

# Lowering $\beta$ -Amyloid Levels Rescues Learning and Memory in a Down Syndrome Mouse Model

William J. Netzer<sup>1\*</sup>, Craig Powell<sup>2,3</sup>, Yi Nong<sup>1</sup>, Jacqueline Blundell<sup>2</sup>, Lili Wong<sup>3</sup>, Karen Duff<sup>3</sup>, Marc Flajolet<sup>1</sup>, Paul Greengard<sup>1</sup>

**1** Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York, New York, United States of America, **2** Departments of Neurology and Psychiatry, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas, United States of America, **3** Department of Psychiatry, Columbia University Medical Center, New York, New York, United States of America

## Abstract

$\beta$ -amyloid levels are elevated in Down syndrome (DS) patients throughout life and are believed to cause Alzheimer's disease (AD) in adult members of this population. However, it is not known if  $\beta$ -amyloid contributes to intellectual disability in younger individuals. We used a  $\gamma$ -secretase inhibitor to lower  $\beta$ -amyloid levels in young mice that model DS. This treatment corrected learning deficits characteristic of these mice, suggesting that  $\beta$ -amyloid-lowering therapies might improve cognitive function in young DS patients.

**Citation:** Netzer WJ, Powell C, Nong Y, Blundell J, Wong L, et al. (2010) Lowering  $\beta$ -Amyloid Levels Rescues Learning and Memory in a Down Syndrome Mouse Model. PLoS ONE 5(6): e10943. doi:10.1371/journal.pone.0010943

**Editor:** Paul A. Adlard, Mental Health Research Institute and the University of Melbourne of Victoria, Australia

**Received:** February 18, 2010; **Accepted:** May 11, 2010; **Published:** June 3, 2010

**Copyright:** © 2010 Netzer et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Funding:** This work was supported by U.S. National Institutes of Health Grants AG09464 (to P.G.), R01MH081164, R21HD065290 (to C.M.P.), The Fisher Center for Alzheimer's Disease Research Foundation (<http://www.alzinfo.org>) and the F.M. Kirby Foundation, Inc. (to P.G.), the Lowe Foundation (Erma Lowe Laboratory for Alzheimer's Disease Research), the Crystal Charity Ball Program for the Prevention of Complications in Children with Down's Syndrome, the Van Beber Family and the David M. Crowley Foundation (to C.M.P.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

\* E-mail: netzerw@rockefeller.edu

These authors contributed equally to this work.

## Introduction

Down syndrome (DS) is a complex genetic disorder that includes varying degrees of intellectual disability [1,2]. Occurring in approximately 1 in 700 births, DS results from trisomy of all or part of human chromosome 21 (trisomy 21) [3], which generally accounts for triplication of at least 100 genes. Among these is the gene encoding APP, as well as genes that upregulate APP expression [4]. Sequential cleavage of APP by  $\beta$ -secretase (BACE 1) and  $\gamma$ -secretase produces peptides of varying lengths (mainly 40 and 42 amino acids), collectively termed  $\beta$ -amyloid, or A $\beta$  [5,6], which is widely believed to be an acute mediator of cognitive impairment [7], as well as a causative factor in Alzheimer's disease (AD). A $\beta$  is over-produced in Down syndrome patients throughout life. Indeed, A $\beta$  serum levels are 200% to 300% higher than in karyotypically normal individuals, and A $\beta$  accumulates within neurons and in amyloid plaques in juvenile and adult DS patients, respectively [8].

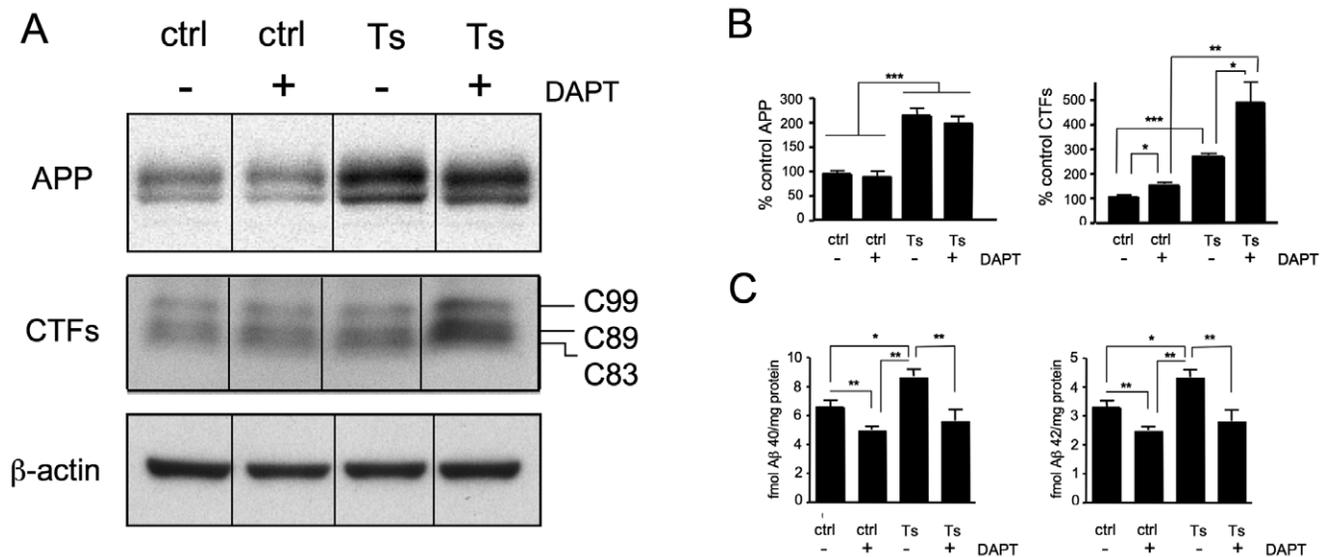
Increased A $\beta$  levels in DS are believed to be responsible for the near universal prevalence of AD in adult DS patients. By age 40, most if not all DS patients have extensive amyloid plaque pathology and degeneration of basal forebrain cholinergic neurons, both characteristic features of AD, and most of these individuals develop AD dementia by the fifth and sixth decades of life [9]. However, it is not known whether elevated A $\beta$  levels in DS children affect intellectual disability. To explore this possibility, we utilized the Ts65Dn mouse, which is widely considered the gold standard of Down syndrome mouse models [10]. Ts65Dn is

characterized by partial trisomy of mouse chromosome 16, which contains genes homologous to the human chromosome 21 genes that are most consistently triplicated in DS, including three copies of *APP*, the gene encoding the amyloid precursor protein [11]. These mice exhibit pronounced cognitive deficits as early as 2–3 months of age [12], as well as other correlates of DS. Additionally, by 6 months of age, Ts65Dn mice begin a progressive, age-related decline in choline acetyltransferase (ChAT) levels and cognitive function [13], features that are common to adult DS and AD patients [14].

## Results

### DAPT alters levels of APP metabolites in 4-month-old Ts65Dn mice

We used 4-month-old Ts65Dn mice and their disomic, colony-mates as controls. We compared APP levels, the  $\beta$ -secretase and  $\alpha$ -secretase cleaved APP C-terminal fragments (C99, C89 and C83) [15], and A $\beta$ 40 and A $\beta$ 42 from hemibrains (lacking cerebellum) of mice that had been treated either with the  $\gamma$ -secretase inhibitor, DAPT [16], or vehicle for four days. Western blot analysis revealed that APP levels in vehicle-treated Ts65Dn mice were elevated to 225% of vehicle-treated controls (Fig. 1a, b), in agreement with some [17] but not all previous studies [18]. Levels of the  $\beta$ -secretase and  $\alpha$ -secretase cleaved APP C-terminal fragments (C99, C89 and C83) in vehicle-treated Ts65Dn mice were elevated to 260% of vehicle-treated controls (Fig. 1a, b) suggesting that increased levels of A $\beta$  might occur as a result of an



**Figure 1. DAPT raises APP-CTF levels and lowers A $\beta$  levels in brains of 4-month-old Ts65Dn mice.** Four-month-old Ts65Dn mice and wild type colony mate controls were treated with vehicle or DAPT (100 mg/kg/day) for 4 days. (A) Representative western blots of APP, CTFs and  $\beta$ -actin from control (ctrl) and Ts65Dn (Ts) mice. (B) Left panel, quantification of APP (Students t-test, mean  $\pm$  s.e.m., unpaired, two-tailed,  $n=8$  per group); Ctrl+Vehicle vs. Ts+Vehicle,  $p=0.0003$ ; Ctrl+DAPT vs. Ts+DAPT,  $p=0.0002$ ; Ctrl+Vehicle vs. Ts+DAPT,  $p=0.0001$ ; Ctrl+DAPT vs. Ts+Vehicle,  $p=0.0006$ . Right panel, combined (C99, C89 and C83) CTFs (all means differ significantly between individual pairs of mean calculated by Students t-test, mean  $\pm$  s.e.m., unpaired, two-tailed). (C) A $\beta$ 40 and A $\beta$ 42 quantification from control and Ts65Dn mice. Left panel, A $\beta$ 40 (Students t-test, mean  $\pm$  s.e.m., unpaired, two-tailed,  $n=6$  per group); Ctrl+Vehicle vs. Ts+Vehicle,  $p=0.0173$ ; Ctrl+Vehicle vs. Ctrl+DAPT,  $p=0.0043$ ; Ctrl+DAPT vs. Ts+Vehicle,  $p=0.0079$ ; Ts+Vehicle vs. Ts+DAPT,  $p=0.0082$ . Right panel, A $\beta$ 42 (Students t-test, mean  $\pm$  s.e.m., unpaired, two-tailed,  $n=6$  per group); Ctrl+Vehicle vs. Ts+Vehicle,  $p=0.0169$ ; Ctrl+DAPT vs. Ts+Vehicle,  $p=0.0003$ ; Ts+Vehicle vs. Ts+DAPT,  $p=0.0052$ . doi:10.1371/journal.pone.0010943.g001

enlarged precursor pool. A $\beta$ 40 and A $\beta$ 42 concentrations were elevated in vehicle-treated Ts65Dn mice to 132% and 139% of vehicle-treated controls, respectively (Fig. 1c).

DAPT treatment lowered A $\beta$ 40 and A $\beta$ 42 concentrations in Ts65Dn mice to 65% and 64% of vehicle-treated Ts65Dn mice, respectively (Fig. 1c). DAPT also decreased A $\beta$ 40 levels in control mice to 76% of vehicle-treated controls (Fig. 1c, left panel) and elevated APP C-terminal fragments to 149% and 180% of vehicle-treated control and vehicle-treated Ts65Dn mice, respectively (Fig. 1b).

### DAPT reverses Ts65Dn Cognitive Deficits in the Morris Water Maze

Ts65Dn mice are characterized by deficits in spatial learning and memory in behavioral tests, including the Morris water maze [19]. Similar cognitive deficits have been described for AD model mice. Since acute treatment with DAPT had previously been shown to rescue cognitive deficits in an AD mouse model [20], we tested the ability of DAPT to improve cognitive function in Ts65Dn mice.

Measuring the time it takes to reach the hidden platform during training (latency), Ts65Dn mice treated with vehicle alone exhibited significantly poorer learning compared to control mice treated with vehicle (Fig. 2a), confirming the previously reported cognitive deficits in these mice [19]. Treatment of Ts65Dn mice with DAPT, however, completely reversed these deficits, such that learning in DAPT-treated Ts65Dn mice was not significantly different from learning in control mice treated with vehicle or DAPT (Fig. 2a). Importantly, average daily swim speed was measured throughout the experiment and was not significantly different among any of the groups (data not shown).

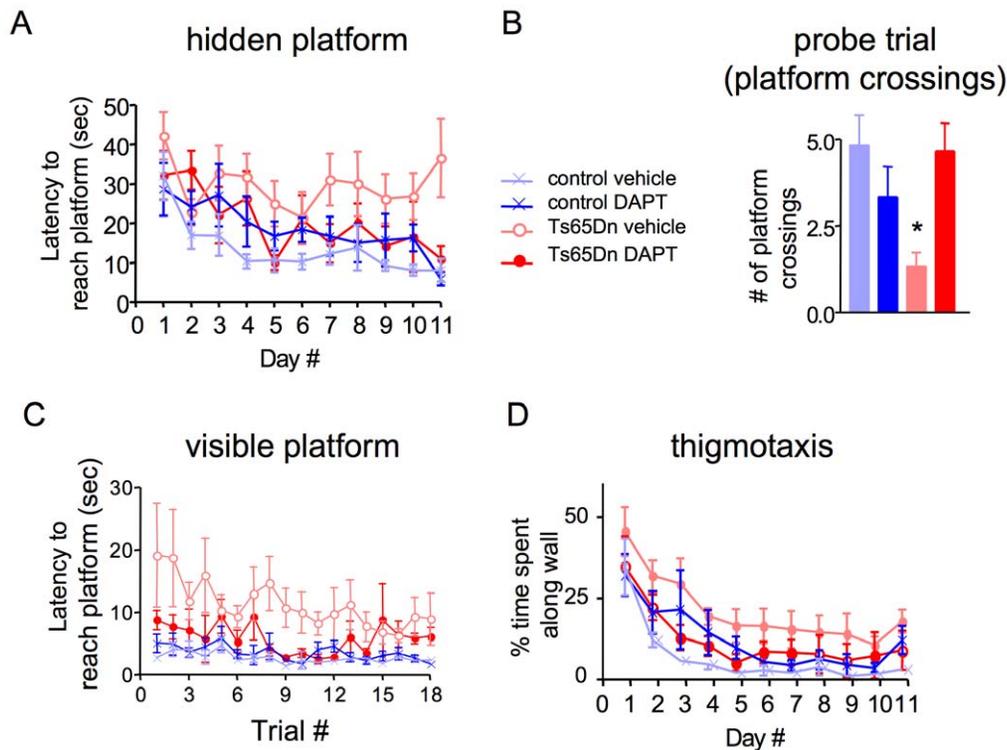
Following 11 days of training, a probe trial was performed on day 12 to assess spatial memory. Using the number of times mice

crossed the target platform location as a measure, Ts65Dn mice treated with vehicle alone showed a dramatically decreased number of crossings compared to vehicle or DAPT-treated control mice. In contrast, the number of target platform crossings for Ts65Dn mice treated with DAPT was equivalent to those of control mice treated with vehicle or DAPT (Fig. 2b). Neither DAPT nor vehicle significantly affected number of crossings for an arbitrary point in the pool (equivalent target location in opposite quadrant; not shown).

In agreement with previous reports [19], Ts65Dn mice exhibited poorer learning on a visible platform version of the water maze. This deficit was also rescued by treatment with DAPT (Fig. 2c). Interestingly, a slight increase in thigmotaxis (tendency to swim near the walls of the water maze) in the Ts65Dn mice was also reversed by DAPT (Fig. 2d), suggesting the complexity of the cognitive and behavioral phenotype that might be affected by A $\beta$ .

### Discussion

The notion that DS symptoms represent an irreversible developmental defect has been challenged recently by demonstrations that cognition in Ts65Dn mice can be improved pharmacologically using either GABA $_A$  antagonists [21], [22], memantine (an NMDA receptor antagonist) [23], or the noradrenergic agonist prodrug, L-DOPS [24]. Together these observations suggest that cognitive improvement in Ts65Dn mice occurs by enhancing or otherwise regulating excitatory synaptic transmission. This is consistent with observations suggesting that GABAergic over-inhibition of excitatory synaptic activity causes loss of synaptic plasticity in Ts65Dn mice [25]. Here we provide evidence that cognitive deficits in DS can be corrected by controlling A $\beta$  production, itself a regulator of glutamatergic transmission. Specifically, we propose that the cognitive improvement we



**Figure 2. DAPT reverses cognitive deficits in 4-month-old Ts65Dn mice in the Morris water maze.** DAPT was administered to Ts65Dn and control mice (100 mg/kg/day) two days prior to, and throughout, the maze testing. (A) Hidden platform test, latency to reach platform during training. (B) Probe trial on day 12, number of platform crossings. (C) Visible platform test, latency to reach platform. (D) Thigmotaxis. Statistical Analysis:  $n=6$  for all groups (A–D). (A) 2-way ANOVA with repeated measures revealed a main effect of genotype  $F_{1,20}=11.31$ ,  $p=0.003$  & Day  $F_{10,200}=4.90$ ,  $p=3.00E-06$  and an interaction between genotype and DAPT  $F_{1,20}=7.73$ ,  $p=0.012$ . Post-hoc planned comparison test between Ts65Dn+vehicle and all 3 other groups (Ts65Dn+vehicle vs. Ts65Dn+DAPT  $p=0.02$ , Ts65Dn+vehicle vs. control+vehicle  $p=0.0003$ , Ts65Dn+vehicle vs. control+DAPT  $p=0.008$ ,  $n=6$  in all groups for all figures). (B) 2-way ANOVA for number of target platform crossings revealed an interaction between genotype and DAPT  $F_{1,20}=8.46$ ,  $p=0.009$ . Post-hoc planned comparison test revealed a significant difference between Ts65Dn+vehicle vs. Ts65Dn+DAPT  $p=0.01$  and between Ts65Dn+vehicle vs. control+vehicle  $p=0.007$ . No significant differences were observed for number of crossings of the analogous, virtual opposite platform location (not shown). (C) 2-way ANOVA with repeated measures revealed significant effects of genotype,  $F_{1,20}=9.91$ ,  $p=0.005$  and day,  $F_{10,200}=21.42$ ,  $p=0.001$ , as well as a significant interaction between genotype and DAPT,  $F_{1,20}=5.43$ ,  $p=0.03$ . Post-hoc planned comparison test revealed significant differences between Ts65Dn+vehicle vs. all 3 other groups (vs. Ts65Dn+DAPT  $p=0.04$ , vs. control+vehicle  $p=0.003$ , and vs. control+DAPT  $p=0.005$ ). (D) 2-way ANOVA with repeated measures revealed main effects of genotype,  $F_{1,20}=5.13$ ,  $p=0.03$  & day  $F_{10,200}=21.94$ ,  $p<1.00E-06$  with an interaction between genotype and DAPT,  $F_{1,20}=5.43$ ,  $p=0.03$ . Post-hoc planned comparison test revealed only a significant difference between Ts65Dn+vehicle vs. control+vehicle  $p=0.004$ . doi:10.1371/journal.pone.0010943.g002

observed in Ts65Dn mice treated with the  $\gamma$ -secretase inhibitor, DAPT, resulted from lowered  $A\beta$  levels [26,27,28].

Our results contribute to a growing body of evidence that supports the hypothesis that cognitive function undergoes rapid change in response to fluctuations in soluble  $A\beta$  levels in cognitively impaired animals. Administration of DAPT to the Alzheimer's mouse model, Tg2576 corrects cognitive deficits characteristic of this strain after exposing the mice to the drug for as little as 3 hours prior to testing [20]. Tg2576 mice express human APP containing the familial Alzheimer's disease (FAD) Swedish mutation, which results in elevated levels of  $A\beta$  peptides [29]. Tg2576 mice begin to develop amyloid plaques at about 12+ months. However, soluble  $A\beta$  levels are elevated by five months and the mice are cognitively impaired at this time. The rapid correction of the cognitive deficit in these mice, ages 5 to 16 months, by DAPT administered only 3 hours prior to testing coincides with the amount of time required to reduce levels of soluble  $A\beta$  by about one half [16].

Other studies have shown that soluble  $A\beta$  rapidly inhibits hippocampal long-term potentiation (LTP) *in vivo* [30] and depresses excitatory synaptic transmission in hippocampal slice

neurons [31]. A mechanism that would account for these effects of  $A\beta$  is suggested by studies in which soluble  $A\beta$  has been shown to induce rapid internalization of NMDA- [26] and AMPA-type glutamate receptors [27,28]. These cellular events suggest a mechanism that could explain reduced cognitive function in the context of  $A\beta$  overexpression as well as the rapid recovery of cognitively impaired animals treated with  $A\beta$ -lowering drugs.

In human DS, children exhibit intellectual disability prior to the development of a neurodegenerative phenotype or the development of amyloid plaques [9]. This does not rule out developmental abnormalities as contributors to intellectual disability. However, given the rapid amelioration of cognitive deficits in Ts65Dn mice by DAPT, we suggest that intellectual disability in young DS patients might also be treatable by  $A\beta$ -lowering drugs.

## Materials and Methods

### Ethics Statement

The care of the animals and sacrifice procedures in this study were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were

approved by the Institutional Animal Care and Use Committee of The Rockefeller University.

### Mouse lines

All mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and maintained at The Comparative Bioscience Center (CBC) at The Rockefeller University. These consisted of Ts65Dn (trisomic) females and normosomic (disomic) colony-mate females as controls. Females were chosen, rather than males, to facilitate housing (5 to a cage). A previous study demonstrated that male and female Ts65Dn mice share equivalent learning deficits [12]. Although onset of estrus cycle in some Ts65Dn females may be delayed by one to two weeks, by eight weeks of age they are cycling the same as their diploid littermates. Since female mice (in general) when housed together, tend to become synchronous, the Ts65Dn mice would likely be synchronous with same-aged controls or littermates (Personal communication, Muriel T. Davisson, PhD, The Jackson Laboratory). Thus, for a given experiment, the mice were assumed to be in similar phases of the estrus cycle. The methods of breeding, genotyping and other pertinent information can be obtained through the Jackson lab web address: <http://www.jax.org/cyto/ts65dn.html>. Briefly, Ts65Dn mice (also designated: Ts(17<sup>16</sup>)65Dn) result from crossing Ts65Dn females to C57BL/6J*Ei*×C3H/HeSnJ (B6*Ei*C3Sn) F1 males. Quantitative PCR is used to identify trisomic mice. Normosomic controls consist of wild type mice (not harboring the segmental trisomy mutation) that are derived from the Ts65Dn colony. The recessive retinal degeneration 1 mutation (*Pde6b<sup>rd1</sup>*) segregates in this colony. Progeny are genotyped by standard PCR to screen out all mice harboring this gene.

### Detection of APP and APP CTFs

Mice were sacrificed by CO<sub>2</sub> asphyxiation and brains were immediately dissected. Cerebellum was removed and a hemibrain (volume approx. 200ml) was homogenized in 600ml of 3% SDS containing protease inhibitor cocktail, then sonicated and heated at 95°C for 10 min, followed by a second round of sonication. The resulting lysates were centrifuged at 13,000×g for 20 minutes at room temperature and supernatants were removed for analysis. After normalizing for protein concentration (bicinchoninic acid method), aliquots of each sample containing approximately 25mg of protein (5ml) were mixed with 20ml SDS sample buffer and resolved by SDS-PAGE on pre-cast 10–20% tricine Novex gels (Invitrogen). After electro-transfer to a PVDF membrane (pore diameter, 0.45μm), western blots were prepared using antibody 369 (which recognizes the C-terminal region of APP and APP-CTFs) or anti- $\beta$ -actin (Santa Cruz Biotechnology, sc-4778). APP, CTFs and actin were resolved by chemiluminescence on Kodak film. There were four groups of mice: Ts65Dn +/- DAPT, controls +/- DAPT. Each group consisted of 8 animals.

### Detection of mouse A $\beta$ by ELISA

Sandwich Elisa was carried out for endogenous mouse A $\beta$  as previously described [32]. The organic solvent, diethyl amine (DEA), was used to extract soluble A $\beta$  [33]. Briefly, hemi-brains were homogenized in 20 mM Tris buffer containing 1 mM EDTA, 1 mM EGTA, 250 mM sucrose and protease inhibitors, pH 7.4. The lysate was further homogenized with 0.4% DEA in 100 mM NaCl and centrifuged at 135,000×g for 60 min. The supernatant was neutralized by adding 0.5 M Tris-HCl, pH 6.8. The ELISA assay was performed as described previously [34]. Briefly, Nunc-immuno plates (Maxisorp; Nunc A/S, Roskilde, Denmark) were coated with 10  $\mu$ g/ml JRF/cA40/10 or JRF/cA42/26 antibodies. Mouse-specific antibody JRF/A1–15/2-

HRPO was used to detect the presence of A $\beta$  peptides. There were four groups of mice: Ts65Dn +/- DAPT, controls +/- DAPT. Each group consisted of 6 animals.

### DAPT administration

(N-[N-(3,5-Difluorophenacetyl-L-alanyl)]-S-phenylglycine *t*-Butyl Ester) was purchased from EMD Biosciences, Inc. and Sigma-Aldrich Co. Formulation and administration were carried out as described [16,35]. Briefly, DAPT was suspended in 100% ethanol (3mg/0.015ml), which was then rapidly mixed with filter-sterilized Mazola corn oil (10 mg/ml suspension in 5% ethanol/95% corn oil) by vortexing. 150 $\mu$ l was injected s.c. twice daily, and 300 $\mu$ l was injected in a single dose on the first and last day, per 30g mouse. Mice received ~100mg DAPT/kg/day. This dose was based on the quantity of DAPT reported to effectively lower A $\beta$  levels in mice, while allowing daily administration for up to two weeks without mortality or significant morbidity [16,35], [36].

### Behavioral testing

Water maze experiments were performed on 4-month-old female Ts65Dn mice and female disomic colony mate controls as previously described except that a single probe trial was conducted on day 12. Visible platform testing, with white vinyl curtains covering external cues, began one day after the probe trial and consisted of 6 trials/day for 3 days. 1.5 mg of DAPT (0.15 ml of a 10 mg/ml suspension in 5% ethanol/95% corn oil) was administered s.c. twice daily 2 days prior to testing and throughout water maze testing. No adverse effects were observed. There were four groups of mice: Ts65Dn +/- DAPT, controls +/- DAPT. Each group consisted of 6 animals. A 1.22 meter diameter, white, plastic, circular pool was filled to a depth of 33 centimeters with 22°C+/-1°C water made opaque with gothic white, non-toxic, liquid tempera paint in a room with prominent extra-maze cues. Mice were placed in one of 4 starting locations facing the pool wall and allowed to swim until finding a 15 centimeter diameter, white platform submerged by 0.75 cm for a maximum of 60 sec. On finding the platform, mice remained on the platform for 20 seconds before being removed to the home cage. If mice did not find the platform within 60 sec, they were guided to the platform by the experimenter and after remaining on the platform for 20 sec were removed to the home cage. Latency to reach the platform, distance traveled to reach the platform, swim speed, time spent in each of 4 quadrants and time spent along the walls were obtained using automated video tracking software from Noldus (Ethovision). Mice were trained with 4 trials/day with an inter-trial interval of 1–1.5 min for 11 consecutive days between 8 AM and 1 PM. A probe trial (free swim with the submerged platform removed) was performed as the first trial of the day on day12. The number of platform location crossings during the probe trial was calculated and analyzed with Student's t-test while latency to platform, swim speed and thigmotaxis (tendency to remain near walls) were analyzed using ANOVA with repeated measures. In separate experiments, a visual cue was attached to the platform and extra-maze cues were covered with white plastic curtains. Latency to reach the visible platform was recorded for 4 different, random platform locations with an inter-trial interval of 1 min. The visible platform test examines the animal's gross visual ability.

### Acknowledgments

We thank Wenjie Luo, Victor Bustos and Gen He for critically reading the manuscript, and Craig Hunter for assisting with animals.

## Author Contributions

Conceived and designed the experiments: WJN PG. Performed the experiments: WJN CP JB LW KD. Analyzed the data: WJN CP KD.

Contributed reagents/materials/analysis tools: WJN CP YN KD. Wrote the paper: WJN. Contributed to the writing of the paper: CP YN PG. Contributed to the design and writing of the paper: MF.

## References

- Epstein CJ (1990) The consequences of chromosome imbalance. *Am J Med Genet Suppl* 7: 31–37.
- Wishart JG (1993) The development of learning difficulties in children with Down's syndrome. *J Intellect Disabil Res* 37 ( Pt 4): 389–403.
- Antonarakis SE, Lyle R, Dermitzakis ET, Reymond A, Deutsch S (2004) Chromosome 21 and down syndrome: from genomics to pathophysiology. *Nat Rev Genet* 5: 725–738.
- Wolvetang EW, Bradfield OM, Tymms M, Zavarek S, Hatzistavrou T, et al. (2003) The chromosome 21 transcription factor ETS2 transactivates the beta-APP promoter: implications for Down syndrome. *Biochim Biophys Acta* 1628: 105–110.
- Selkoe DJ, Yamazaki T, Citron M, Podlisny MB, Koo EH, et al. (1996) The role of APP processing and trafficking pathways in the formation of amyloid beta-protein. *Ann N Y Acad Sci* 777: 57–64.
- Tanzi RE, Bertram L (2005) Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. *Cell* 120: 545–555.
- Walsh DM, Selkoe DJ (2004) Deciphering the molecular basis of memory failure in Alzheimer's disease. *Neuron* 44: 181–193.
- Gyure KA, Durham R, Stewart WF, Smialek JE, Troncoso JC (2001) Intraneuronal abeta-amyloid precedes development of amyloid plaques in Down syndrome. *Arch Pathol Lab Med* 125: 489–492.
- Hyman BT (1992) Down syndrome and Alzheimer disease. *Prog Clin Biol Res* 379: 123–142.
- Davisson MT, Schmidt C, Reeves RH, Irving NG, Akeson EC, et al. (1993) Segmental trisomy as a mouse model for Down syndrome. *Prog Clin Biol Res* 384: 117–133.
- Akeson EC, Lambert JP, Narayanswami S, Gardiner K, Bechtel LJ, et al. (2001) Ts65Dn – localization of the translocation breakpoint and trisomic gene content in a mouse model for Down syndrome. *Cytogenet Cell Genet* 93: 270–276.
- Martinez-Cue C, Baamonde C, Lumberras M, Paz J, Davisson MT, et al. (2002) Differential effects of environmental enrichment on behavior and learning of male and female Ts65Dn mice, a model for Down syndrome. *Behav Brain Res* 134: 185–200.
- Granhölm AC, Sanders LA, Crnic LS (2000) Loss of cholinergic phenotype in basal forebrain coincides with cognitive decline in a mouse model of Down's syndrome. *Exp Neurol* 161: 647–663.
- Godridge H, Reynolds GP, Czudek C, Calcutt NA, Benton M (1987) Alzheimer-like neurotransmitter deficits in adult Down's syndrome brain tissue. *J Neurol Neurosurg Psychiatry* 50: 775–778.
- Naruse S, Thinakaran G, Luo JJ, Kusiak JW, Tomita T, et al. (1998) Effects of PS1 deficiency on membrane protein trafficking in neurons. *Neuron* 21: 1213–1221.
- Dovey HF, John V, Anderson JP, Chen LZ, de Saint Andrieu P, et al. (2001) Functional gamma-secretase inhibitors reduce beta-amyloid peptide levels in brain. *J Neurochem* 76: 173–181.
- Reeves RH, Irving NG, Moran TH, Wohn A, Kitt C, et al. (1995) A mouse model for Down syndrome exhibits learning and behaviour deficits. *Nat Genet* 11: 177–184.
- Choi JH, Berger JD, Mazzella MJ, Morales-Corraliza J, Cataldo AM, et al. (2009) Age-dependent dysregulation of brain amyloid precursor protein in the Ts65Dn Down syndrome mouse model. *J Neurochem*.
- Holtzman DM, Santucci D, Kilbridge J, Chua-Couzens J, Fontana DJ, et al. (1996) Developmental abnormalities and age-related neurodegeneration in a mouse model of Down syndrome. *Proc Natl Acad Sci U S A* 93: 13333–13338.
- Comery TA, Martone RL, Aschmies S, Atchison KP, Diamantidis G, et al. (2005) Acute gamma-secretase inhibition improves contextual fear conditioning in the Tg2576 mouse model of Alzheimer's disease. *J Neurosci* 25: 8898–8902.
- Rueda N, Florez J, Martinez-Cue C (2008) Chronic pentylentetrazole but not donepezil treatment rescues spatial cognition in Ts65Dn mice, a model for Down syndrome. *Neurosci Lett* 433: 22–27.
- Fernandez F, Morishita W, Zuniga E, Nguyen J, Blank M, et al. (2007) Pharmacotherapy for cognitive impairment in a mouse model of Down syndrome. *Nat Neurosci* 10: 411–413.
- Costa AC, Scott-McKean JJ, Stasko MR (2008) Acute injections of the NMDA receptor antagonist memantine rescue performance deficits of the Ts65Dn mouse model of Down syndrome on a fear conditioning test. *Neuropsychopharmacology* 33: 1624–1632.
- Salehi A, Faizi M, Colas D, Valletta J, Laguna J, et al. (2009) Restoration of norepinephrine-modulated contextual memory in a mouse model of Down syndrome. *Sci Transl Med* 1: 7ra17.
- Kleschevnikov AM, Belichenko PV, Villar AJ, Epstein CJ, Malenka RC, et al. (2004) Hippocampal long-term potentiation suppressed by increased inhibition in the Ts65Dn mouse, a genetic model of Down syndrome. *J Neurosci* 24: 8153–8160.
- Snyder EM, Nong Y, Almeida CG, Paul S, Moran T, et al. (2005) Regulation of NMDA receptor trafficking by amyloid-beta. *Nat Neurosci* 8: 1051–1058.
- Hsieh H, Boehm J, Sato C, Iwatsubo T, Tomita T, et al. (2006) AMPAR removal underlies Abeta-induced synaptic depression and dendritic spine loss. *Neuron* 52: 831–843.
- Almeida CG, Tampellini D, Takahashi RH, Greengard P, Lin MT, et al. (2005) Beta-amyloid accumulation in APP mutant neurons reduces PSD-95 and GluR1 in synapses. *Neurobiol Dis* 20: 187–198.
- Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, et al. (1996) Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science* 274: 99–102.
- Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, et al. (2002) Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature* 416: 535–539.
- Kamenetz F, Tomita T, Hsieh H, Seabrook G, Borchelt D, et al. (2003) APP processing and synaptic function. *Neuron* 37: 925–937.
- Horikoshi Y, Sakaguchi G, Becker AG, Gray AJ, Duff K, et al. (2004) Development of Abeta terminal end-specific antibodies and sensitive ELISA for Abeta variant. *Biochem Biophys Res Commun* 319: 733–737.
- Savage MJ, Trusko SP, Howland DS, Pinsker LR, Mistretta S, et al. (1998) Turnover of amyloid beta-protein in mouse brain and acute reduction of its level by phorbol ester. *J Neurosci* 18: 1743–1752.
- Refolo LM, Pappolla MA, LaFrancois J, Malester B, Schmidt SD, et al. (2001) A cholesterol-lowering drug reduces beta-amyloid pathology in a transgenic mouse model of Alzheimer's disease. *Neurobiol Dis* 8: 890–899.
- Lanz TA, Himes CS, Pallante G, Adams L, Yamazaki S, et al. (2003) The gamma-secretase inhibitor N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester reduces A beta levels in vivo in plasma and cerebrospinal fluid in young (plaque-free) and aged (plaque-bearing) Tg2576 mice. *J Pharmacol Exp Ther* 305: 864–871.
- Imbimbo BP (2008) Therapeutic potential of gamma-secretase inhibitors and modulators. *Curr Top Med Chem* 8: 54–61.