Potential memory restorative effects of a neurotrophic factor mimetic in an aged, transgenic mouse model of Alzheimer's disease

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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 and the projected prevalence is only expected 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 therapuetic role to reduce the cognitive symptoms present in patients with AD [6].

Question: Is the APP_{770} transgenic mouse strain a viable model of the spatial learning and memory deficits common in patients with AD?

Question: Does the growth factor mimetic BTX-1039 restore spatial memory deficits in this aged mouse model of AD?

Question: Do the transgenes or the drug ifluence hippocampal cell proliferation?

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.
- Subjects were divided into 4 treatment groups:
  AD/BTX-1039: AD mice injected with BTX-1039
  AD/saline: AD mice injected with 0.2 mL saline
  WT/BTX-1039: WT mice injected with BTX-1039
  WT/saline: WT mice injected with 0.2 mL saline
- The age of mice and drug dose varied among experiments:
  Experiment 1: 6-month-old mice,injected with 60 mg/kg BTX-1039 (n=14-15/group).
  Experiment 2: 9-month-old mice,injected with 60 mg/kg BTX-1039 (n=7-15/group).
  Experiment 3: 8-month-old mice, injected with 100 mg/kg BTX-1039 (n=3/group).

Water maze testing:

- 14 days of blind intraperitoneal injections
- 6 days of submerged trials; 4 trials per day; platform in same quadrant for all trails.
- 1 day of probe trials; 1 trial per mouse; platform removed from maze.
- 3 days cued trials; 4 trials per day; platform in new location each day.

Cell proliferation assay:

- Following behavioral testing, all mice were euthanized and their brains were perfused and extracted.
- Tissue was sectioned and then stained for Ki67 using ABC peroxidase immunohistochemistry. Incubation in rabbit monoclonal antibody against Ki67 (Vector Laboratories; 1:1000) for 24 h was followed by a 2 h incubation in goat anti-rabbit secondary antibodies (Vector Laboratories; 1:500). Sections were then incubated for 1.5 h in avidin-biotin horseradish peroxidase complex (ABC Elite Kit; Vector Labs; 1:100) and reacted for 5 min in 0.05% diaminobenzidine (DAB).
- A light microscope was used to count Ki67-labeled cells at 1000x magnification in every 10th section of the dentate gyrus, specifically within the granule cell layer (GCL), subgranular zone (SGZ), and hilus.

Figure 1. Experimental timeline for behavioral testing.

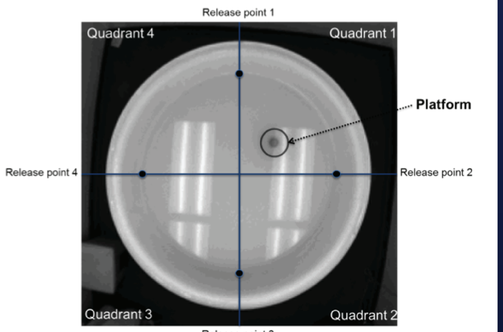


Figure 2. Morris water maze. Mice were released from all four release points in a random order during submerged and cued trials. All trials lasted for a maximum of 90 s.

BTX-1039 (60 mg/kg) reduced some of the deficits in spatial learning and memory displayed by 6-mo AD mice

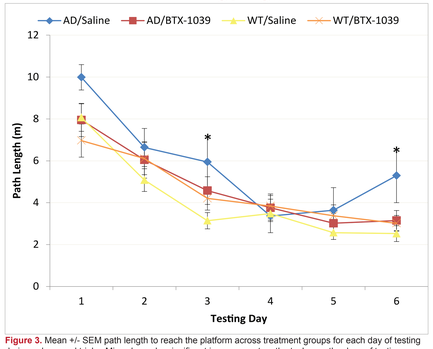


Figure 3. Mean +/- SEM path length to reach the platform across treatment groups for each day of testing during submerged trials. Mice showed a significant improvement on the task over the days of testing ($p<0.0005$) and WT mice had signficantly shorter path lengths than AD mice ($p=0.031$). The main effect of drug was not significant, but there was a marginally significant strain x drug interaction ($p=0.073$) potentially driven by the AD/saline group performing worse than all other groups on certain days . All other interaction effects were not significant. Analyses within days showed that the AD/saline group performed significantly worse than all other groups on days 3 and 6 (*$p<0.044$).

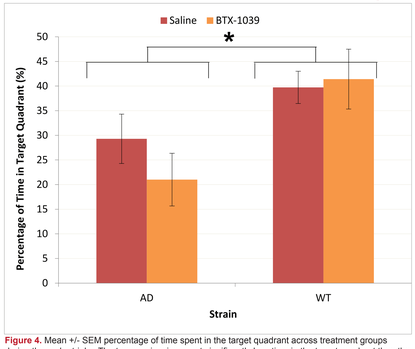


Figure 4. Mean +/- SEM percentage of time spent in the target quadrant across treatment groups during the probe trials. The transgenic mice spent significantly less time in the target quadrant than the wildtype mice (*$p=0.007$). There were no other significant main effects or interactions.

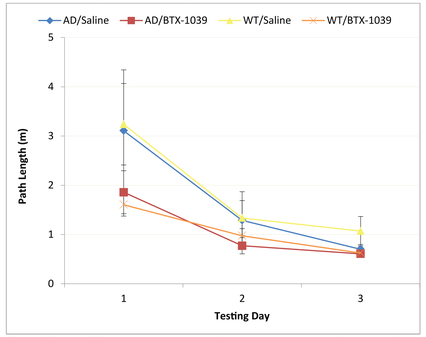


Figure 5. Mean +/- SEM path length to reach the platform across treatment groups for each day of testing during cued trials. Mice showed a significant improvement on the task over the days of testing ($p<.0005$). The drug-injected mice performed better than the control mice, creating a nearly significant effect of drug ($p=0.060$) and a marginally significant day x drug interaction ($p=0.095$). There were no other significant main effects or interactions . Analyses within days showed that there were no signficant differences between groups on individual days of testing ($p>0.05$).

BTX-1039 (60 mg/kg) reduced some of the deficits in spatial learning and memory displayed by 9-mo AD mice

Figure 6. Mean +/- SEM path length to reach the platform across treatment groups or each day of testing during submerged trials. There was a significant decrease in path length across days of testing ($p<0.0005$) and a marginally significant main effect of strain with transgenic mice displaying longer path lengths ($p=0.064$). Analyses within days showed that the AD/saline group performed significantly worse than all other groups on the final two days of testing (*$p<0.0005$).

Figure 7. Mean +/- SEM percentage of time spent in the target quadrant across treatment groups during the probe trial. There were no significant main effects or interactions.

Figure 8. Mean +/- SEM path length to reach the platform across treatment groups for each day of testing during cued trials. There was a significant decrease in path length across days of testing ($p<0.0005$) as well as a marginally significant day × strain × drug interaction ($p=0.098$). There were, however, no other significant effects. Analyses within days showed that there were no signficant differences between groups on individual days of testing ($p>0.05$).

BTX-1039 (100 mg/kg) was effective in reducing deficits in spatial learning and memory displayed by 8-mo AD mice

Figure 9. Mean +/- SEM path length to reach the platform across treatment groups for each day of testing during submerged trials. There was a significant decrease in path length across days of testing ($p<0.0005$), although there were no other significant main effects or interactions. Analyses within days showed that the AD/saline group performed significantly worse than all other groups on days 2-4 of testing (*$p<0.005$).

Figure 10. Mean +/- SEM percentage of time spent in the target quadrant across treatment groups during the probe trial. The transgenic mice spent significantly less time in the target quadrant than the wildtype mice (*$p=0.023$). There were no other significant main effects or interactions.

Figure 11. Mean +/- SEM path length to reach the platform across treatment groups for each day of testing during cued trials. There were no significant main effects or interactions. Analyses within days showed that there were no signficant differences between groups on individual days of testing ($p>0.05$).

AD mice demonstrated significantly increased hilar cell proliferation

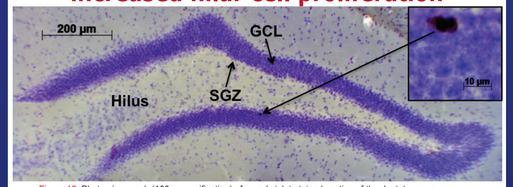
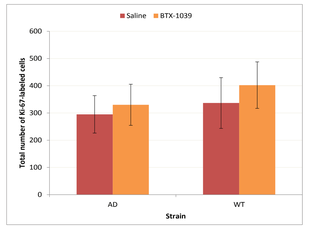


Figure 12. Photomicrograph (100× magnification) of cresyl violet-stained section of the dentate gyrus, including the subgranular zone (SGZ), granule cell layer (GCL), and hilus. Inset (1000× magnification) displays a pair of Ki67-labeled cells along the SGZ. This tissue section is from a 9-mo WT mouse treated with saline.

A



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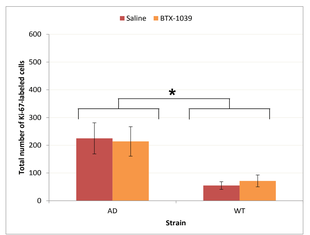

Figure 13. Mean +/- SEM total number of Ki67-labeled cells within the GCL+SGZ (A) and hilus (B) of the dentate gyrus across treatment groups (AD/saline, AD/BTX-1039, WT/saline, and WT/BTX-1039). Ki67-labeled cells were counted for every 10th section of each subject's brain at 1000× magnification under a light microscope. There was a significant main effect of strain with AD mice having significantly greater total number of Ki67-labeled cells in the hilus compared to WT mice (*$p = 0.001$). No other main effects or interactions were significant.

Conclusions

1) The APP_{770} transgenic mouse strain is a viable behavioral model of AD. The APP_{770} mice had spatial learning and memory deficits, as demonstrated by their poor performance during probe and submerged trials. Differences between strains were clearest at 8 months of age, and we are in the process of replicating Experiment 3.

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, but had no clear effects on probe trials or cued trials. This suggests that the drug may have its strongest effects on learning of hippocampus-dependent tasks.

3) Hilar cell proliferation was significantly increased in the transgenic mice. Increased hilar cell proliferation is present in other neurological disorders such as epilepsy, for which there is a high comorbidity in patients with AD [7, 8]. However, BTX-1039 had no effect on cell proliferation in the dentate gyrus.

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