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Efficacy Measures of Tolfenamic Acid in a Tau Knock Out Model: Relevance to Alzheimer's Disease

Allison Leso
University of Rhode Island, allisonleso@gmail.com

EFFICACY MEASURES OF TOLFENAMIC ACID IN A TAU KNOCK OUT MODEL: RELEVANCE TO ALZHEIMER'S DISEASE

BY

ALLISON LESO

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ALLISON LESO

APPROVED:

Thesis Committee:

Major Professor Nasser Zawia

Leslie Mahler

Kristina Ward

Andrea Rusnock
ASSOCIATE DEAN OF THE GRADUATE SCHOOL

UNIVERSITY OF RHODE ISLAND
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ABSTRACT

In the healthy human brain, the protein tau serves the essential function of stabilizing microtubules. However, in a diseased state, tau becomes destabilized and aggregates into a pathogenic form that ultimately creates one of the two major hallmarks of Alzheimer's disease (AD), amyloid-beta (Aβ) plaques and tau tangles. Multiple neurodegenerative diseases, termed tauopathies, such as Pick's disease, and progressive supranuclear palsy (PSP), are also linked to mutations in tau. While AD does include a second hallmark in the form of Aβ plaques, to date all therapeutics aimed at this hallmark have failed. However, the nonsteroidal anti-inflammatory drug (NSAID) tolfenamic acid (TA) has been shown to reduce the levels of multiple neurodegenerative endpoints, and improve cognitive function, in various murine models. Of the murine models tested with TA, all contained some form of the tau gene and the amyloid precursor protein (APP) gene, the precursor of Aβ. The experimental model utilized in this paper, unlike others, tested whether the same positive effects of TA can take place after removal of endogenous murine tau. The impacts of TA, both molecular and behavioral, were no longer significant in the absence of tau. Mice treated with TA, and lacking the tau gene performed no better than their counterparts that were untreated. Additionally, mice treated with TA exhibited no change in levels of neurodegenerative endpoints over those mice that were untreated. Only those mice that were treated with TA while concurrently possessing the tau gene exhibited improved cognitive function and lower pathological burden. This project better identifies links between tau and known neurodegenerative endpoints, and proposes that tau is essential for the action of TA.

Keywords: Alzheimer’s disease; tau; tolfenamic acid; Sp1; neurodegeneration

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Lastly, to my parents and my brother Alden, thank you for standing by me through this chapter of my life and all the others, and pushing me to become the scientist you have always believed in me to be. Your steadfast support and love has made all the difference.

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Efficacy Measures of Tolfenamic Acid in a Tau Knock Out Model: Relevance to Alzheimer's Disease

Allison Leso[1], Syed Waseem Bihaqi[2], Anwar Masoud[3], Joanna K. Chang[4], Asma Lahouel[5], Nasser Zawia[1,4*]

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[1]Interdisciplinary Neuroscience Program, University of Rhode Island, Kingston, RI, USA
[2]George and Anne Ryan Institute for Neuroscience, University of Rhode Island, Kingston, RI, USA
[3]Biochemical Technology Program, Faculty of Applied Science, Thamar University, Thamar, Yemen
[4]Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI, USA
[5]Department of Molecular and Cellular Biology, Jijel University, Algeria

*Corresponding authors:

Address correspondence to Nasser H. Zawia at the Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI, 02881, USA; Tel: +1-401-874-2663; Fax: +1-401-874-5491; Email: nzawia@uri.edu

1. INTRODUCTION

Neurodegenerative diseases as a whole are defined as disorders with a selective loss of neurons and distinct involvement of functional systems defining a clinical presentation (Moodley and Chan, 2014). Research has demonstrated that proteins with altered physicochemical properties may tend to deposit in definitive regions of the brain, such as the frontal cortex (Irvine et al., 2008). Among various neurodegenerative disorders, Alzheimer's disease (AD) is perhaps the most widely studied. The deposition of proteins such as hyperphosphorylated tau and plaques of amyloid are the two major biological hallmarks of AD, and the official markers of postmortem diagnosis of AD (Reitz and Mayeux, 2014). Tau belongs to a family of microtubule-associated proteins (MAPs) that undergoes hyperphosphorylation in the AD state, and ultimately forms neurofibrillary tangles (NFTs). This improper phosphorylation of tau may alter its conformation, allowing it to detach from microtubules, abnormally aggregate, and form the diagnostic insoluble NFTs (Fischer et al., 2009; Ishihara et al., 2001; Jho et al., 2010).

In spite of years of research on a wide spectrum of neurodegenerative disorders, the primary and decisive pathway under dysregulation in AD is unresolved. Previously established research has found that in AD, both amyloid and tau become insoluble and form plaques and NFTs, respectively (Bloom, 2014). Though normal phosphorylation occurs through kinase and phosphatase mechanisms at a large variety of sites, NFT's hyperphosphorylation is site-specific (Duka et al., 2013). These specific sites have been identified as serine or threonine residues, acting as substrates for kinase activity (Larson et al., 2012). The most notable family of kinases capable of tau phosphorylation are those of the mitogen-activated protein kinase (MAPK) family, specifically cyclin-dependent

kinase-5 (CDK5) (Wagner et al., 1996). CDK5's activation is supported largely by p35/25, a complex of regulatory subunits abundantly found in post mitotic neurons (Patrick et al., 1999; Tomizawa et al., 2002). Hyperphosphorylation of tau has shown to be responsible for the neurofibrillary lesions and thus any interference in this pathway would be expected to provide therapeutic benefits (Iqbal et al., 2010).

Specificity protein 1 (Sp1) is a zinc-finger transcription factor essential for the regulation of tau and CDK5 genes, among others (Valin et al., 2009). CDK5 is responsible for the phosphorylation of tau on sites that are unusually hyperphosphorylated in tauopathies (Bu et al., 2002). Sp1 also regulates the expression of tau, thus, mutations on the Sp1 binding regions on the tau promoter may affect tau expression (Santpere et al., 2006). Previous reports from our lab have provided convincing evidence that either silencing of the Sp1 gene using small interfering RNA (Basha et al. 2005), or treatment of animals with Tolfenamic acid (TA) lowers the expression of Sp1 target genes (Adwan et al. 2011). Sp1 binding motifs were also found on the promoter regions of p35 and p39 (Ohshima et al., 1996, 1995; Ross et al., 2002; Valin et al., 2009). Therefore, targeting Sp1 is an ideal approach to reduce tau levels, and such reduction is likely to impact any post-translational modifications of tau, thereby providing a mechanistic approach to reduce the pathological features of neurodegenerative diseases while providing cognitive improvement.

TA, known as (Clotam® Rapid), possesses the ability to reduce Sp1, and has been used as a migraine medication for decades in Europe due to its anti-inflammatory properties associated with COX inhibition (Sidhu et al., 2006). However, TA is not approved for any human indication in the United States. The European Medicine Agency

and the US Food and Drug Administration (FDA) have designated TA as a potential treatment for frontotemporal dementia (FTD) and progressive supranuclear palsy (PSP). The present study was conducted to assess two specific aims which would aid in determining the ability of TA to modulate the overexpression of tau-related biomarkers and the severity of cognitive deficits and tauopathy in animal models. These two aims are stated as follows: 1) to determine the relevance of the tau pathway to the efficacy of TA as it may effect and improve memory retention and learning; 2) to determine the ability of TA to downregulate tau-related biomarkers in both the presence and absence of the tau pathway. These aims will be evaluated through the usage of: 1) mice that are non-carriers for tau (NC); 2) mice that are carriers for human tau and knockout for murine tau (hTau +/-) gene (C). Furthermore, a cellular model of SH-SY5Y neuroblastoma cells exposed to a series of concentrations of TA was used to provide further confirmation of treatment efficacy in conjunction with animal models. Studies aim to build a better understanding of the mechanisms at play when tau is present, and perhaps more illuminating, when it has been removed.

2. MATERIAL AND METHODS

2.1. Animal exposure

Transgenic (Tg) mice hemizygous for hTau, strain B6.Cg-Mapttm1(EGFP)Klt Tg(MAPT)8cPdav/J, were obtained from Jackson Laboratory; Stock No: 005491 (Bar Harbor, ME). These Tg mice are knock-in for hTau, and express all six isoforms of the human tau (3R and 4R), and are homozygous knockouts for murine tau. As these mice are carriers of the hTau gene, they will be known as carriers (C, +/-). Included in the transgene are the coding sequences, intronic regions, and regulatory elements of the endogenous human promoter region. Mice that are homozygous knockouts for murine tau will be referred to as non-carriers (NC, -/-). The control group received only vehicle (corn oil), while treatment group received TA (dissolved in corn oil) daily for 34 days at a dose of 5 mg/kg via oral gavage. All mice were bred and genotyped in house, on a 12:12 light-dark cycle at the University of Rhode Island (URI). Food and water were made available for all mice *ad-libitum,* and room temperature was maintained at 22+/-2°C. All animal procedures and protocols were approved by the University of Rhode Island Institutional Animal Care and Use Committee (IACUC), and all animals were under the constant supervision of a URI veterinarian for the duration of the study.

2.2. Behavioral Studies

Mice were tested for learning and memory using the Morris Water Maze (MWM) task, following the method of Morris *et al*. (Morris et al., 1982).The maze apparatus consisted of a white pool (48" diameter, 30" height) filled with water to a depth of 14".

In order to make the water opaque, white, non-toxic paint (Crayola, New York City, NY, USA) was used. The pool was virtually divided into four quadrants (NW, NE, SW, and SE), and distinct visual cues were placed along the sides of the pool. A clear Plexiglas escape platform ($10cm^2$) was submerged in one of the quadrants, 0.5 cm below the surface of the water. This quadrant was then known as the platform zone (PFZ). Water temperature was maintained at 25+/-2°C during all procedures.

Mice were allowed to acclimate to the experiment on day 0, in what is known as a habituation trial, by swimming freely for 60 s. Over the following 7 days, mice received 3 daily training sessions with a 20-minute inter-trial interval. The starting position was randomly assigned between four possible positions while the platform, and associated platform zone (PFZ), remained fixed for all trials. Mice were given a maximum of 60 s of swimming to find the platform. For the first 3 days of the trial, any mouse that failed to locate the platform in time was gently guided towards the platform by the experimenter and allowed to remain on the platform for 30 s. For the remaining 4 days of the trial, mice were removed from the maze if they failed to locate the platform within 60 s. Following the 7^{th} day of training, probe trials were performed on days 8 and 18 in order to assess memory retention. In probe trials, the platform was removed and mice were allowed to swim for 60 s. A predilection for the platform location would be an indication that the mice had developed memory of the correct quadrant (the quadrant that contained the hidden platform during the previous training sessions). The swim paths and latencies were videotaped and analyzed with a computerized video-tracking system (ObjectScan, Clever Sys. Inc., Reston, VA, USA).

2.3. Cell Culture:

Human neuroblastoma (SH-SY5Y) cells were procured from American Type Culture Collection (ATCC, Manassas, VA), and were cultured in 6 well plates (CellTreat, Pepperell, MA) at a density of 0.3 x 10^6 cells/well containing Dulbecco's Modified Eagle Medium(DMEM)/F12 medium (Sigma-Aldrich, MO) with 10% fetal bovine serum (FBS) (Sigma-Aldrich, MO), 100 μg/mL penicillin, and 100 μg/mL streptomycin in a carbon dioxide (CO_2) incubator maintained at 5% CO_2 and37°C. In order to differentiate, SH-SY5Y cells were stimulated with 10 μM all-trans retinoic acid (Sigma-Aldrich, MO), as described previously (Bihaqi et al., 2017). The cells were then examined for neurite outgrowth for 48 h, 72 h, and 6 days, with media changes occurring every 3 days. A 20X objective lens on a Nikon ECLIPSE camera (TE2000-E), adapted to the microscope, was used to examine the morphology of cultured cells. Differentiated neuroblastoma cells were exposed to TA according to conditions previously established by our lab (Adwan et al., 2014). Cells were incubated with 0, 5, or 25 μM of TA for 72 hours at 37°C.

2.4. Protein extraction and Western blotting:

Euthanization of mice was performed by CO_2 inhalation 24 h after the final dose of TA was administered. Brains were dissected and stored at -80°C, awaiting analysis. Brain cortices and cells collected following exposure were homogenized or lysed in radio-immunoprecipitation assay (RIPA) lysis buffer (Sigma Aldrich, MO) supplemented with 0.1% protease inhibitor cocktail, and 5μl phosphatase inhibitor per 1mL lysis buffer (Sigma-Aldrich, MO). The samples were incubated on ice for 30 min in order to allow

efficient lysis of the samples. The samples were sonicated and vortexed for 5 min before centrifugation at 10,000 × g for 20 min at 4°C and supernatants were collected and stored at −80°C until further use. Protein concentration was determined by Pierce bicinchoninic assay (BCA) kit (Thermo Scientific, Waltham, MA). For the determination of protein expression of various biomarkers 40μg of the total protein samples were loaded onto an SDS-Page gel and run for 2 h at 100 mV, and then transferred to Polyvinylidene Fluoride (PVDF) membranes (GE, Piscataway, NJ). Non-specific binding was blocked by incubation with 5% BSA in Tris buffer saline + 0.1% Tween 20 (TBST) at room temperature for 1h. Membranes were incubated overnight at 4°C with following primary antibodies at a dilution of 1:1000 [Mouse Anti-APP, Rabbit Anti-CDK5, Rabbit Anti-COX2, Rabbit Anti-GAPDH, Rabbit Anti-p35/25, Mouse Anti-SP1] (Cell Signaling Technology, Danvers, MA). On the following day, membranes were washed and exposed for 1 h to IRDye®800LT Infrared Dye (LI-COR Biotechnology, NE), goat anti-mouse/goat anti-rabbit diluted at 1:10000. The images were developed using Odyssey infrared imaging system (LI-COR Biotechnology, NE). As a control for equal protein loading, membranes were stripped and re-probed with rabbit GAPDH antibody (diluted at 1:2500, Cell Signaling Tech, MA) and exposed to anti rabbit IRDye® 680LT Infrared Dye. After transferring to a PVDF membrane, the gel was stained with Bio-safe Coomassie blue stain (Bio-Rad, CA) to assess the loading of the samples.

2.5. Statistical treatment:

Data for molecular tests was analyzed by students t-test followed by Bonferonni's post-hoc test to compare the effects among various treatments. GraphPad Prism 3.0 computer software (La Jolla, CA, USA) was used for analysis, $P<0.05$ was considered significant. Behavioral data was analyzed by two-way analysis of variance (ANOVA) followed by Duncan's post-hoc test to compare the effects among various treatments. GraphPad Prism 3.0 computer software (La Jolla, CA, USA) was used for analysis. $P<0.05$ is considered significant. Table 1 displays the power produced by each comparison in both the molecular and behavioral end points of this paper. In behavioral studies, an "n" of 5 was used, and in molecular studies, an "n" of 4 was utilized. These numbers are adequate to predict differences between groups with statistical power while also considering the inter-individual variation presented among animals.

3. RESULTS

3.1. Treatment with TA improves memory retention only in the presence of the tau gene

Distance travelled reaching the platform allows for assessment of memory retention, as those mice that remember the location of the platform will cover less distance in reaching it. Results indicated that only those mice treated with TA and possessing the tau gene swam a significantly lower distance than their untreated counterparts (Fig. 1 A, B). Differences in cognitive function between NC's treated with 5 mg/kg of TA and their respective controls were assessed utilizing two probe trials spanning 10 days. Probe trials measured the amount of time spent inside of or outside of the correct PFZ after a latency period, which can allow for assessment of reference and working memory. Statistical analysis revealed no significant difference between the treatment group and aged matched control group, demonstrating the drug as ineffective in this experimental paradigm (Fig. 1C). However, this finding should be compared against the results of the same trial reported in a previous study by Chang et. al. in which hTau carriers treated with TA performed significantly better compared to their untreated, control counterparts (Chang et al., 2018).

There were no significant differences between swim speeds in both groups during probe trials.

3.2. TA alters the protein levels of APP only in the presence of the tau gene

Antibodies directed against APP were used to study the protein expression via western blot in differentiated SHSY-5Y cells treated with TA at 5 μM and 25 μM for 72 consecutive hours. Our results indicated a significant decrease in the normalized protein levels of APP in cells treated with 5 μM of TA ($p=0.007$) and 25 μM of TA ($p=0.05$) compared to control (Fig.2A).

In aged 18-month-old NC mice, TA loses its ability to alter APP levels. NC-C showed no significant difference in levels of APP protein expression, relative to GAPDH, when compared to NC-TA (Fig. 2B).

3.3. TA impacts CDK5 and p25/35 only in the presence of the tau gene

Quantification of protein expression relative to GAPDH by western blotting across the four groups of mice revealed that only those murine models which possessed hTau and were treated with TA saw significant decreases in levels of CDK5 ($p=0.03$) (Fig.3A) and p25 ($p=0.05$) (Fig.3C). On the other hand, those NC-TA mice did not demonstrate the same downregulation of CDK5 and p25, even though they had received identical treatment with TA. No significant difference between controls and respective treatment groups (C and NC) was seen for p35 (Fig.3B).

3.4. TA alters COX2 levels regardless of the presence or absence of tau

To confirm that TA still retains its COX related anti-inflammatory activity, COX2 protein levels were measured across all four groups relative to GAPDH. Results indicated a significant decrease in the protein levels of COX2 in both the C ($p=0.03$) and NC ($p=0.003$) treated groups (Fig. 4).

4. DISCUSSION

By the year 2050, the prevalence of AD is expected to quadruple, leaving approximately 1 in 85 people afflicted with the disease (Rocca et al., 2011). Considering that the nature of the disease carries with it extreme phenotypic changes in the form of varying and progressive cognitive deficits, these numbers are particularly alarming. Trials of approved prescriptions for AD such as donepezil (Aricept) and rivastigmine (Exelon) showed a positive impact on cognitive functions after usage of these drugs and others in their class (Farlow, 2002). These drugs, among others, work to alleviate the symptomatic aspects of AD, rather than obstruct the progressive cellular death that underlies the phenotype. Consequently, there is no disease modifying drug available for AD.

Going forward, an ideal drug candidate for AD should also possess the ability to attenuate this widespread cell death, and the ability to halt or restore cognitive decline. As a drug candidate, TA shows the promise of halting neuronal loss in addition to improving cognitive functions in murine models in which it is applied. In addition, TA, unlike other failed therapeutics, possesses the ability to target an upstream factor (Sp1) of APP, CDK5, and p25, all of which are found upregulated in AD patients. TA has demonstrated its capability in attenuating the cognitive deficits in a transgenic mouse model of AD, R1.40, through a study published by Subaiea and colleagues (Subaiea et al., 2013). Mnemonic improvements in cognitive tasks occurred in parallel to reductions in APP expression, and soluble and insoluble $A\beta_{40-42}$ levels, which correlated with reductions in Sp1 protein expression. Seminal work by Adwan and coworkers used the identical R1.40 model, and after treatment with TA, the model showed a reduction in total murine tau

protein levels by 46%, concordant with a 50% reduction in the levels of CDK5 (Adwan et al., 2015). Together, these results suggest that TA is capable of concurrent action on both the amyloid and tau pathways as both were present in these experimental models.

Recently, Chang and colleagues reported that TA treated hTau knockin mice displayed a significant improvement in cognitive function and long-term memory compared to their non-treated counterparts (Chang et al., 2018). In addition, TA was shown to reduce site specific hyperphosphorylation of tau at threonine-181 (THR181) and serine-396 (SER396), known sites of hyperphosphorylation in AD (Gong and Iqbal, 2008; Chang et al., 2018).

All of these studies have explored the effect of TA on both wild type and transgenic mice, both of which possessed the *MAPT* and the *APP* gene. The findings of the above studies as a collective, however, did not make it clear whether TA had simultaneous effects on the amyloid and tau pathways, or whether it selectively targeted one of these pathways in a way which impacted the other. The present study has built on these findings to demonstrate that the cognitive improvements and reduction in AD-associated biomarkers after TA treatment is contingent on the presence of tau (Fig. 1).

The calpain driven transformation of p35 to p25 is responsible for the activation of CDK5, thus any aberrations to CDK5 are subsequent to aberrations in p25 (Patrick et al., 1999). Studies have found accumulations of p25 in the neurons of post mortem brains of AD patients, directly correlated with an increase in CDK5 activity (Patrick et al., 1999). Thus, there is need for a therapeutic agent with an ability to degrade p25, which would eventually slow the phosphorylative activity of CDK5 and demand it contribute

less to overall phosphorylation. The ability of TA to attenuate hyperphosphorylation via degradation of CDK5 and p25 is lost in concordance with the loss of the tau gene (Fig.3 A &C).

The success of TA binding to Sp1 would initiate a cascade of events that would lower levels of proteins transcribed from genes rich in GC regions, of which would include APP, CDK5, and p35/25. Previous published work by our lab has shown that administration of TA to wildtype mice or human APP knock-in mice results in a significant reduction in Sp1 levels (Adwan et al., 2014; Subaiea et al., 2013). This suggests that Sp1might act as a target of TA, and any alterations in its levels would have an impact on its ability to drive its target genes. The presence of tau may be necessary for this action to be productive, particularly when it relates to TA-induced downregulation of the amyloid pathway. As seen in Figure 2, only in an experimental model in which the tau pathway is present (Fig. 2A), is TA able to work on the amyloid pathway and downregulate levels of APP protein. When the tau pathway has been removed, (Fig. 2B), TA is no longer able to degrade the protein.

TA presents itself as promising in other modes, as neurodegenerative diseases are highly inflammatory in nature. In AD alone, increases in levels of neuronal COX2, microglial COX1, mPGES1, and parenchymal PGE2 have been observed (Yagami et al., 2016). All of the aforementioned are markers of inflammation, and as such, an effective therapeutic for AD should possess some anti-inflammatory properties, which TA possesses (Fig.4). This study clearly shows that the anti-inflammatory activity of TA was

independent of the neurodegenerative process, as the absence of tau seemed to impact the tau and amyloid pathways, but not the inflammatory process.

TA exhibits itself as a drug with a broad spectrum of activity, as it is capable of lowering several AD associated proteins and inflammation associated pathways. Central nervous system (CNS) drugs are rarely successful, no matter if their application is neurodegenerative in nature. Though around 98% of all small molecules are incapable of transport across the blood-brain barrier (BBB), TA crosses the BBB, and the validity of such was established in previous literature utilizing multiple methodologies. In 2011, Subaiea et al. studied the extent of TA to reach the brain via *in silico*, *in vitro*, and *in vivo* methodologies (Subaiea et al., 2011). All three methodologies supported the conclusion that TA is able to reach the CNS in concentrations that would allow it to exert pharmacological effects.

5. CONCLUSIONS

In summary, the ability of TA to decrease AD-related biomarkers is only present in models which contain either the endogenous or human form of the tau gene. This ability is strongly attenuated when the gene in question has been removed. Increases in APP, SP1, CDK5, and p25 are seen in AD patients. Increases in the aforementioned are associated with neuronal death, and as such an effective treatment may aim to inhibit their overproduction. Previous studies have shown decreases in these proteins and others in models in which both human and murine tau and APP are present (Adwan et al., 2014, 2011; Subaiea et al., 2013). However, this study aims to elucidate the mechanisms by which this effective treatment may work, and as the results show, treatment with TA is unsupported when tau has been removed. Altogether, these results suggest that tau is a requirement for the action of TA in an AD paradigm.

CONFLICT OF INTEREST AND ACKNOWLEDGEMENT STATEMENT:

Conflict of Interest: The authors declare no conflict of interest, financial or otherwise.

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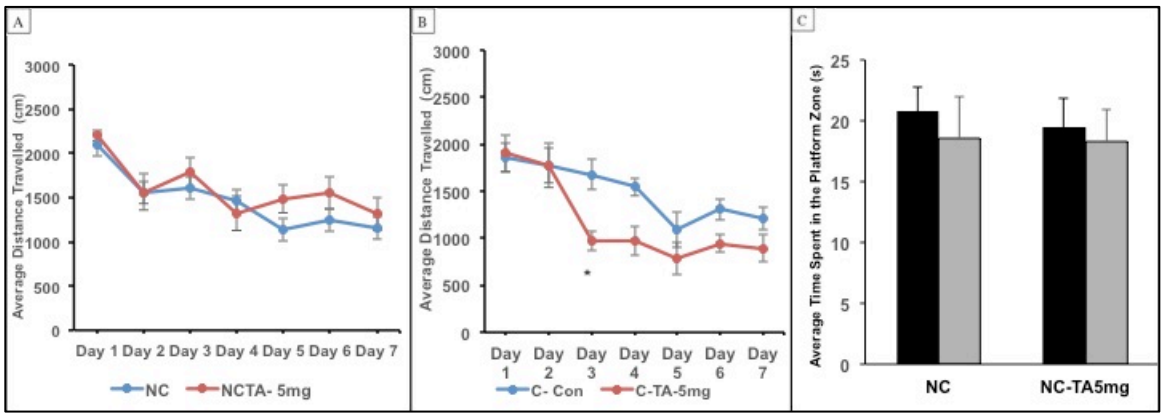
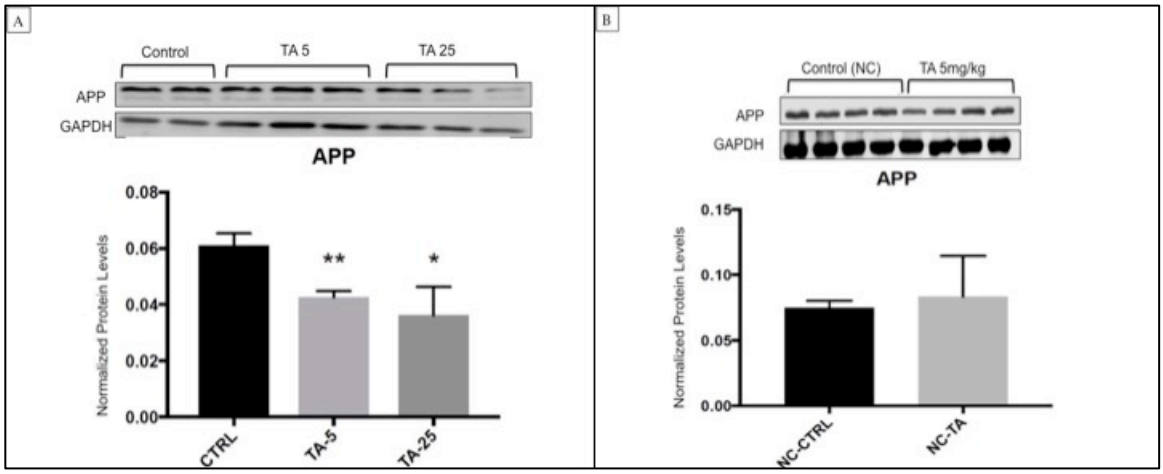
FIGURES:

Figure 1: Memory retention in 18 mo. knock out (NC) and hTau carrier (C) mice after treatment with TA.



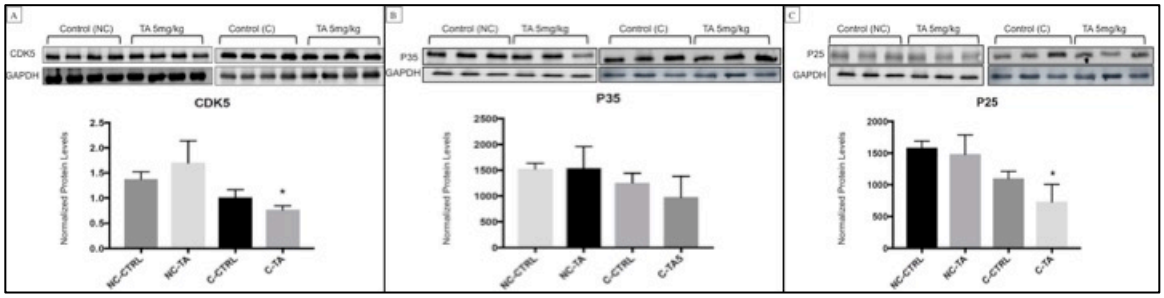
(A,B) Average distance swam in centimeters before reaching platform during 7 days of acquisition period. Each data point represents average distance per day. Values are expressed as mean ± standard error of the mean. $P<0.05$ considered significant by ANOVA, noted by "*" (C-CTRL v. C-TA). (C) Memory retention was assessed by a 60-second probe trial on day 8 (Probe trial 1), following the last day of acquisition testing and repeated after day 18 (Probe trial 2). Each data point in the bar diagram represents the average total time in seconds spent by mice in the PFZ.Values are expressed as mean ± standard error of the mean. No significance was observed.

Figure 2: APP protein expression after exposure to TA:



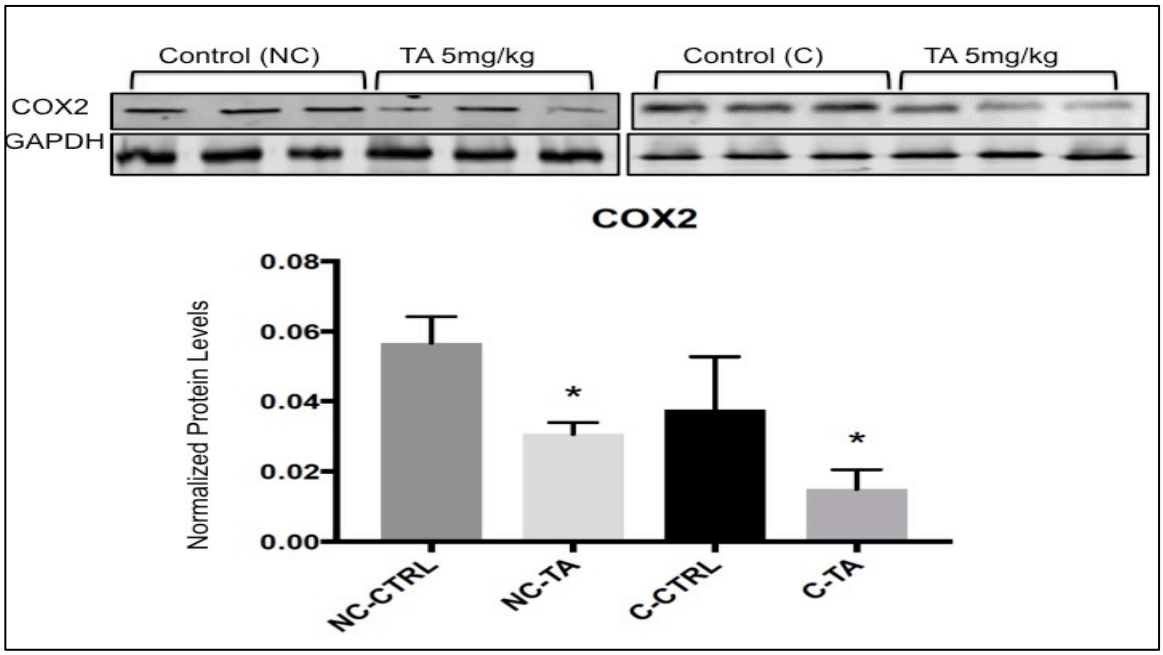
(A) SHSY-5Y neuroblastoma cells after 72 h exposure to 0, 5, or 25 µM of TA. Quantification of APP normalized against GAPDH, n = 3. $P<0.05$ considered significant by students t-test, noted by "*". For 5 µM, p=0.007, for 25 µM, p=0.05. (B) Aged 17-18 month old mice, homozygous knockouts for tau, mice were administered with vehicle (corn oil), or 5 mg/kg TA (dissolved in corn oil) for 34 consecutive days. Quantification of APP in the frontal cortex was normalized against GAPDH, n=4, no significance observed. All values (Fig. 3A, B) are expressed as mean ± standard error of the mean.

Figure 3: (A) CDK5, (B) p35, and (C) p25 protein expression in frontal cortex after exposure to TA:



(A-C) Quantification of protein in the frontal cortex of mice aged 17-18 months normalized against GAPDH. Mean ± SEM. $P<0.05$ is considered significant via students t-test, noted by "*". Mice were administered with vehicle (corn oil), or 5 mg/kg TA dissolved in corn oil for 34 consecutive days. (A) Quantification of CDK5normalized against GAPDH, n = 4. (NC-CTRL, NC-TA) $P>0.05$, (C-CTRL, C-TA) $P=0.03$. (B) Quantification of p35 normalized against GAPDH. Mean ± SEM, n = 3, $P>0.05$ for all groups. (C) Quantification of p25 normalized against GAPDH. Mean ± SEM, n = 3. (NC-CTRL, NC-TA) $P>0.05$, (C-CTRL, C-TA) $P=0.05$.

Figure 4: COX2 protein expression in frontal cortex after exposure to TA:



Quantification of COX2 in the frontal cortex normalized against GAPDH. Mean $\pm$ SEM, n = 3. $P<0.05$ is considered significant via students t-test, noted by "*".(NC-CTRL, NC-TA) P=0.003, (C-CTRL, C-TA) P=0.006.

Table 1: Power Analysis

BIOMARKER	**POWER: TA 5uM**	**POWER: TA 25uM**
APP	0.9	0.9
BIOMARKER	**POWER: NON CARRIERS**	**POWER: CARRIERS**
APP	0.12	N/A
CDK5	0.65	0.9
P35	0.05	0.5
P25	0.2	0.9
COX2	1	0.9

Analysis of power across all comparisons. For molecular tests, n=4. For behavioral tests, n=4. An alpha error probability of 0.05 was used, and effect size for each comparison was calculated based on mean and standard deviation utilizing formulas from http://www.socstatistics.com.

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