

BACKGROUND

- Alzheimer's disease (AD) is an irreversible and progressive neurodegenerative disorder affecting >6 million Americans aged 65 and older.
- Elevated circulating branched-chain amino acids (BCAAs) have been positively associated with AD pathology and cognitive decline.
- Previous studies from our laboratory demonstrated that high BCAA levels impair neuronal function, while BCAA-lowering interventions alleviate AD-related pathology.
- BT2 is a pharmacological compound that enhances BCAA catabolism and reduces circulating BCAA levels.
- The optimal dose of BT2 for improving cognitive function and reducing AD pathology in female mice remains unknown.
- Therefore, this study investigated the dose-dependent effects of BT2 on plasma BCAA levels and cognitive performance in 5xFAD mice.

OBJECTIVE



To determine the optimal dose of BT2 for reducing circulating BCAA levels and improving cognitive function in female 5xFAD mice, a well-established mouse model of AD.

METHODS

ANIMALS

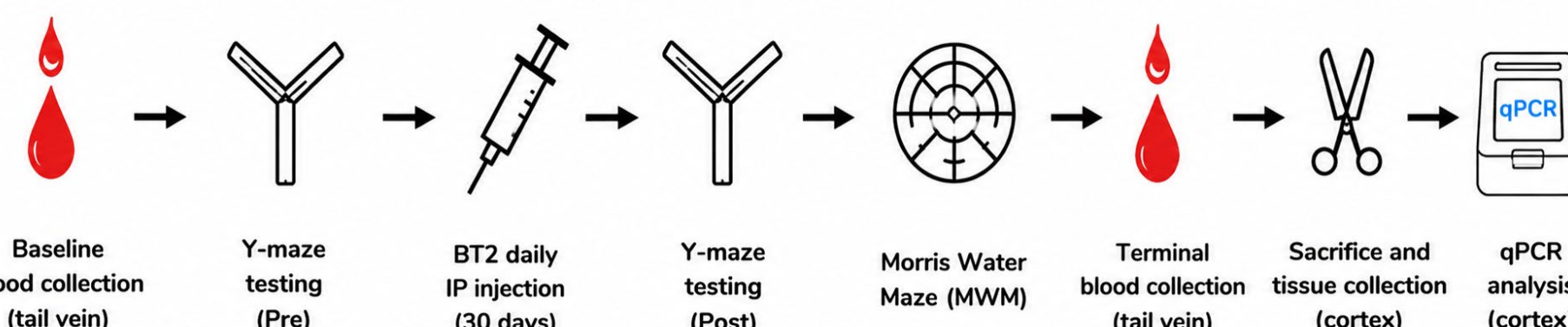
- 3-month-old female mice
- Wildtype littermate (WT) & 5xFAD (AD model)
- n = 7 per group



EXPERIMENTAL DESIGN

WT GROUP	AD GROUP
WT Control	5XFAD Control
WT+ BT2 (20mg/kg BW)	5XFAD+ BT2 (20mg/kg BW)
WT+ BT2 (40mg/kg BW)	5XFAD+ BT2 (40mg/kg BW)
WT+ BT2 (80mg/kg BW)	5XFAD+ BT2 (80mg/kg BW)
WT + 50% BCAA Restriction	5XFAD + 50% BCAA Restriction

PROCEDURES



RESULTS

1 BT2 Injection Reduces Plasma BCAA Levels in Female 5xFAD Mice

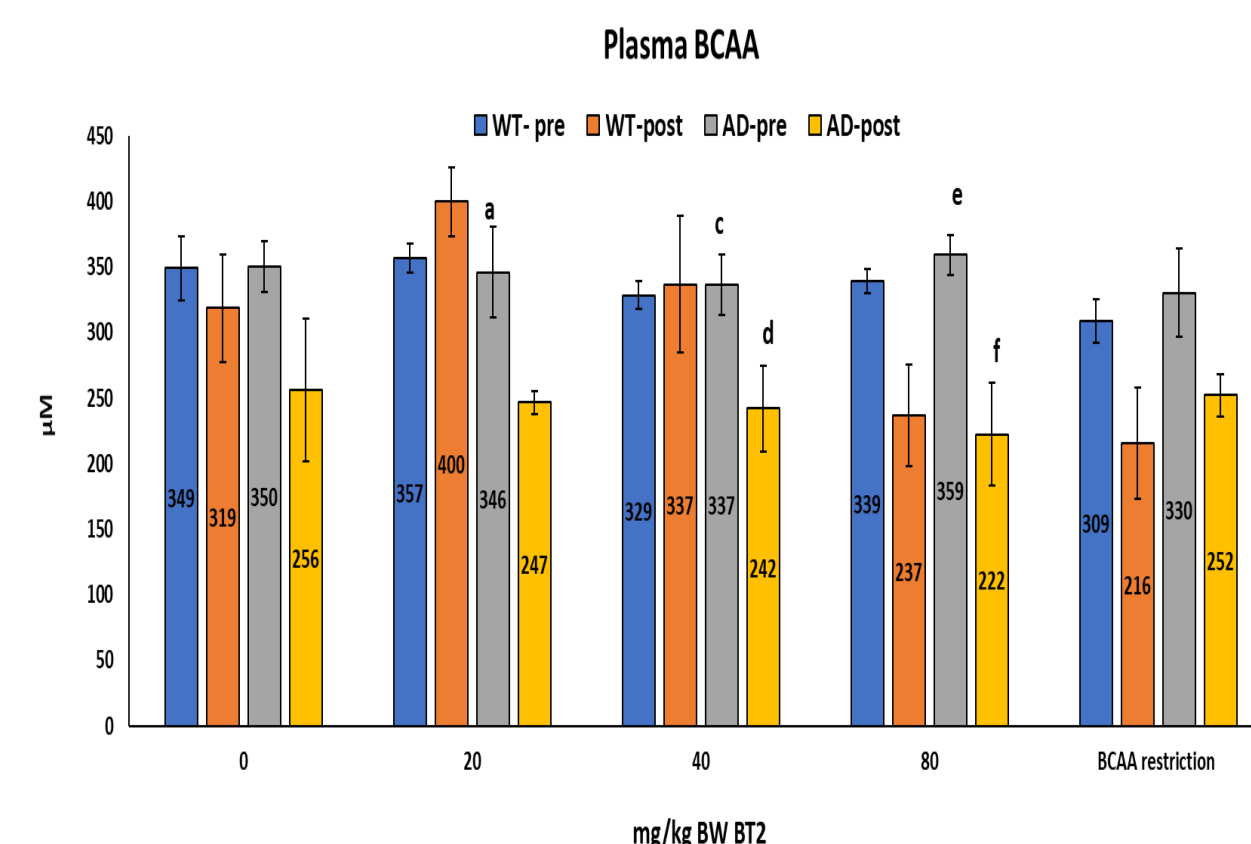


Figure 1. BT2 injection reduces plasma BCAA levels in female 5xFAD mice.

Plasma BCAA concentrations were measured before and after treatment. BT2 significantly reduced plasma BCAA levels at doses of 20 mg/kg body weight (BW) ($p = 0.0403$), 40 mg/kg BW ($p = 0.0195$), and 80 mg/kg BW ($p = 0.0121$), as determined by paired t -tests.

BT2 significantly reduced plasma BCAA levels in 5xFAD mice at all doses tested.

2 BCAA Reduction Improves Cognitive Function by Reducing Escape Latency in the Morris Water Maze in Female 5xFAD Mice

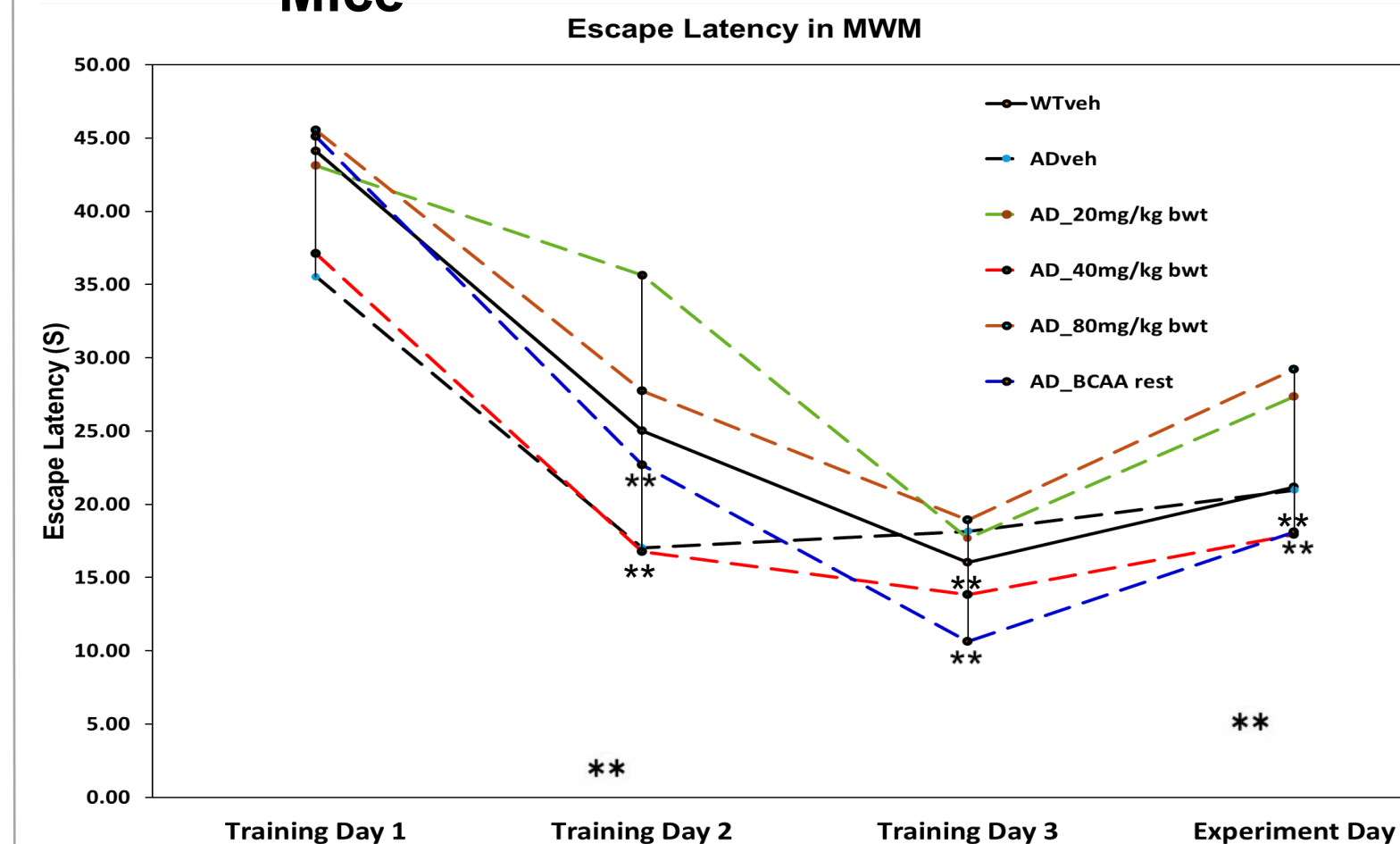


Figure 2. BT2 injection improves cognitive function by significantly reducing escape latency in the Morris Water Maze (MWM). Data were analyzed using two-way repeated-measures ANOVA followed by Bonferroni's multiple-comparisons test. BT2 treatment at 40 mg/kg BW and BCAA-restriction diet significantly reduced escape latency on training days 2 and 3, as well as on the experimental day, compared with day 1 ($p < 0.01$).

40 mg/kg BT2 produced the greatest improvement in cognitive function through escape latency

3 BT2 Treatment Preserves Cognitive Function in Female 5xFAD Mice in the Y-Maze

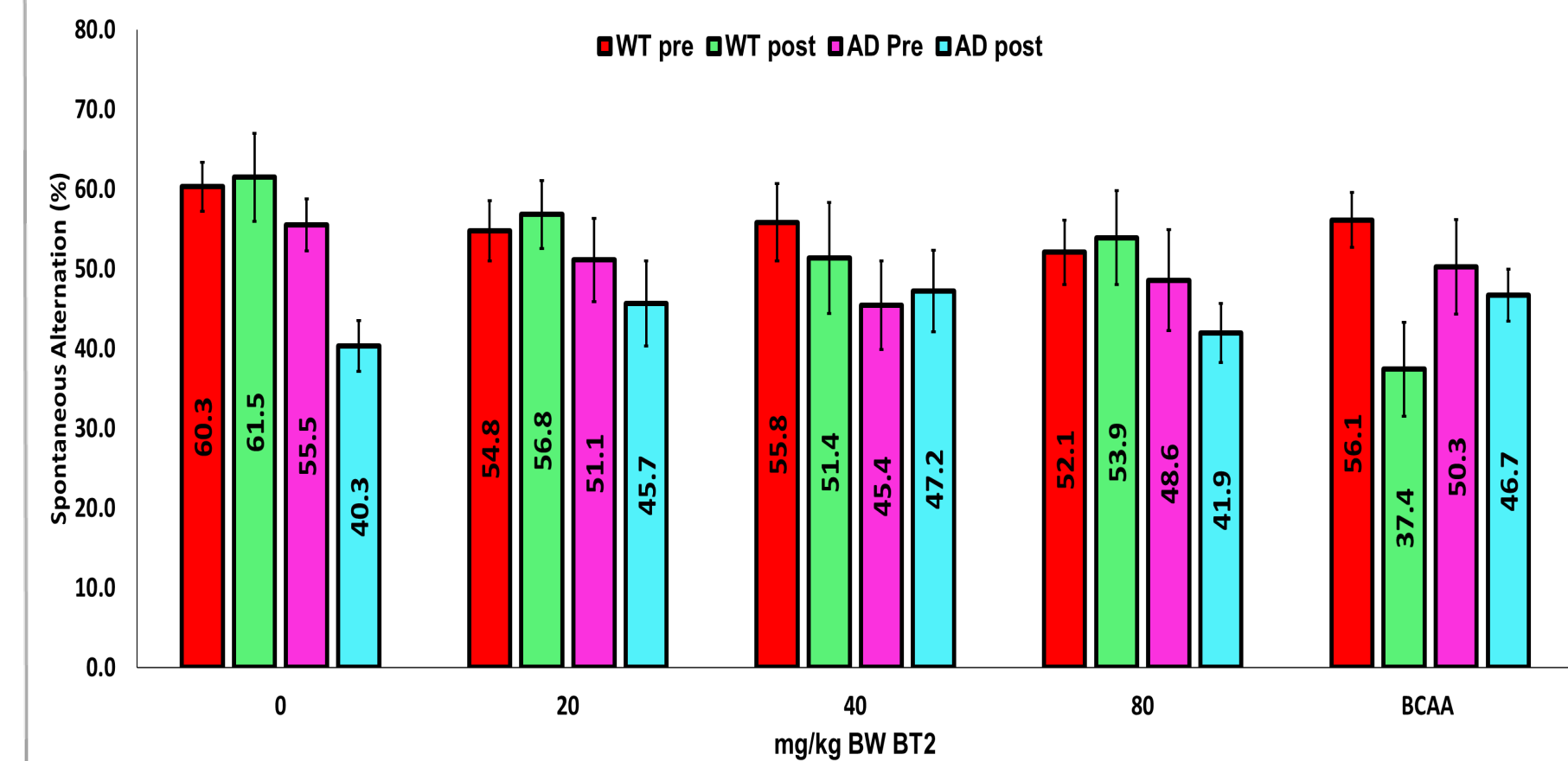


Figure 3. BT2 treatment preserves cognitive function in female 5xFAD mice. Spontaneous alternation (SA%) in the Y-maze was assessed before and after treatment. Mice treated with 40 mg/kg BT2 maintained their SA%, whereas untreated AD mice and mice receiving other BT2 doses exhibited a decline in SA%. Data were analyzed using two-way ANOVA followed by Tukey's post hoc test.

BT2 at 40 mg/kg preserved spontaneous alternation, indicating maintained working memory.

SUMMARY OF FINDING

Outcome	Effect of BT2 in 5XFAD Mice
Plasma BCAA Levels	Significant reduction at 20, 40, and 80 mg/kg
Morris Water Maze	↓ Escape latency (best at 40 mg/kg)
Y-Maze (SA%)	Preserved spontaneous alternation (best at 40 mg/kg)
Optimal Dose	40mg/kg BW

CONCLUSION

- ✓ Pharmacological reduction of circulations BCAAs with BT2 improves cognitive outcomes in an AD mouse model.
- ✓ BT2 significantly lowers plasma BCAA levels.
- ✓ A dose of 40 mg/kg BW produces the greatest cognitive benefit.
- ✓ Targeting BCAA metabolism may represent a novel therapeutic strategy for slowing AD progression.

FUTURE DIRECTION



- Investigate molecular mechanisms (autophagy, mitochondrial function, AD pathology).
- Evaluate translational relevance in preclinical studies.

