed by incubation for 2 h at 37°C, and washed with PBST. Biotinylated 1E8 mouse monoclonal antibody (2 ng/μL), which binds to Aβ17-22, was added per well, followed by standards and samples in triplicate at 50 μL per well. Standards and samples were diluted in a solution containing 50% PBST/50% casein.

Following overnight incubation at 4°C, the plate was washed with PBST, and Streptavidin-Europium (1:1000 dilution) was added to each well and the plate was allowed to incubate for 1 h at room temperature. Following washes with PBST, the plate was developed with enhancement solution (Perkin Elmer, Melbourne). Fluorescence intensities were measured on Victor² 1420 Multilabel Plate Reader (PerkinElmer, Melbourne, Australia) with excitation at 340 nm and emission at 613 nm.

Statistical analyses

Statistics were generated using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com, and values are represented as mean ± standard error of the mean (SEM) unless otherwise indicated. Results were considered statistically significant at $p < 0.05$. Fear conditioning and electrophysiological data was analysed by one-way ANOVA and analyses of significant differences between specific groups were made using Newman-Keuls *post hoc* comparisons. The Y Maze experiments were analysed using Student's t-test. Criterion attainment versus training day in the Barnes maze trials was analysed by Chi-square test for trend.

RESULTS

In order to determine if reduction of levels of p75 could ameliorate the cognitive decline in AD, we crossed the AD transgenic mice, Tg2576, with p75^{-/-} mice (Lee et al., 1992) to

generate Tg2576/p75^{+/-} mice. We chose to study Tg2576 which were heterozygous for the p75 deletion because p75^{+/-} mice appear indistinguishable from their wild type parental strain, whereas homozygous p75^{-/-} mice have developmental phenotypes (Lee et al., 1992), including deficits in the peripheral nervous system. The background strains for all mice were 129T2/Sv, and the Tg2576/p75^{+/-} mice were compared with this wild type strain as well as the Tg2576 and p75^{+/-} mice.

Western blot analyses of whole brain extracts probed with antibodies to p75 were conducted to determine the levels of p75 protein in these mice strains. Both p75^{+/-} mice and Tg2576/p75^{+/-} mice show a reduction of ~50% in the levels of p75 compared with both 129T2/Sv and Tg2576 mice (Fig. 1). A short form of p75, still present in this p75^{-/-} mouse, has also been reported (von Schack et al., 2001). This short form incorporates the intracellular domain of p75 and was found in Schwann cell extracts of both wild type and p75^{-/-} mice (von Schack et al., 2001). However, in our Western blot analyses of brain extracts of either wild type or p75^{+/-} mice, we did not detect this form of p75 using antibodies to either full length p75, or two different antibodies which were specific for the intracellular domain of p75.

It was possible that our Western blots were not sensitive enough to detect the short form of p75, if it was present. We thus undertook reverse transcriptase PCR of RNA isolated from brain using primers specific for the short form of p75 (von Schack et al., 2001). A PCR product was sporadically detected which corresponded to that previously described (von Schack et al., 2001), however this could only be achieved using high amounts of input RNA (3 µg) and high PCR cycle number (45 – 60 cycles; data not shown). These data suggest that the short form p75 transcript is present in very low amounts in the brain. Correspondingly the short form protein is undetectable in whole brain extracts. It is thus unlikely that this form of p75 has any functional role in the phenotype of the Tg2576/p75^{+/-} mice described below. The

phenotypic effects we describe are most likely due to a reduction in the levels of full length p75.

Effects of reduction in p75 on context fear conditioning in Tg2576 mice

The mice were tested in a series of different learning models from ages 5-8 months and in one test at 12-14 months. Separate cohorts of mice were used for all behavioural tests. All genotypes of mice were analysed using context fear conditioning. This fear conditioning paradigm measures the degree to which an animal can learn an association between a neutral stimulus (the context of the shock box) and an aversive stimulus (the footshock). One day after the mice were trained in context fear conditioning, they were placed back into the shock box and tested for fear memory by measuring their level of freezing, a strong indicator of fear-related behaviour in mice (Blanchard and Blanchard, 1988).

The freezing levels of the mice were determined immediately after footshock as a measure of their shock response. Both wild type and p75^{+/-} mice showed essentially identical freezing levels of ~13% post shock, indicating that both of these strains have the same shock response (Fig. 2A). The post shock freezing levels of the Tg2576 mice were significantly reduced by almost 50% compared with the wild type mice ($p < 0.05$), indicating that Tg2576 mice have a deficit in their shock response. However, there was no significant decrease ($p > 0.05$) in post shock freezing level of the Tg2576/p75^{+/-} mice compared with wild type mice. This indicates that the Tg2576/p75^{+/-} do not have a significant deficit in their shock response.

During testing for fear conditioning, wild type mice showed high levels of freezing, and froze an average of 45% of the time (Fig. 2B). In clear contrast, the Tg2576 mice froze much less compared with the wild type mice (Fig. 2B; $p < 0.001$), averaging 13% freezing over the testing period, or less than 30% of that of the wild type mice. This demonstrates that the Tg2576 mice have a deficit in context fear conditioning. Part of this deficit may be due to the

reduced shock response and consequent decreased fear learning, but there may be an additional deficit in recall of the fear memory. In comparison, the Tg2576/p75^{+/-} mice froze to a similar albeit slightly lower level (35%) compared with the wild type mice; there was no significant difference in freezing between the Tg2576/p75^{+/-} and the wild type mice (Fig. 2B; $p > 0.05$). The Tg2576/p75^{+/-} mice froze to a significantly greater level compared with the Tg2576 mice ($p < 0.01$). These findings demonstrate that reduction of p75 has effectively rescued the deficits of the Tg2576 mice in context fear conditioning.

Y Maze

A modified Y Maze with visual cues was used as a test of short term memory and response to novelty. The Y maze had three equally spaced arms, one of which was blocked from entry by a removable divider during the training trial. After a 2 h interval each mouse was again placed in the Y Maze, with the previously inaccessible third 'novel' arm available for exploration. Time spent in the novel arm was compared with the average time spent in each of the familiar arms; a preference for spending time in the novel arm indicates the mice remembered the previously explored familiar arms.

Wild type and p75^{+/-} mice both showed a clear preference for the novel arm during testing, spending more than twice the amount of time in the novel arm compared with the familiar arms (Fig. 3, $p < 0.001$). In contrast, Tg2576 mice showed no preference for the novel arm (Fig. 3, $p > 0.05$), indicating an impairment in their memory retention. The performance of Tg2576/p75^{+/-} mice in the Y maze was similar to that of the control groups with a significant preference for the novel arm (Fig. 3, $p < 0.001$). This indicates that the Tg2576/p75^{+/-} mice perform well in the Y Maze and suggests that p75 reduction rescues the short term memory deficits seen in Tg2576 mice.

Barnes maze

The Barnes Maze is a sensitive test for long term spatial, hippocampal dependent memory. For this test, the mouse is placed in the centre of a brightly lit platform and in order to escape to a safe environment, it needs to locate a single target hole, which has an escape tunnel, out of a total of 40 holes around the periphery of the platform. The trial is repeated three times per day until either the mouse solves the test by attaining criterion or undergoes 30 trials. The criterion for solving the maze was defined as using a spatial strategy and searching three holes or less on three consecutive trials to find the escape tunnel (Bach et al., 1995).

The performance of the Tg2576 mice was relatively poor and worse than that of the wild type mice. This can be seen in the criterion attainment curve for the mice which shows that fewer of the Tg2576 mice reached criterion compared with the wild type mice, and those that did took more trials to reach criterion (Fig. 4A). This difference in performance between the Tg2576 and wild type mice was significant from day 6 (Chi-square test for trend = 4.645, $p = 0.0311$). However, this deficit in solving of the Barnes maze was eliminated in the Tg2576/p75^{+/-} mice (Fig. 4A). From day 6, a significantly higher proportion of Tg2576/p75^{+/-} mice attained criterion compared to the Tg2576 mice (Chi-square test for trend = 9.232, $p = 0.0024$).

The number of holes searched in error (errors) prior to finding the escape tunnel was also measured for each mouse. In early trials, all mice averaged 10 – 12 errors (Fig. 4B), which is essentially a random distance from the escape tunnel. In the mid trials, Tg2576/p75^{+/-} mice made significantly fewer errors ($p < 0.05$) compared with Tg2576 mice. This difference in performance was more pronounced in the final trials of the Barnes maze, when Tg2576/p75^{+/-} mice made half the errors compared with Tg2576 mice (Fig. 4B; $p < 0.001$).

The overall search strategies employed by the mice to find the escape tunnel were also investigated, as outlined in methods. All mice strains began using a mix of random and serial strategies to find the escape tunnel. As the mice did more trials, the frequency of spatial search strategies to find the escape tunnel. As the mice did more trials, the frequency of spatial search strategies also increased for each of the strains. By the end of training, the majority of wt, p75^{+/-} and Tg2576/p75^{+/-} mice were using spatial search strategies to solve the maze (Fig. 4C-E). However the Tg2576 mice showed a different pattern, with a similar percentage of mice using the serial search strategy compared with the spatial search strategy (Fig. 4F). This indicates that the Tg2576 mice have a reduced ability to employ a spatial search strategy in the Barnes maze, which is clearly enhanced by reduction of p75. Overall, Barnes maze testing showed enhancement of spatial memory in Tg2576/p75^{+/-} mice compared to their Tg2576 littermates.

Barnes maze at 13 months of age

The tests described above revealed that p75 reduction reduced impairment in fear conditioning, Y maze and Barnes maze. All of these tests were performed around 6 months of age, which is relatively early in the progression of the disease in the Tg2576 model. We also wanted to know whether p75 reduction was effective well into the progression of the AD. Therefore, the Barnes maze performance was also tested at 12 - 14 months of age (Fig. 5). At this age, the Tg2576 mice have evidence of micro and macro amyloid deposits, in addition to higher levels of A β compared to 6 months of age (Kawarabayashi et al., 2001).

The rescue of Tg2576 cognitive impairment by p75 reduction was more pronounced at 13 months than in the younger mice. Tg2576 mice performed poorly at 13 months, with only 27% of these mice reaching criterion (Fig. 5A). In comparison, 73% of Tg2576/p75^{+/-} mice reached criterion, a significantly higher proportion (Chi-square test for trend = 5.602, $p = 0.0179$; Fig. 5A). Increasing age and disease progression did not diminish the protective effect

of p75 reduction. The criterion attainment curves of Tg2576/p75^{+/-} mice were essentially the same as those of the p75^{+/-} mice (Fig. 5A), suggesting that p75 reduction resulted in complete rescue of the memory deficits in the Tg2576 mice.

Analysis of search errors in the Barnes maze confirmed that the Tg2576/p75^{+/-} mice performed significantly better compared with Tg2576 mice at 13 months. By the mid trials of the maze, Tg2576/p75^{+/-} mice made approximately half the errors compared with Tg2576 mice (Fig. 5B; $p < 0.001$). By the final trials, Tg2576/p75^{+/-} mice made less than half the errors compared with Tg2576 mice (Fig. 5B; $p < 0.001$). Comparison of search strategies used by the different strains also showed large differences between Tg2576 and Tg2576/p75^{+/-} mice at 13 months. At this age, the Tg2576 mice used a spatial search strategy less than 40% of the time by the end of the trial period (Fig. 5E), whereas over 80% of the Tg2576/p75^{+/-} mice searched spatially by the final trials (Fig. 5F).

Hippocampal CA1 synaptic properties

To determine if there were differences in synaptic transmission, we investigated the fEPSC input/output relationship and paired pulse facilitation in the different mice groups. Hippocampal CA1 recordings in slices from Tg2576 mice showed an obvious reduction in fEPSC amplitude measured at all stimulation strengths compared to wild type mice (Fig. 6A, B). For example, at 0.04 mA, the wild type mice had a slope of $42.92 \pm 6.16 \mu\text{V}/\text{ms}$ compared to $18.20 \pm 4.31 \mu\text{V}/\text{ms}$ in Tg2576 mice (Fig. 6B; $p < 0.05$). Most notably, Tg2576/p75^{+/-} mice showed no reduction in fEPSP input/output relationship compared with wild type mice (Fig. 6A, B). This suggests that reduction of p75 has rescued the deficit in synaptic transmission in the Tg2576 CA1 area. p75^{+/-} mice also showed no difference compared with wild type mice (Fig. 6A, B).

It was possible that the hippocampal slices from the Tg2576 mice were more susceptible to glutamate induced toxicity during isolation, and this may have contributed to the observed reduction in fEPSP amplitude. Several studies have demonstrated that addition of the broad-spectrum glutamate receptor antagonist, kynurenic acid, during isolation reduces glutamate induced toxicity and can enhance basal synaptic transmission (Chapman et al., 1999; Fitzjohn et al., 2010). We thus tested slices isolated in kynurenic acid (Fig. 6C). However, the fEPSP input/output relationship from slices isolated in kynurenic acid was not different to those isolated without it (Fig. 6C), suggesting a true deficit in synaptic transmission in the Tg2576 mice. We conclude that the Tg2576 transgene results in a basal deficit in transmission within the Schafer collateral pathway similar to that previously described (Chapman et al., 1999; Jacobsen et al., 2006) and that this deficit is reversed by reduction of p75.

We also tested paired pulse facilitation to investigate if the Tg2576 or p75 mutations had any effect on this measure of presynaptic function (Zucker and Regehr, 2002). There was no difference across all genotypes in paired-pulse facilitation (Fig. 6D).

Synaptic plasticity

Long-term potentiation (LTP) is a model for a cellular mechanism of learning and memory and is known to be reduced in animal models of AD (Chapman et al., 1999; Jacobsen et al., 2006). We tested this form of synaptic plasticity in all genotypes using a conventional LTP induction protocol and found LTP could be evoked in all hippocampal slices. A significant reduction in LTP was observed for the Tg2576 mice (Fig. 7A, D; $p < 0.01$) as previously reported (Jacobsen et al., 2006). Importantly and mirroring the changes seen in basal synaptic properties, no significant reduction in LTP magnitude was found in the Tg2576/p75^{+/-} compared with wild type slices (Fig. 7B, D). This result suggests that reduction of p75 can rescue the deficits in synaptic plasticity in the Tg2576 mice. Also, confirming a

previous study (Barrett et al., 2010), LTP in the p75^{+/-} mice showed a significant increase ($p < 0.01$) compared with wild type mice.

Levels of A β

It was possible that downregulation of p75 may have some effect on the level of A β in the brains of the Tg2576 mice. To measure levels of the human A β protein which is regulated by the Tg2576 transgene, brains were homogenised and solubilised with formic acid. Total human A β levels were measured using ELISA. At 8 - 9 months of age, there was a trend for an increase in the level of A β in brains of the Tg2576/p75^{+/-} mice compared with Tg2576 mice (Fig. 8A), however at this age the trend was not significant. In older age groups (11 - 16 months), this trend persisted and showed a significant increase in levels of A β in Tg2576/p75^{+/-} mice compared with Tg2576 mice (Fig. 8A; $p < 0.01$). Wild type brains, as expected, did not contain the human form of A β (Fig. 8A). These findings indicate that reduction of p75 results in an increase in the levels of A β in Tg2576 mice.

Levels of soluble A β were also measured in Tg2576 and Tg2576/p75^{+/-} mice from 5 months as well as the later ages (Fig. 8B). At 5 months, at the time many of the behavioural tests were undertaken, low levels of soluble A β were present, but there was no significant difference between the levels in Tg2576 and Tg2576/p75^{+/-} mice (Fig. 8B; $p > 0.05$). At later times, the levels of soluble A β increased to similar extents in both strains of mice, with no observable difference between Tg2576 and Tg2576/p75^{+/-} mice (Fig. 8B; $p > 0.05$).

DISCUSSION

Our study asked if reduction of p75 can affect the primary symptoms of AD in a well established mouse model of this disease. The Tg2576 mouse expresses the human APP gene,

containing the Familial AD Swedish mutation K670N/M671L, throughout the brain and duplicates many facets of the human disease (Hsiao et al., 1996; Kawarabayashi et al., 2001). The onset of spatial learning impairment occurs at 4 - 5 months (Jacobsen et al., 2006) with appearance of insoluble or aggregated forms of A β from 6 months (Westerman et al., 2002). Plaques are not observed until at least 10 months of age (Kawarabayashi et al., 2001). We have undertaken behavioural testing, electrophysiology and analysis of levels of A β in Tg2576 mice with reduced p75, Tg2576/p75^{+/-} mice. These mice show a reduction of 50% in levels of full length p75 compared with Tg2576 or wild type mice, with no evidence of any substantial levels of any other form of p75. Our findings suggest that reduction of p75 results in amelioration of the cognitive decline associated with AD.

Effects of reduction of p75 on memory and synaptic function

Context fear conditioning is a form of classical conditioning requiring the animal to learn and remember a given context and associate it with the negative stimulus of a footshock. Multiple areas of the brain are most likely involved in this learning and memory event, in particular the hippocampus and amygdala (Fanselow and Poulos, 2005; Kim and Jung, 2006). A number of studies have shown impaired contextual fear conditioning in Tg2576 mice from 4 months of age (Jacobsen et al., 2006; Rustay et al., 2010). Our results support these findings. First, the Tg2576 mice show an impaired shock response, with freezing levels post shock of ~50% of the 129 wild type mice. Related findings have been previously reported (Barnes and Good, 2005), and suggest that Tg2576 mice either have a sensory deficit with reduced shock sensitivity and/or a deficit in forming an association between the shock and the context. Further, during testing for fear conditioning, the Tg2576 mice showing freezing responses to the training context of only 30% of that seen in the wild type mice. The

impairments both in shock response and in fear conditioning memory were effectively rescued in the Tg2576/p75^{+/-} mice.

Similar results were found when mice were tested in the exploratory Y maze, a simple test of short term memory which utilises the animal's response to novelty (Dellu et al., 2000). The Y maze has been used to assess short term memory in rodents in models of disease and injury including AD (Carvalho et al., 2013). Tg2576 mice did not show a preference for the newly available novel arm over the previously explored familiar arms, on average spending the same amount of time in each. This Y maze result is representative of the learning impairments known to occur in Tg2576 mice from 5 months of age (Jacobsen et al., 2006). In contrast, the behaviour of Tg2576/p75^{+/-} mice was very similar to the wild type and p75^{+/-} control groups, indicating that p75 reduction has prevented or delayed the Tg2576 associated memory deficits in 5 - 7 month old mice.

We also tested the mice using the Barnes spatial maze. The Barnes maze requires learning of a relatively precise spatial memory in order for the animal to directly locate the correct hole out of a possible 40 holes on the platform. This task requires the use of hippocampal dependent spatial reference memory (Bach et al., 1995; Fox et al., 1998). The Tg2576 mice showed a trend of poor performance on the Barnes maze when compared to wild type controls. The p75 reduction in these mice resulted in a large increase in the proportion of mice which successfully solved the Barnes maze as demonstrated by higher criterion attainment. This demonstrates that reduction of p75 can rescue the spatial learning deficits in the Tg2576 mice.

The mice were also tested at 12 – 14 months, when there are with high levels of A β and significant numbers of plaques in the brains of the Tg2576 mice (Kawarabayashi et al., 2001). We used the Barnes maze, which we have previously used on older mice (Barrett et al., 2010; Greferath et al., 2000). We did not undertake fear conditioning at this age because in

preliminary testing all strains of mice showed significant freezing before being shocked, making it difficult to determine if the freezing after they were trained had anything to do with fear memory. Similarly, in preliminary testing of the Y maze, 12 – 14 month old mice did not investigate the maze; they simply stayed within their home arm without investigating. For mice tested in the Barnes maze at 12 – 14 months, reduction of p75 resulted in effectively full rescue of the learning and memory deficits present in the Tg2576 mice. Our findings that reduction of p75 can effectively rescue the deficits in the Tg2576 mice in 3 independent learning and memory tasks suggests that p75 reduction has generally ameliorated the memory deficits in this model of AD at 5 – 8 months. Further, Barnes maze testing at 12 – 14 months suggests that the effect of p75 reduction persists well into the progression of the disease.

In contrast to our previous studies of p75 deficient mice on the Barnes maze, there was only an insignificant trend of improved performance of the p75^{+/-} mice compared to wild type mice (Barrett et al., 2010; Greferath et al., 2000). This was due in part to better than expected performance of wild type controls, which had previously performed consistently poorly on the Barnes maze, and not as good performance of p75^{+/-} mice. A number of factors may have contributed to these different findings. Firstly, in the previous study, all mice had been derived by backcrossing repeatedly with 129T2/SvEMsJ mice bred at the Walter and Eliza Hall Institute. Subsequently, the 129 mice for backcrossing were obtained from an ABR substrain bred at the Garvan Institute, due to unavailability of stock from our first source. These different substrains of 129T2/SvEMsJ may have diverged genetically and phenotypically over the years of separation. Secondly, new housing conditions have been developed at our facility, eliminating most pathogens and resulting in generally healthier mice. Thirdly, the proximal visual cues were identical in the two studies, but the Barnes maze room itself had undergone refurbishment, allowing the possibility of new spatial cues. Finally, the present study included a mixture of males and females whereas the earlier study was done

on females only. Any or all of these factors may have contributed to the difference in performance of wild type and $p75^{+/-}$ mice.

The electrophysiological analyses showed there were pronounced deficits in hippocampal function and synaptic plasticity in the Tg2576 mice which were rescued by reduction of p75. Analysis of fEPSP in the CA1 region showed a deficit in synaptic transmission in the Tg2576 mice, consistent with previous studies (Chapman et al., 1999; Jacobsen et al., 2006). A reduction in spine number (and hence excitatory synapses) is a likely basis for this deficit (Jacobsen et al., 2006; Wei et al., 2010). No change in the paired-pulse facilitation argues that presynaptic function of remaining synapses is normal.

Impaired synaptic plasticity is highly correlated with cognitive deficits in young (Jacobsen et al., 2006) and aged Tg2576 mice (Chapman et al., 1999) and it has been shown that hippocampal LTP is enhanced in $p75^{-/-}$ mice compared to wild type littermates (Barrett et al., 2010). Our findings show that reduction of p75 not only enhances LTP in wild type mice, but is also effective in rescuing this form of synaptic plasticity in the Tg2576 mice. The rescue of hippocampal function and synaptic plasticity by p75 reduction is likely to provide at least part of a cellular mechanism for the rescue of behavioural deficits we find in the Tg2576/ $p75^{+/-}$ mice. Both of these effects could contribute to the improved hippocampal function and synaptic plasticity we observe.

Effects of reduction of p75 on A β

Our results show that p75 reduction results in significantly increased levels of insoluble formic acid extractable A β in Tg2576 mice from one year of age, but no significant change in levels of soluble A β . A previous study reported that p75 deletion in another model of AD, the *APP^{Swe}/PS1^{dE9}* mouse, resulted in increased levels of insoluble but decreased soluble A β (Wang et al., 2011). These transgenic mice bear AD related mutations in two genes: a

chimeric mouse/human APP695, with mutations linked to familial AD, and human Presenilin 1 carrying the exon-9-deleted variant associated with familial AD (Jankowsky et al., 2003). This model has quite severe brain pathology with the mice beginning to develop plaques as early as 3 months of age. Nevertheless, in this study there was no effect of either the *APP^{Swe}/PS1^{dE9}* transgene or of p75 deletion in performance on the Morris water maze (Wang et al., 2011). In the study of Wang et al (2011), it was concluded that deletion of p75 decreases A β production but increases A β deposition in the brain.

It was further suggested that p75 may play a protective role against amyloid plaque formation and A β deposition in AD (Wang et al., 2011). However, increasing evidence suggests that amyloid plaque is not the causative agent in AD but that soluble, or oligomeric, A β is more directly involved (Benilova et al., 2012; Mucke and Selkoe, 2012). In our studies, p75 reduction had no significant effect on levels of soluble A β and the increased levels of insoluble A β would not be expected to affect the progression of the disease.

Possible mechanisms

Any effect of p75 on A β production and deposition is one of a number of possibilities to explain how reduction of p75 could ameliorate the affects of AD. It has also been shown that A β interacts with p75 to induce neurodegeneration and cell death *in vitro* and *in vivo* (Coulson, 2006; Knowles et al., 2009; Sotthibundhu et al., 2008; Yaar et al., 1997). A β could also indirectly influence p75 function via its affects on synaptic plasticity (Selkoe, 2008). Thus, it is possible that the benefit of p75 reduction is due to decreased A β induced neurodegeneration.

The primary targets of any A β induced neurodegeneration mediated by p75 are the cholinergic neurons of the basal forebrain, the only brain neurons which constitutively express p75 (Schattenman et al., 1988). Our previous data and that of others showed that p75 is

involved in degeneration of these cholinergic neurons in wild type mice (Barrett et al., 2010; Greferath et al., 2000; Yeo et al., 1997). In $p75^{-/-}$ mice compared with wild type mice there was an increase in the number of cholinergic axons in CA1 region of hippocampus, increased hippocampal ChAT levels, and an increase in the number of ChAT neurons in basal forebrain. For each of these measures, $p75^{+/-}$ mice were intermediate between $p75^{-/-}$ and wild type mice (Barrett et al., 2010; Greferath et al., 2012). In the Thy1-hAPP^{Lond/Swe} AD mice with genetic ablation of $p75$, there was reversal of cholinergic neurite degeneration in the forebrain and decreased hippocampal neuritic dystrophy compared with Thy1-hAPP^{Lond/Swe} mice expressing normal levels of $p75$ (Knowles et al., 2009). These findings thus indicate that $p75$ is involved in cholinergic neurodegeneration in AD.

These findings may also help to explain how changes in $p75$ levels mediate changes in hippocampus. Cholinergic input to hippocampus regulates neuronal excitability and memory processing (Bartus et al., 1982; Bartus et al., 1981; Cole and Nicoll, 1984). Ablation or reduction in $p75$ levels leads to both improved cholinergic function as well as decreased hippocampal neuritic dystrophy in AD, both of which should result in improved hippocampal function and memory.

The cholinergic neurons may not be the only targets of $p75$ mediated neurodegeneration in AD as it is known that $p75$ is upregulated elsewhere in the brain in AD, in particular in frontal cortex and hippocampus (Chakravarthy et al., 2012; Mufson and Kordower, 1992; Wang et al., 2011). There is also a well established connection between $p75$ and $A\beta$ in that they share a common processing enzyme, γ -secretase. Cleavage by γ -secretase produces the pathogenic $A\beta$ peptide from the C99 fragment, which is derived from amyloid precursor protein (APP); cleavage by γ -secretase also inactivates the pro-apoptotic C-terminal fragment of $p75$ (Underwood et al., 2008). It is feasible that the presence of high levels of the C99

fragment, such as occur in AD, could competitively inhibit inactivation of pro-apoptotic p75 C-terminal fragment by γ -secretase.

Yet another possibility is that the beneficial effects of p75 reduction are independent of the detrimental effects of A β . If this were the case, p75 reduction would be expected to enhance performance of wild type mice, there would be a deficit in the Tg2576 mice, and Tg2576/p75^{+/-} mice would have an intermediate phenotype. There is evidence for this; e.g. in the Barnes maze in the older mice, where the p75^{+/-} mice learn the maze faster than wild type mice. However, Tg2576/p75^{+/-} do not show an intermediate phenotype in the Barnes maze. Further, in context fear conditioning and the Y-maze, the only effect of p75 reduction is seen in the Tg2576 mice. These findings suggest that some of the effects of p75 reduction are more directly associated with A β .

The present work provides good evidence that downregulation of p75 can lead to amelioration of cognitive decline in AD. If this is the case, therapies that downregulate or block p75 may help patients afflicted with AD. Neural administration of NGF, a p75 ligand, also produces enhanced cognition (Fischer et al., 1991). However, therapies increasing NGF may not be safe considering that NGF has multiple effects throughout the nervous system via its other receptor, TrkA, such as increased peripheral pain perception (McMahon et al., 1995). Thus, targeting p75 is an attractive option because of its limited expression. Indeed there is some promising data supporting the use of synthetic p75 ligands in AD (Knowles et al., 2013; Yaar et al., 2008). Identifying and interrupting keypoints in p75 mechanisms may aid in the development of pharmacological agents capable of stopping or delaying the cognitive decline of AD patients.

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FIGURE LEGENDS

Figure 1. p75 expression is reduced in p75^{+/-} mice. A. Whole brain extracts were prepared from the mice strains (n = 3 for each strain) used in this study and were subjected to Western blot analysis with a p75 antibody. p75 full length (p75-FL) protein was detected in all samples with reduced levels in p75^{+/-} and Tg2576/p75^{+/-} mice compared with wt and Tg2576 mice. Blots were also probed with Actin antibodies. B. Levels of immunoreactivity of p75 relative to Actin were determined by optical density and show a ~50% reduction of p75 in p75^{+/-} and Tg2576/p75^{+/-} mice compared to wt and Tg2576 mice. **p* < 0.05.

Figure 2. p75 reduction rescues fear conditioning deficit in Tg2576 mice. Mice were fear conditioned as described in methods and tested for fear conditioning memory one day later. (A) shows the freezing response of the different strains of mice immediately after they received the shock. (B) shows the freezing response of the mice when they were returned to the shock chamber 24 h after receiving the shock. Shown are mean freezing levels ± SEM for 19 wild type, 19 Tg2576, 21 p75^{+/-} and 16 Tg2576/p75^{+/-} mice. **p* < 0.01, ***p* < 0.01.

Figure 3. p75 reduction improves Y-maze performance of Tg2576 mice. Time spent in the novel arm compared to the familiar arms of a Y-maze was determined in a 5 min test. Wild type, p75^{+/-} and Tg2576/p75^{+/-} mice all showed a significant preference for the novel arm over the familiar arms. Only the Tg2576 mice did not show a significant preference for the novel arm over the familiar arms (*p* > 0.05). Results show mean ± SEM for 23 wild type, 33 Tg2576, 26 p75^{+/-} and 24 Tg2576/p75^{+/-} mice. **p* < 0.001.

Figure 4. Performance in the Barnes Maze at early ages. Mice between 5 and 8 months were trained on the Barnes spatial maze 3 times per day until they had reached criterion or for a maximum of 10 days. (A) shows the fraction of mice of each strain which attained search

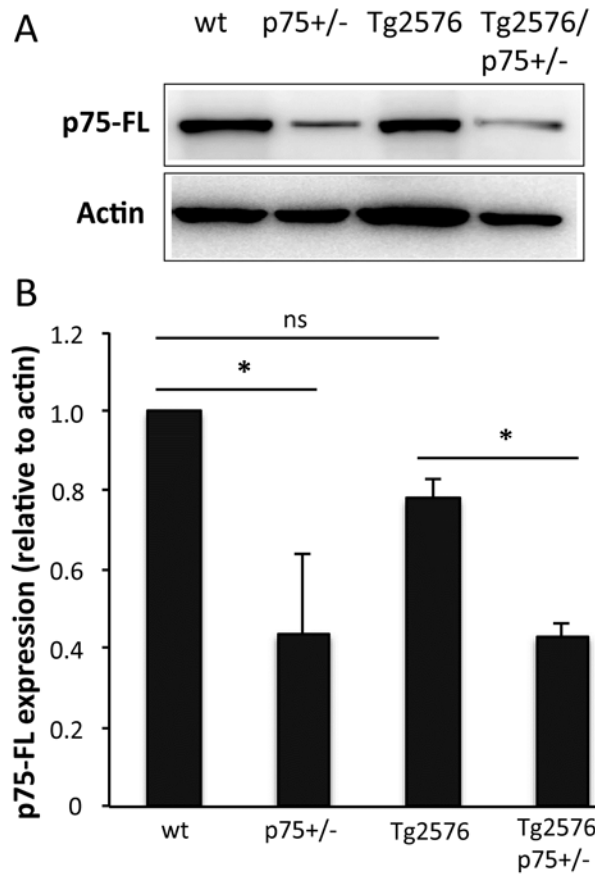
criterion for each training day. The mice reached criterion when they made 3 errors or less before they found the escape hole, over 3 consecutive trials. (B) shows mean errors \pm SEM for each strain from pooled data of trials undertaken on days 1 – 3, 5 – 7 and 8 – 10. (C – F) show the average search strategy used by mice from each strain from pooled data of the 3 trials taken on each day. Data were obtained from 14 wild type, 14 p75^{+/-}, 14 Tg2576 and 12 Tg2576/p75^{+/-} mice tested on the Barnes maze. * $p < 0.05$; *** $p < 0.001$.

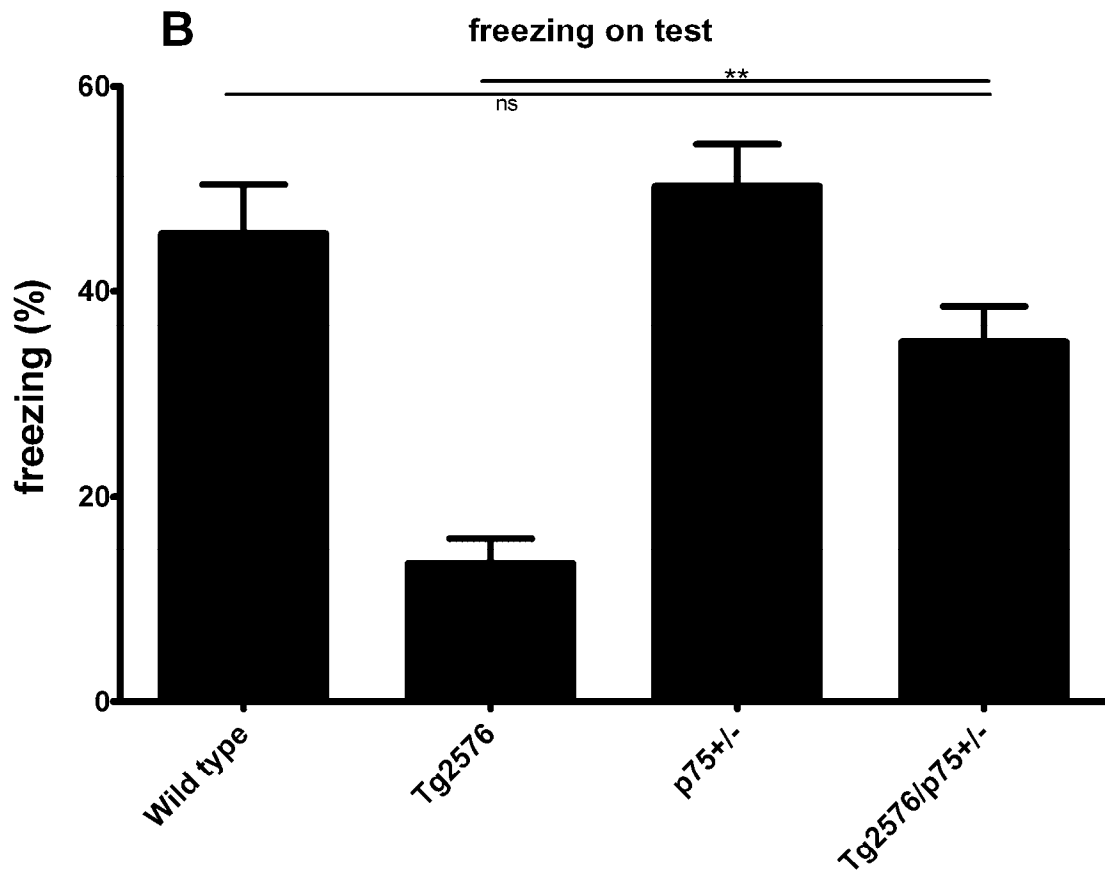
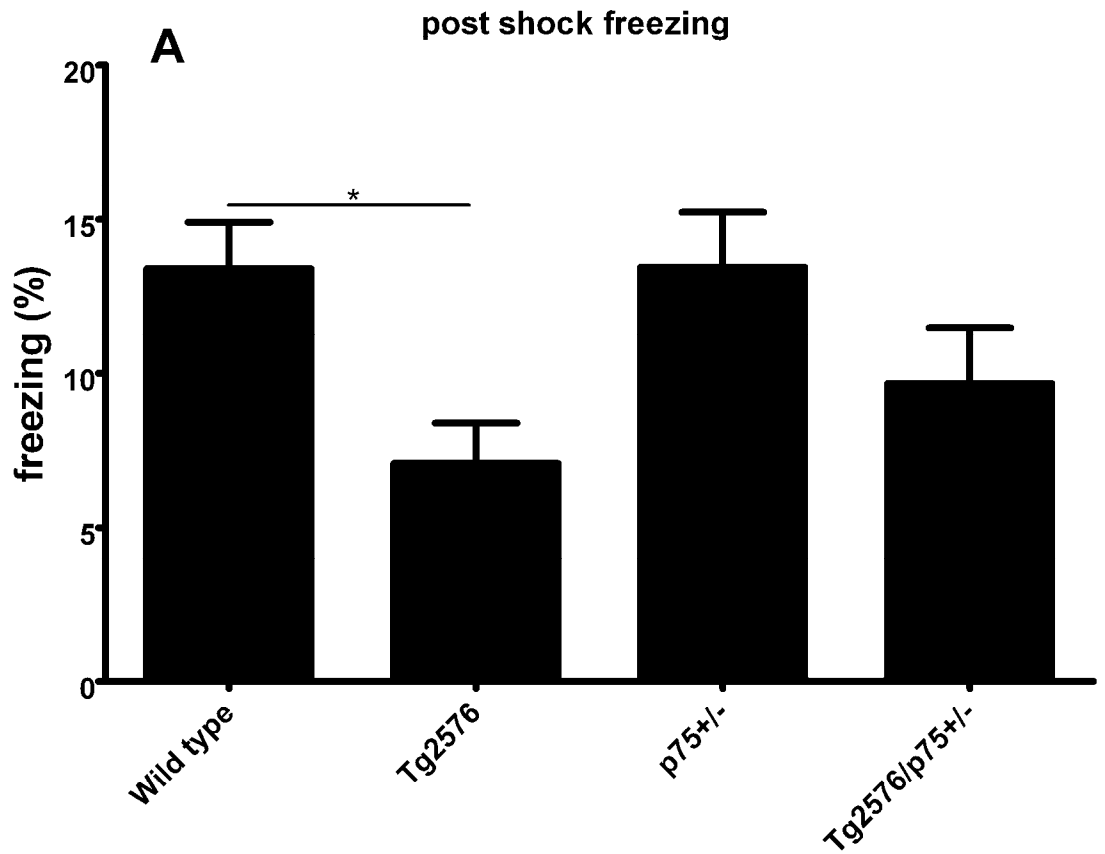
Figure 5. Performance in the Barnes Maze at older ages. B. Mice were tested at 12 – 14 months of age. (A) shows the fraction of mice of each strain which attained search criterion for each training day. (B) shows mean errors \pm SEM for each strain from pooled data of trials undertaken on days 1 – 3, 5 – 7 and 8 – 10. (C – F) show the average search strategy used by mice from each strain from pooled data of the 3 trials taken on each day. Data were obtained from 8 wild type, 6 p75^{+/-}, 15 Tg2576 and 15 Tg2576/p75^{+/-} mice tested on the Barnes maze. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

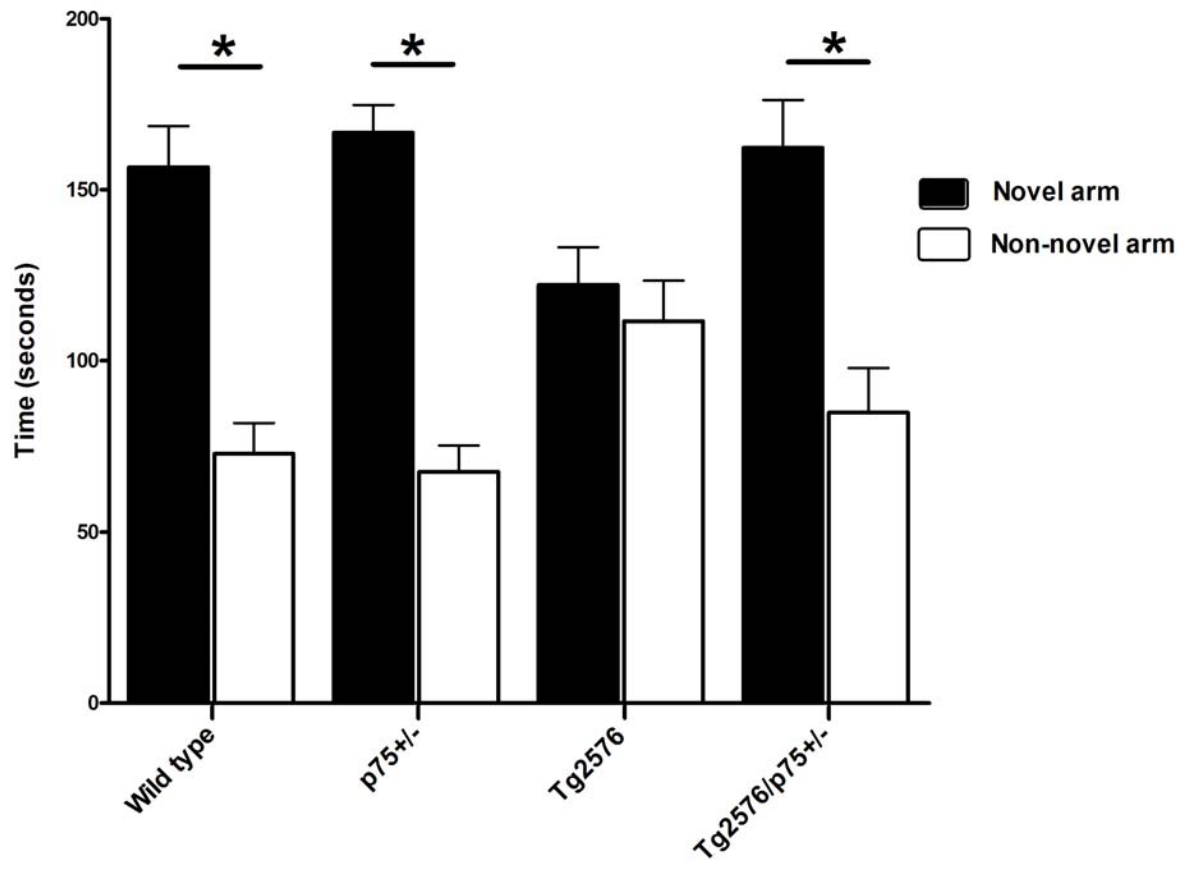
Figure 6. Tg2576 mice show a synaptic deficit that is rescued by p75 reduction. A. Examples of fEPSPs recorded from hippocampal CA1 region of the different strains of mice with a stimulus of 0.12 mA. Scale bars are 200 μ V and 30 ms. B. Relationship between input stimulation and the slope of fEPSPs recorded from hippocampal CA1 region for each strain. Data are expressed as mean \pm SEM for 26 wild type, 7 Tg2576, 15 Tg2576/p75^{+/-} and 19 p75^{+/-} mice. C. Input/output curves of fEPSPs recorded from CA1 of Tg2576 mice in which the slices have been cut in the presence (open squares; $n = 5$) or absence (open circles; $n = 7$) of 1 mM kynurenic acid. D. Paired-pulse facilitation ratios determined for different strains of mice. Data are expressed as mean \pm SEM for 9 wild type, 4 Tg2576, 11 Tg2576/p75^{+/-} and 15 p75^{+/-} mice.

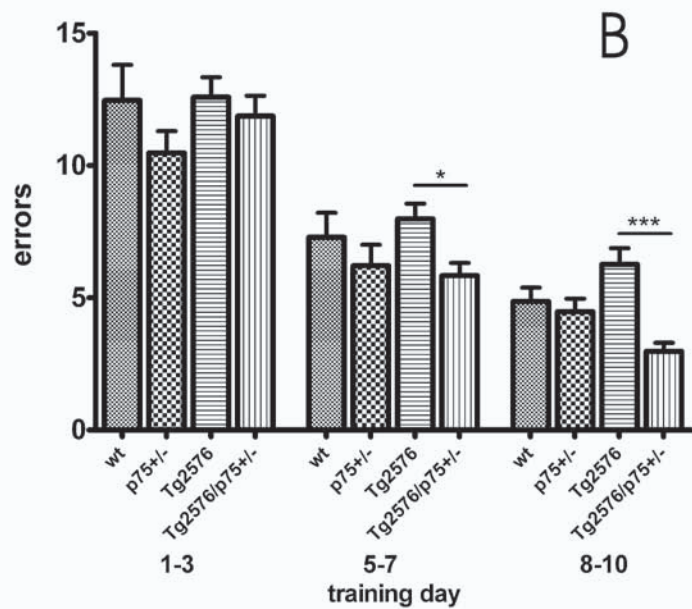
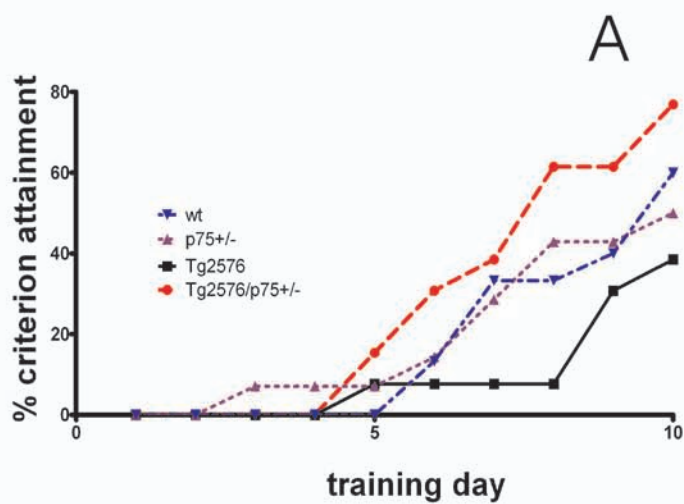
Figure 7. LTP in Tg2576 mice is rescued by reduction of p75. A - C. Time course of LTP experiments. Shown are normalized fEPSP slopes for separate pairs of strains to enable unambiguous visualization of LTP for each strain. Sample numbers for each strain were: 16 wild type; 9 Tg2576; 15 Tg2576/p75^{+/-} and 19 p75^{+/-}. Bars indicate SEM D. Normalised fEPSP slope averaged over the last 15 min of recording for each mouse strain, expressed as mean \pm SEM. Examples of fEPSPs immediately before and 60 minutes after tetanic stimulation are shown above each bar; scale bars are 200 μ V and 10 ms. * $p < 0.05$, ** $p < 0.01$.

Figure 8. A β levels in Tg2576 mice with reduced p75. A. Increase in total A β levels in Tg2576/p75^{+/-} mice. Brains from mice at the indicated age range were extracted with formic acid and total levels of A β were measured by ELISA. Sample numbers for each strain were: 8 - 9 months: 3 wild type, 5 Tg2576, 4 Tg2576/p75^{+/-} and 6 p75^{+/-}; 11 - 16 months: 5 wild type, 7 Tg2576, 6 Tg2576/p75^{+/-} and 7 p75^{+/-}. B. No change in soluble A β levels. Soluble extracts were prepared from mice brains at the indicated ages and levels of soluble A β were measured by ELISA. Sample numbers for each strain were: 5 months: 5 Tg2576 and 6 Tg2576/p75^{+/-}; 8 - 9 months: 5 Tg2576 and 4 Tg2576/p75^{+/-}; 11 - 16 months: 7 Tg2576 and 7 Tg2576/p75^{+/-}. Levels are expressed as mean ng A β per mg brain protein \pm SEM. * $p < 0.05$.

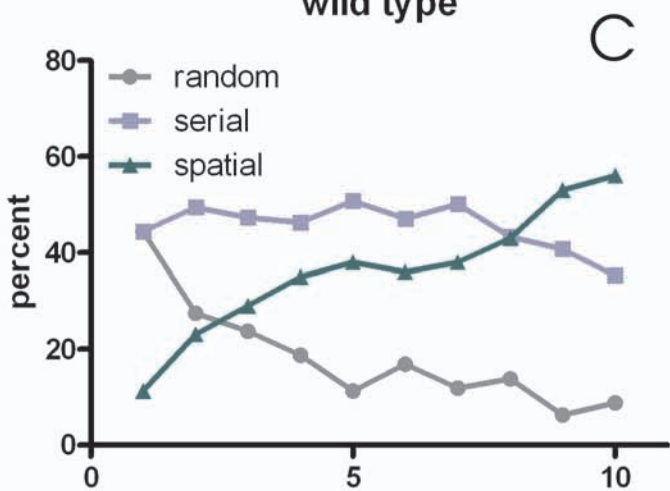




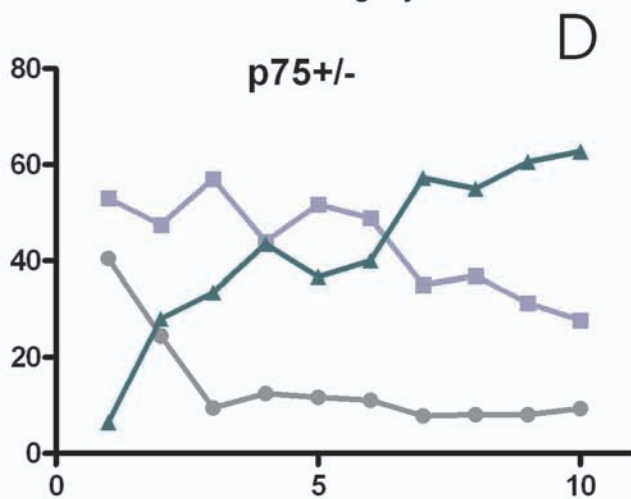




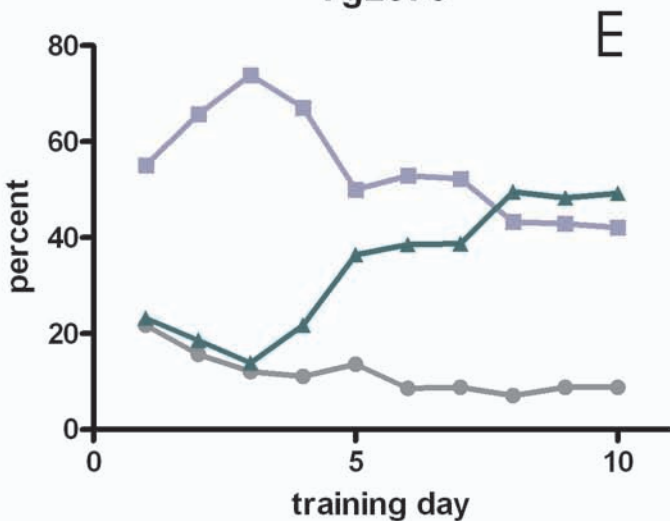
wild type



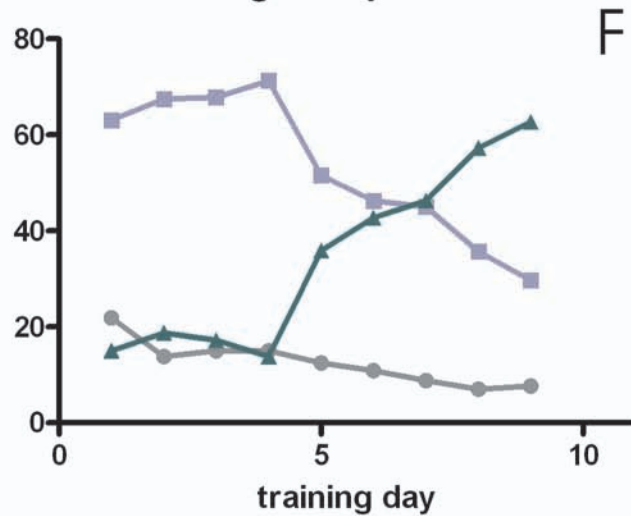
p75+/-

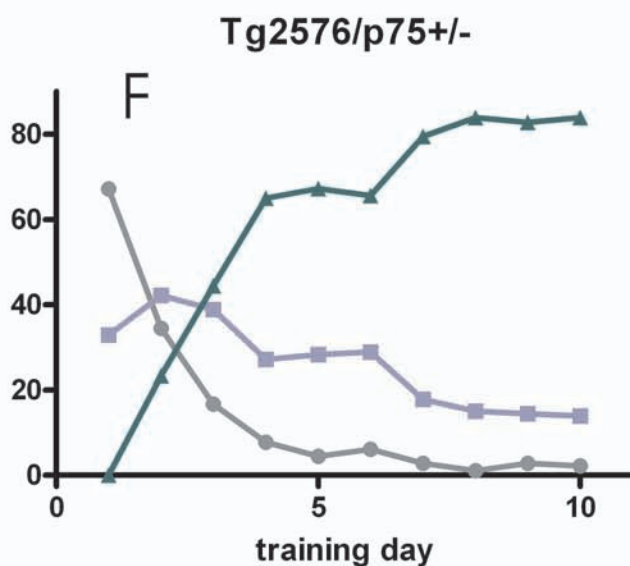
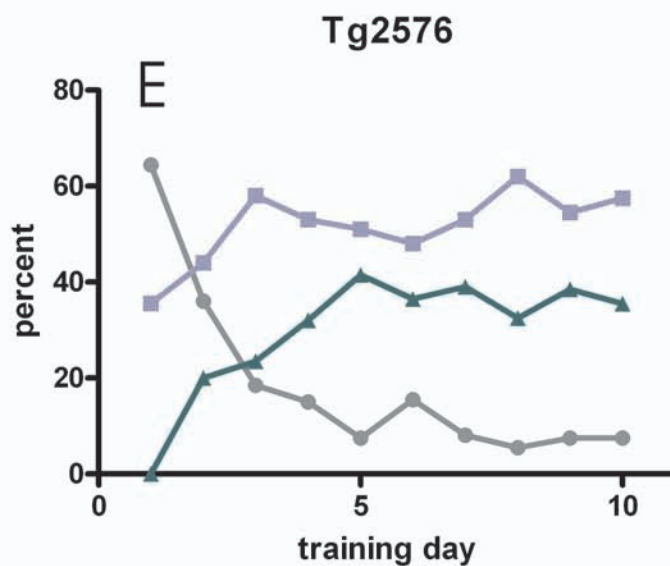
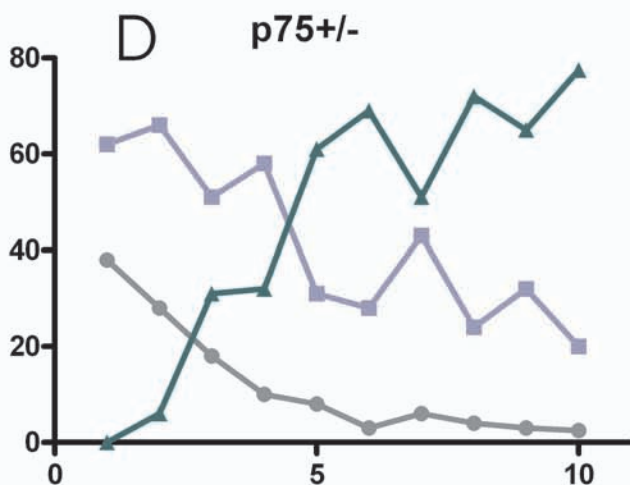
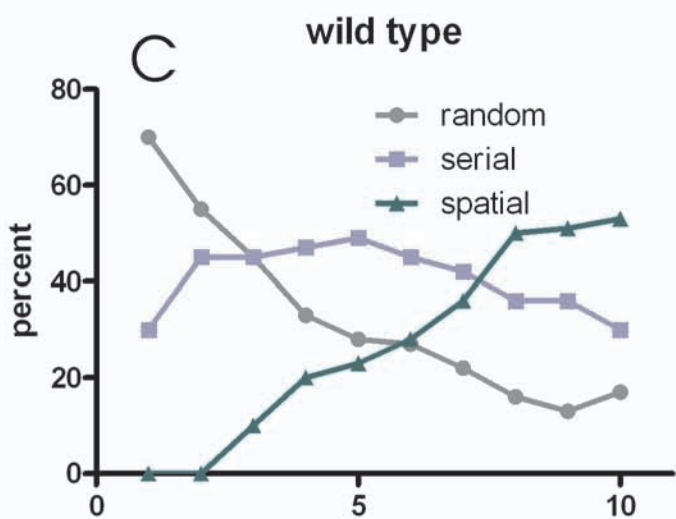
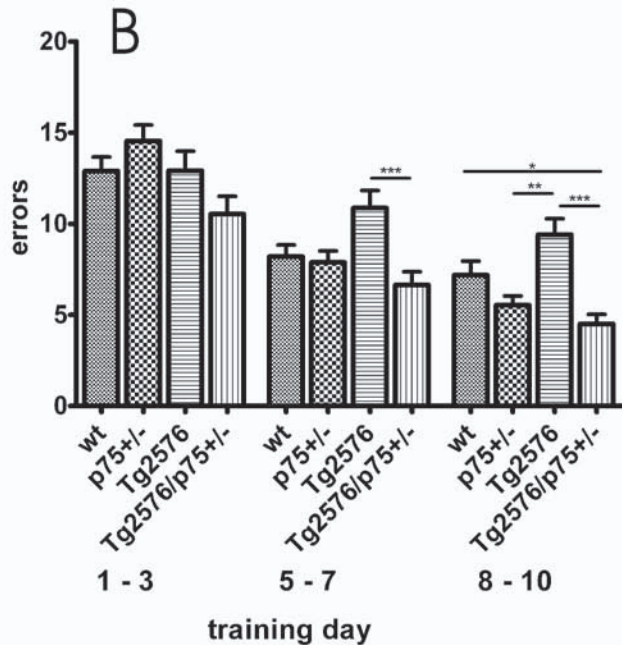
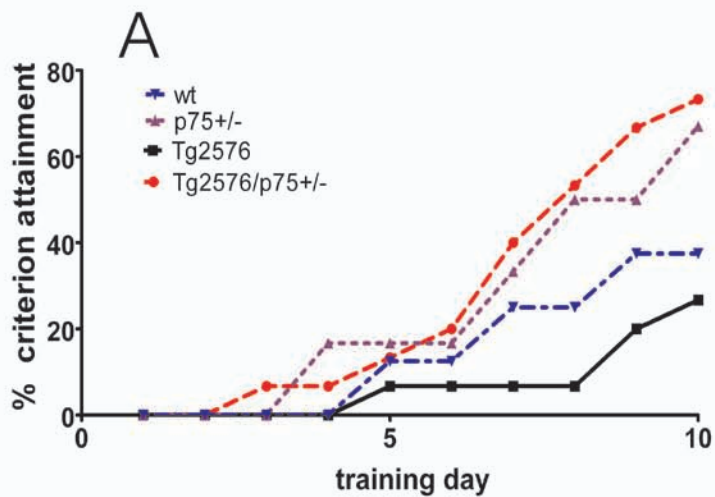


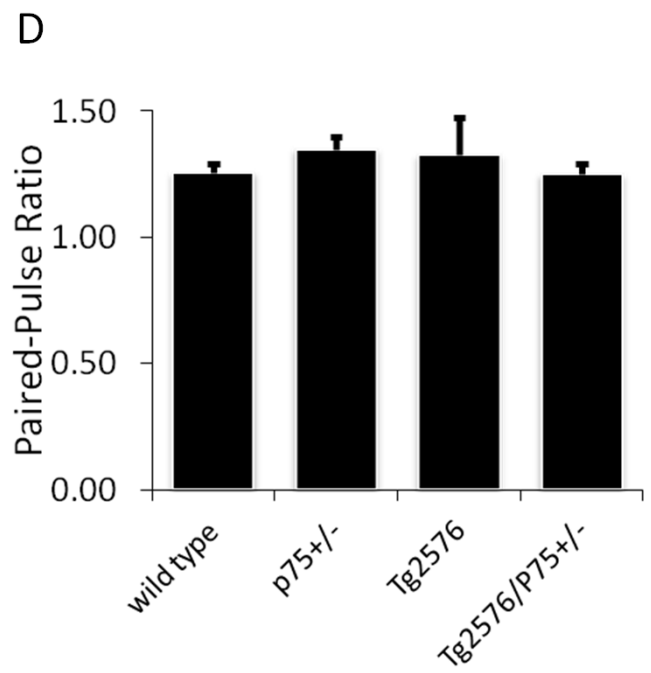
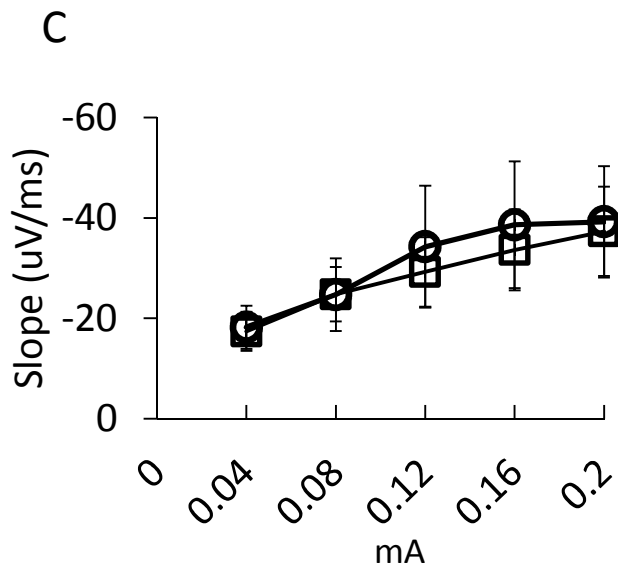
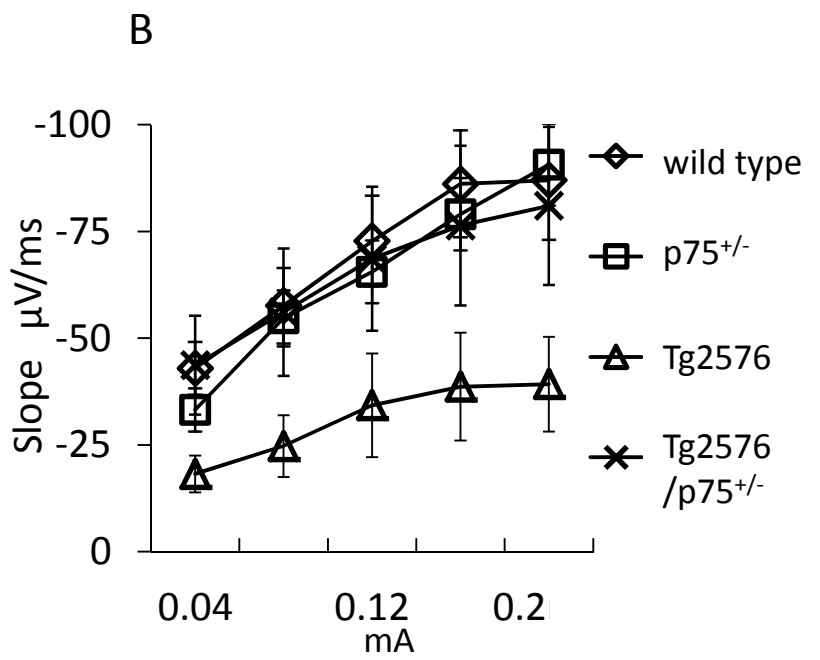
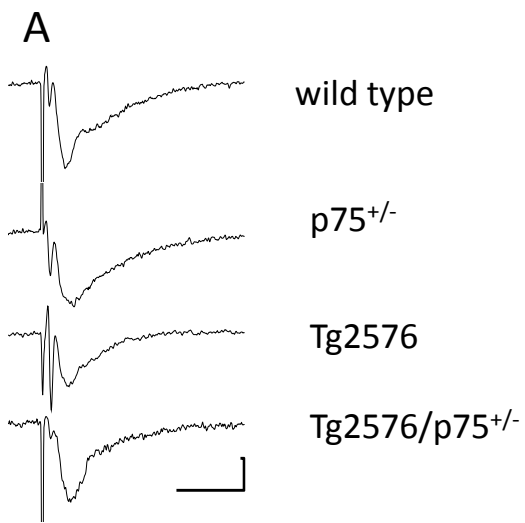
Tg2576

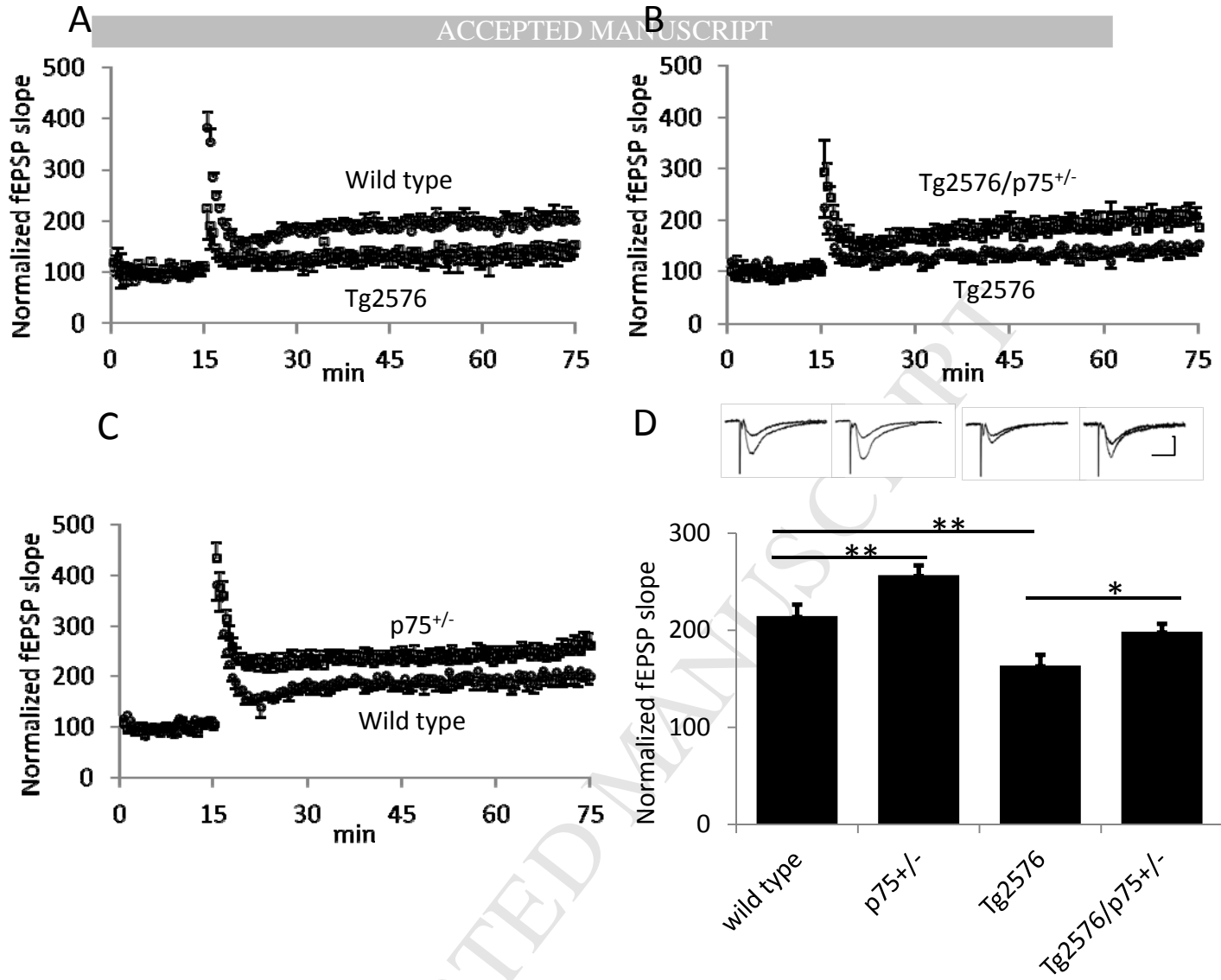


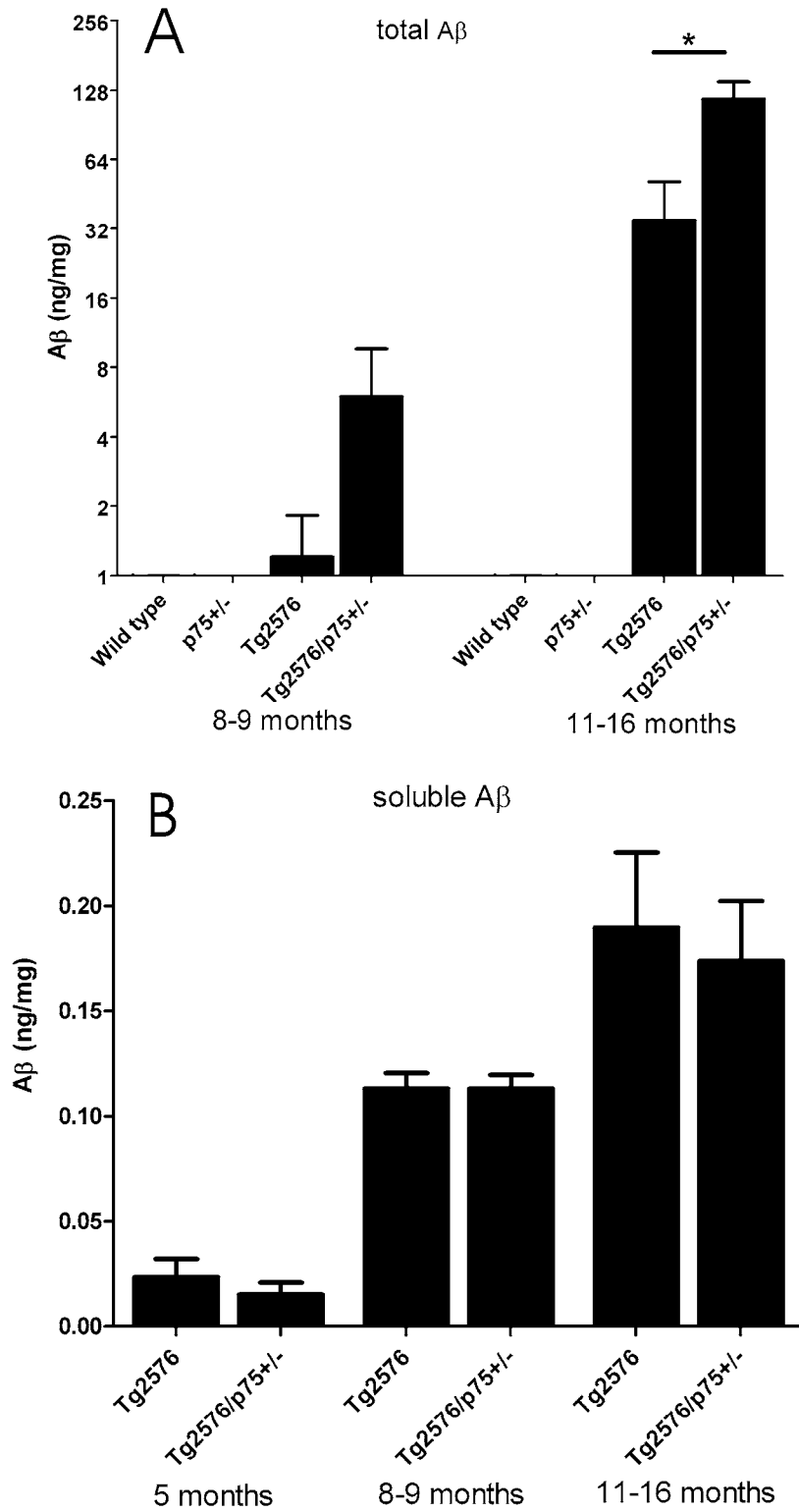
Tg2576/p75+/-











Highlights of manuscript “p75 reduction ameliorates the cognitive deficits in a model of Alzheimer's disease” by Murphy et al.

- Alzheimer's disease is a major cause of dementia with no known cure
- The p75 neurotrophin receptor may be involved in Alzheimer's disease
- We reduced levels of p75 by 50% in an Alzheimer's disease model
- All cognitive deficits we examined were rescued
- Our findings suggest p75 is a good target for treatment of Alzheimer's disease