A Neurotrophic Factor Mimetic Improves Spatial Memory and Reduces Hippocampal Caspase-3 in a Transgenic Mouse Model of Alzheimer's Disease

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Background

- Alzheimer's disease (AD) is a neurodegenerative disorder in which one progressively loses their memory and other critical brain functions. Studies show that it's prevalency will increase drastically by the year 2050.[1,2]
- The amyloid cascade hypothesis posits that the accumulation of amyloid-β (Aβ) leads to a buildup of Aβ 'senile' plaques, neurofibrillary tangles (NFTs), followed by neuron death and subsequent cognitive/behavioral deficits.[3]
- One of several groups of molecules that have been investigated for their therapeutic properties against AD are growth factors, such as BDNF and NGF.[4,5]
- Previous studies have suggested that a growth factor called ependymin (EPN) is impli cated in neuronal plasticity and memory[6] and could have potential restorative effects on AD patients.

Questions

1. Is the APP770 mouse strain a relevant and appropriate model of the behavioral and physiological effects of human AD?
2. Does the ependymin growth factor mimetic, BTX-1039, function to improve spatial memory deficits in 6, 8, and 12 month-old mouse models of AD?
3. Does BTX-1039 affect some of the physiological changes that occur in mouse models of AD?

Methods

Subjects

- Subjects were male and female C57BL/6 background strain mice. Compared to wild-type mice (WT), AD mice were transgenic for the Swedish K670N/M671L, Dutch E693Q, and Iowa D694N making them homozygous for the human APP770 allele.
- Experiments were conducted with three different age classes: 6-months-old, 8-months-old, and 12-months-old.
- All subjects received 14 days of I.p. injections prior to testing:
  - WT Saline: wild type mice injected with saline (n=14-16)
  - WT 60 mg/kg: wild type mice injected with 60 mg/kg (n=13-16)
  - WT 100 mg/kg: wild type mice injected with 100 mg/kg (n=13-16)
  - AD Saline: transgenic mice injected with saline a(n=14-16)
  - AD 60 mg/kg: transgenic mice injected with 60 mg/kg (n=13-16)
  - AD 100 mg/kg: transgenic mice injected with 100 mg/kg (n=13-16)

Water Maze Testing

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

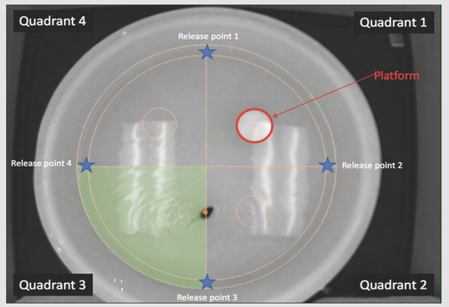
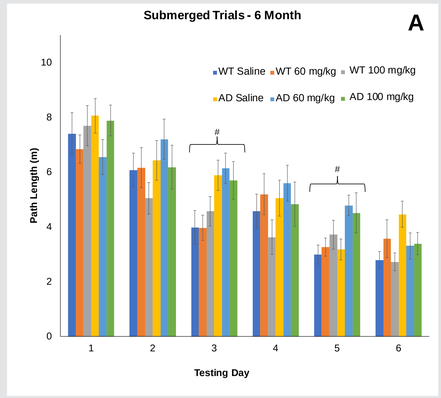


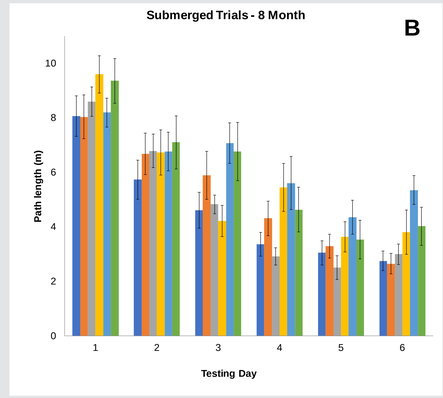
Figure 1. Morris Water Maze. Mice were released from all four release points in a random order during submerged and cued trials. All trials lasted for a maxium of 90s.

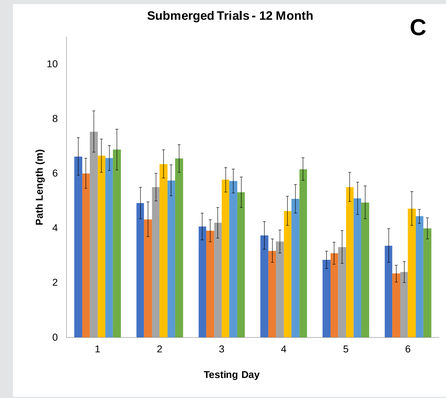
Tissue Collection and Histology

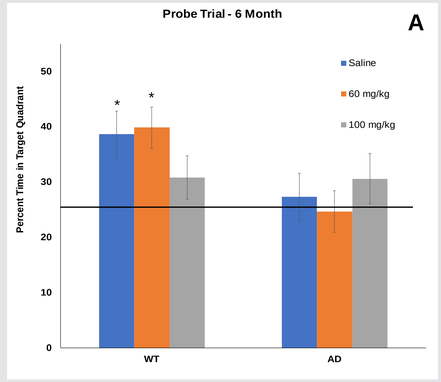
- Mice were euthanized and perfused with 0.9% NaCl; and 4% paraformaldehyde (PFA).
- Once brains were extracted, they were post-fixed with 4% PFA, placed in a 30% sucrose solution for cryoprotection, and finally kept in a tris buffered saline (TBS) solution until sectioning. Brains were sectioned using a vibrating blade microtome into 40 µm coronal sections. Sections were subsequently stored at -20°C in a TBS-antifreeze solution before perox idase IHC.
- 12-months old aged sections were incubated in either rabbit anti-Aβ primary antibody (Ab camb2539) or rabbit anti-cleaved caspase-3 primary antibody (Rabbit mAb #9664) at a con centration of 1:1000 overnight to tag for Aβ plaques or cleaved caspase-3 proteins, respec tively. Tissue was then incubated in a secondary antibody (goat anti-rabbit biotinylated IgG, Vector Labs) at a concentration of 1:500 for signal amplification.
- Sections were then reacted with ImmPACT DAB (Vector Labs) followed by mounting onto Superfrost+ microscope slides.
- Tissue was subsequently dehydrated and coverslipped with Permount.
- Quantification of plaques and cleaved caspase-3 was performed with light microscopy and the ImageJ program.

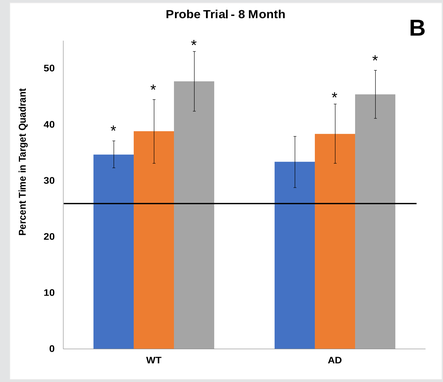
BTX-1039 reduced deficits in spatial learning and improved memory in older mice











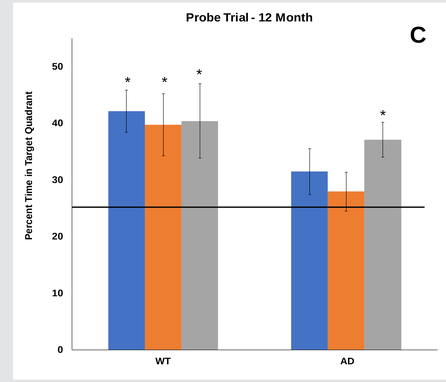
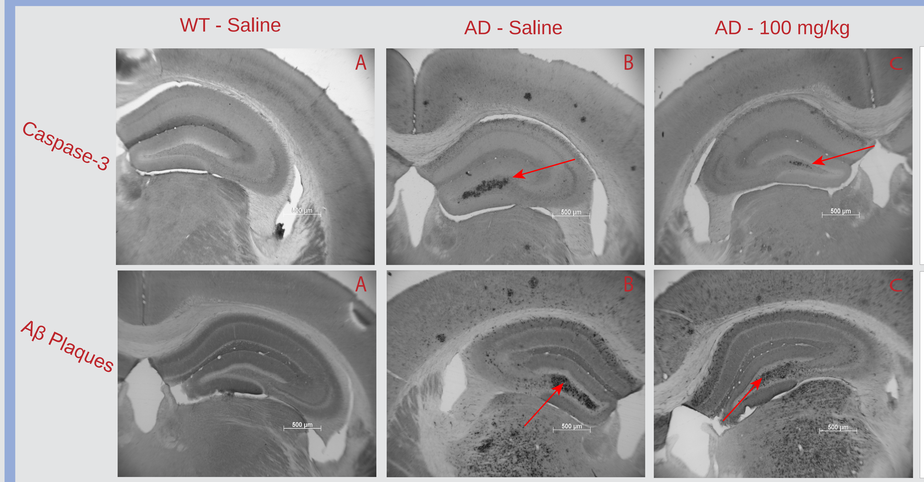


Figure 2. Path length (mean +/- SEM) to reach the platform across treatment groups for each day of testing during the submerged trials. (A) For 6-month-old mice, there was a significant decrease in path length across all days of testing ($p<.0005$). There was a significant main effect of strain ($p<.007$), but not for drug or an interaction of both on path length (all $p>.82$). Analyses within days revealed that there was a significant main effect of strain on days 3 and 5. There were no other significant main effects or interactions. (B) For 8-month-old mice, there was a significant decrease in path length across all days of testing ($p<.0005$). There were no other significant main effects or interactions (all $p>.10$). Analyses within days showed that the AD saline group performed significantly worse than all other groups on days 2-4 of testing (*$p<.005$). There were no other significant main effects or interactions. (C) For 12-month-old mice, path length also decreased significantly over the six days ($p<.0005$). There was a significant main effect of strain showing longer path length to the platform in the AD mice group ($p=.002$). There were no other significant main effects or interactions.

Figure 3. Percentage of time spent in the target quadrant (mean +/- SEM) across all treatment groups during the probe trials. Wild type (WT) and transgenic (AD) are grouped by treatment with either saline or BTX-1039 (60 mg/kg or 100 mg/kg). (A) For 6-month-old mice, only the WT saline and BTX-1039 60 mg/kg groups spent significantly longer than 25% of the probe day trial in the target quadrant (*$p<.006$). There were no other significant main effects or interactions. (B) For 8-month-old mice, all groups except the AD saline spent significantly more than 25% of the alotted time in the target quadrant (*$p<.05$). There were no other significant main effects or interactions. (C) For 12-month-old mice, some groups spent significantly more than 25% of the alotted time in the target quadrant (*$p<.05$) (FIX THIS try to add to this). There were no other significant main effects or interactions.



BTX-1039 reduced Caspase-3

Figure 4. Examples of IHC staining of caspase-3 in 12-month-old WT and AD mice with various drug treatments and accompanying density analysis. (A) WT mouse given saline. (B) AD mouse given saline. (C) AD mouse given the 100 mg/kg dose of BTX-1039. (D) Mean (+/- SEM) of percent area of caspase-3 in the dorsal hippocampus. Significantly higher levels of activated caspase-3 were found in AD mice compared to that of WT ($p<.0005$). Significantly less caspase-3 expression in BTX-1039 treated AD mice compared to that of the saline group ($p<.006$). Images depict hippocampus and cortical sections at 4X magnifications. Red arrows indicate positive staining.

Figure 5. Examples of IHC staining of Aβ plaques in12-month-old WT and AD mice with various drug treatments and accompanying density analysis. (A) WT mouse given saline. (B) AD mouse given saline. (C) AD mouse given the 100 mg/kg dose of BTX-1039. (D) Mean (+/- SEM) of percent area of Aβ plaques in the dorsal hippocampus. No significant differences in percent area of Aβ plaques were found among all three treatment groups of AD mice. Images depict hippocampus and cortical sections at 4X magnifications. Red arrows indicate positive staining.

Conclusions

1.) The APP_{770} transgenic mouse strain used in this experiment is an appropriate model of the behavioral signs of human AD. Differences between strains are clear in all three age classes showing that AD mice performed significantly worse on spatial memory tasks. For the submerged trials, the 6, 8, and 12-month-old AD mice took significantly longer to reach the platform. Performance differences in the probe trials were more apparent in the 8 and 12-month -old mice.

2.) BTX-1039 has more of an effect on cognitive symptoms of AD on older age classes. There was only a trending significant interaction between the day and the drug group in the submerged trials of the 6-month-old mice. Treatment with 60 or 100 mg/kg did seem to reduce some of the cognitive impairments of AD on the 8-month-old mice in several of the submerged trial testing days, in addition to improving memory on the probe day. Regarding the 12-month-old mice, higher doses of the drug significantly improved memory on probe day, especially for that of transgenic mice.

3.) The APP_{770} transgenic mouse strain is an appropriate model of the physiological changes of human AD. Caspase-3 and Aβ plaque staining revealed that transgenic mice treated with saline have significantly higher concentrations of either protein compared to that of WT subjects. These results indicate that the transgenic strain used is a reasonable model of AD.

4.) BTX-1039 altered some of the physiological changes of 12-month-old transgenic mice. Treatment with either dosage of BTX-1039 significantly reduced activated caspase-3 in transgenic older mice. However, BTX-1039 did not affect the amount of Aβ plaque aggregation. These results suggest that the growth factor mimetic is ameliorating spatial memory deficits not through reduction of Aβ, but rather through the reduction of hippocampal cell death.

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