A Neurotrophic Factor Mimetic Improves Spatial Memory Retention In An Aged, Transgenic Mouse Model of Alzheimer's Disease

Middlebury College

D.E. Morrison, K. Zhang, E. Ferrari, O. Artaiz, M.D. Spritzer

Department of Biology and Program in Neuroscience, Middlebury College

National Institute on Aging

Background

- Alzheimer's Disease (AD) is a progressive neurodegenerative disorder that manifests as impaired cognition. Today there are over 5 million people in the U.S. suffering from AD, a number that is projected to increase in the coming years [1].
- The amyloid cascade hypothesis details the relationship between AD neuropathology and clinical symptomology [2]. The increased accumulation of amyloid-beta (Aβ) peptide results in the formation of senile plaques that are toxic to the neuronal microenvironment, contributing to vascular dysfunction and neurodegeneration [3].
- AD neuropathology appears several decades prior to the onset of mild cognitive impairment, specifically impairing the dorsolateral prefrontal cortex and medial temporal lobe [4]. Atrophy of these brain regions impairs the retrieval of declarative memories, executive function, and ability for spatial orientation [5].
- Neurotrophic factors, which enhance neuronal growth and survival, have demonstrated a potential therapeutic role in reducing the cognitive symptoms seen in AD patients [6].
- Ependymin(EPN) is a naturally secretory glycoprotein found in goldfish, monkeys, mice and humans, that functions similarly to neurotrophic factors. EPN is implicated in the treatment of AD due to its enhancement of memory and learning [7,8].

Questions:

1. Is the APP_{770} mouse strain is a viable model for the spatial learning and memory deficits of AD patients?
2. Does the growth factor mimetic, BTX-1039, restore spatial memory and learning deficits in an aged mouse model of AD?
3. Do the transgenes appropriately model of the neurological brain changes associated with AD?

Methods

Subjects:

- Subjects were male and female C57BL/6-wild type mice (WT) and C57BL/6-Tg(Thy1-APPSwDutIowa)BWevn/Mmjax mice (AD), which expressed the human 770 isoform of the APP gene with the Swedish, Dutch, and Iowa mutations.
- Experiments were conducted with two age classes: **8-months old and 12-months old**
- Subjects received 14 day of i.p. injections prior to behavioral testing:

WT Saline: wildtype mice injected with saline (n=14-16)
WT 60mg/kg: wildtype mice injected with 0.2 mL of 60 mg/kg BTX-1039 (n=13-16)
WT 100mg/kg: wildtype mice injected with 0.2mL of 100 mg/kg BTX-1039 (n=14-16)
AD saline: transgenic mice injected with 0.2 mL saline (n=12-18)
AD/60mg/kg: transgenic mice injected with 0.2mL of 60 mg/kg BTX-1039 (n=12-17)
AD/100mg/kg: transgenic mice injected with 0.2 mL of 100 mg/kg BTX-1039 (n=10-18)

Injections (Days 1-14)	Submerged Trials (Days 15-20)	Probe (Day 21)	Cued Trials (Days 22-24)	Perfusion & Collection (Day 26)

Water maze testing:

- 6 days submerged trials; 4 trials/day; platform in same quadrant on all trials.
- 1 day probe trials; 1 trial/day; no platform.
- 3 days cued trials; 4 trials/day; platform in new quadrant each day.

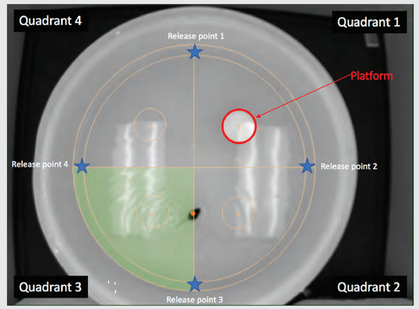


Figure 1. Morris water maze. Mice were released from all four release points in a random order during submerged and cued trials. All trials lasted for a maximum of 90 s.

Tissue Collection and Histology:

- Mice were euthanized and perfused with 0.9% NaCl and 4% parafprmaldahyde in a phosphate buffer.
- Brains were extracted from perfused mice and post-fixed with 4% PFA solution and then placed in a tris buffered saline (TBS) solution until sectioning. Brains were sectioned in to 40 µm coronal sections using a vibrating blade microtome. Slices were stored at -20°C in cryoprotectant (tris-buffered antifreeze solution) prior to peroxidase Immunohistochemistry.
  - Every tenth brain slices were incubated in a rabbit anti-Aβ primary antibody (Abcamab2539) at a concentration of 1:1000 overnight to tag Aβ plaques. Tissue was then incubated in a secondary antibody (goat anti-rabbit bioinylated igG, Vector Labs) at a concentration of 1:500 to amplify the signal.
  - Sections were then reacted with ImPACT DAB (Vector Labs) followed by mounting on to Superfrost+ microscope slides.
  - Tissue was dehydrated, cleared, and coverslipped with Permount.
  - Quantification of plaques will be conducted using light microscopy.

BTX-1039 reduced deficits in spatial learning in 8-month and 12-month old mice

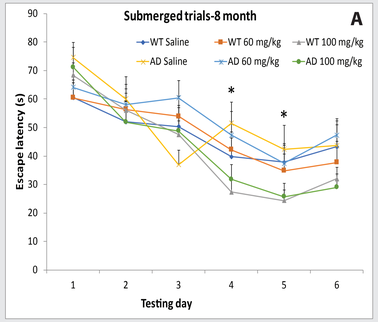


Figure 2. Escape latency (mean +/- SEM) to reach the platform for mice across treatment groups for each day of testing during submerged trials. **(A)** For 8-month old mice, there was a significant decrease in path length across all days of testing (p<0.0005). There was a significant interaction of day by drug (p=0.023). Within days analyses revealed that the 100 mg/kg group performed significantly better than both the 60 mg/kg and the saline groups on days 4 and 5 of testing (*p<0.042). There were no other significant main effects or interactions. **(B)** For 12-month-old mice, escape latency decreased significantly over the six testing days (p<0.0005), but there were no other significant main effects or interactions.

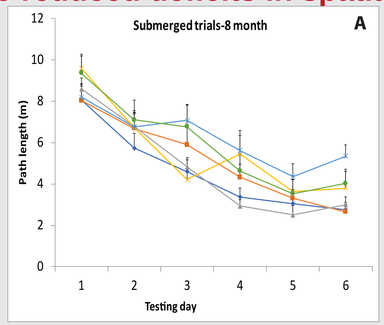


Figure 3. Path length (mean +/- SEM) to reach the platform across treatment groups for each day of testing during submerged trials. **(A)** For 8-month-old mice, there was a significant decrease in path length across all days of testing (p<0.0005). There were no other significant main effects or interactions (all p>0.10). Analyses within days showed that the AD/saline group performed significantly worse than all other groups on days 2-4 of testing (*p<0.005). **(B)** For 12-month-old mice, path length decreased significantly over the six days (p<0.0005). There was a significant main effect of strain showing longer path length to the platform in the AD mice (p=0.002). There were no other significant main effects or interactions.

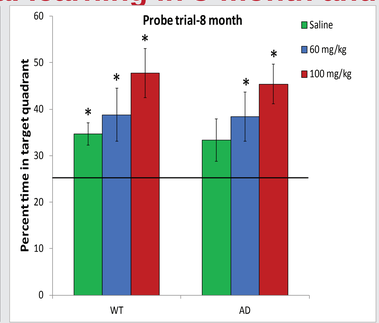


Figure 4. Percentage of time spent in the target quadrant (mean +/- SEM) across treatment groups during the probe trial. Transgenic (AD) and wild type mice (WT) are grouped by treatment with either saline or BTX-1039 (60 mg/kg or 100 mg/kg). **(A)** For 8-month-old mice, all groups except the AD/saline spent significantly more than 25% of the allotted time in the target quadrant (*p<0.05). There were no other significant main effects or interactions (all p>0.10). **(B)** For 12-month-old mice, some groups spent significantly more than 25% of the allotted time in the target quadrant (*p< 0.05).

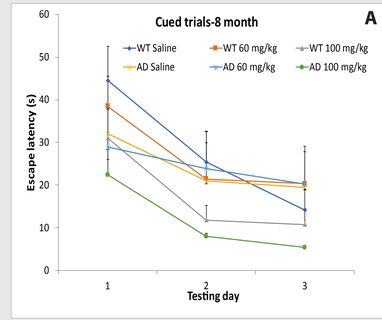


Figure 5. Escape Latency (mean +/- SEM) to reach the visible platform from the release point across treatment groups for each cued trial day. **(A)** For 8-month-old mice, escape latency decreased significantly over the three days (p<0.0005). There were no other significant effects. **(B)** For 12-month-old mice, escape latency decreased significantly over the three days (p<0.0005). There were no other significant effects.

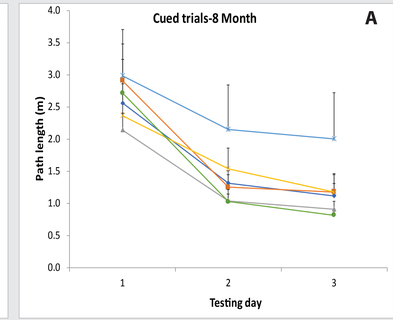
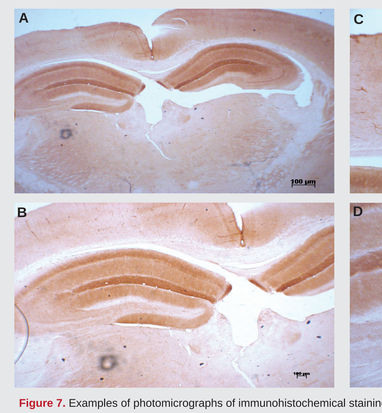
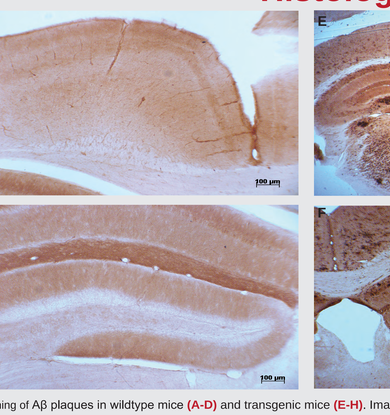
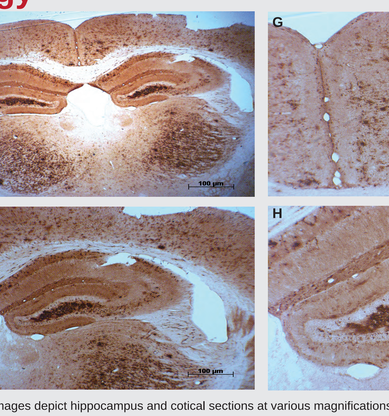
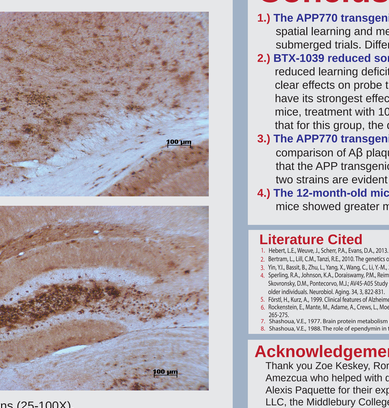


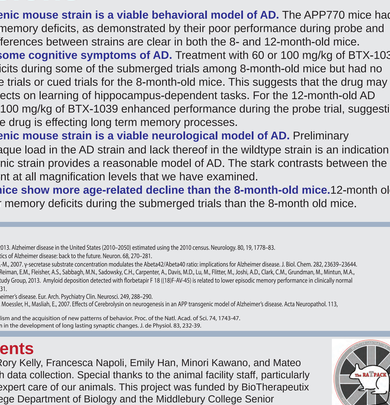
Figure 6. Path length (mean +/- SEM) to reach the visible platform from the release point across treatment groups for each day of testing there during cued trials. **(A)** For 8-month-old mice, there were no significant main effects or interactions (all p >0.20). Analyses within days showed that were no significant differences between groups on individual days of testing (p>0.05). **(B)** For 12-month-old mice, path length significantly decreased over the three days (p<0.0005). There were no other significant effects.











Histology

Figure 7. Examples of photomicrographs of immunohistochemical staining of Aβ plaques in wildtype mice **(A-D)** and transgenic mice **(E-H)**. Images depict hippocampus and cotical sections at various magnifications (25-100X).

Conclusions

1.) **The APP770 transgenic mouse strain is a viable behavioral model of AD.** The APP770 mice had spatial learning and memory deficits, as demonstrated by their poor performance during probe and submerged trials. Differences between strains are clear in both the 8- and 12-month-old mice.

2.) **BTX-1039 reduced some cognitive symptoms of AD.** Treatment with 60 or 100 mg/kg of BTX-1039 reduced learning deficits during some of the submerged trials among 8-month-old mice but had no clear effects on probe trials or cued trials for the 8-month-old mice. This suggests that the drug may have its strongest effects on learning of hippocampus-dependent tasks. For the 12-month-old AD mice, treatment with 100 mg/kg of BTX-1039 enhanced performance during the probe trial, suggesting that for this group, the drug is effecting long term memory processes.

3.) **The APP770 transgenic mouse strain is a viable neurological model of AD.** Preliminary comparison of Aβ plaque load in the AD strain and lack thereof in the wildtype strain is an indication that the APP transgenic strain provides a reasonable model of AD. The stark contrasts between the two strains are evident at all magnification levels that we have examined.

4.) **The 12-month-old mice show more age-related decline than the 8-month-old mice.** 12-month old mice showed greater memory deficits during the submerged trials than the 8-month old mice.

Literature Cited

1. Hebert, L.E., Weuve, J., Scherr, P.A., Evans, D.A., 2013. Alzheimer disease in the United States (2010–2050) estimated using the 2010 census. Neurology. 80, 19, 1778–83.
2. Bertram, L., Lill, C.M., Tanzi, R.E., 2010. The genetics of Alzheimer disease: back to the future. Neuron. 68, 270–281.
3. Yin, Yi., Bassit, B., Zhu, L., Yang, X., Wang, C., Li, Y.-M., 2007. γ-secretase substrate concentration modulates the Abeta42/Abeta40 ratio: implications for Alzheimer disease. J. Biol. Chem. 282, 23639–23644.
4. Sperling, R.A., Johnson, K.A., Doraiswamy, P.M., Reiman, E.M., Fleisher, A.S., Sabbagh, M.N., Sadowsky, C.H., Carpenter, A., Davis, M.D., Lu, M., Flitter, M., Joshi, A.D., Clark, C.M., Grundman, M., Mintun, M.A., Skovronsky, D.M., Pontecorvo, M.J., AV45-A05 Study Group, 2013. Amyloid deposition detected with florbetapir F 18 (18F-AV-45) is related to lower episodic memory performance in clinically normal older individuals. Neurobiol. Aging. 34, 3, 822–831.
5. Fjell, H., Kurz, A., 1999. Clinical features of Alzheimer's disease. Eur. Arch. Psychiatry Clin. Neurosci. 249, 288–290.
6. Rockenstein, E., Mante, M., Adame, A., Crews, L., Moessler, H., Masliah, E., 2007. Effects of Cerebrolysin on neurogenesis in an APP transgenic model of Alzheimer's disease. Acta Neuropathol. 113, 265-275.
7. Shashoua, V.E., 1977. Brain protein metabolism and the acquisition of new patterns of behavior. Proc. of the Natl. Acad. of Sci. 74, 1743-47.
8. Shashoua, V.E., 1988. The role of ependymin in the development of long lasting synaptic changes. J. de Physiol. 83, 232-39.

Acknowledgements

Thank you Zoe Keskey, Rory Kelly, Francesca Napoli, Emily Han, Minori Kawano, and Mateo Amezcua who helped with data collection. Special thanks to the animal facility staff, particularly Alexis Paquette for their expert care of our animals. This project was funded by BioTherapeutix LLC, the Middlebury College Department of Biology and the Middlebury College Senior Research Project Supplement.