

Soluble Aβ-Oligomer-Selective Antibody ACU-3B3 Reduces Amyloid Pathology and Improves Multiple Behavioral Domains in a Mouse Model of Alzheimer’s Disease

Keran Ma^{1,2}, Maria M Martinez-Losa^{1,2}, YueQiang Xue³, Abdullah Khan¹, Kaitlyn Ho¹, Jean-Cosme Dodart⁴, Jasna Jerecic⁴, Inma Cobos³, Jorge J Palop^{1,2}

¹Gladstone Institute of Neurological Disease, San Francisco, USA; ²Department of Neurology, University of California, San Francisco, USA;

³Department of Pathology, Stanford University, Palo Alto, USA; ⁴Acumen Pharmaceuticals Inc., Charlottesville, USA

Abstract

Background: Considerable evidence suggests that amyloid-beta (Aβ) contributes to Alzheimer’s disease (AD) pathogenesis. Despite initial failures, some clinical trials targeting Aβ by immunotherapy showed reduced Aβ pathology and moderate beneficial effects on cognitive functions. Because Aβ exists in multiple assembly states, we tested the in vivo efficacy of the Aβ-oligomer selective antibody ACU-3B3, the murine precursor to the clinical candidate antibody ACU-193, which targets a broad range of soluble Aβ oligomers with low affinity for monomeric and fibrillar Aβ.

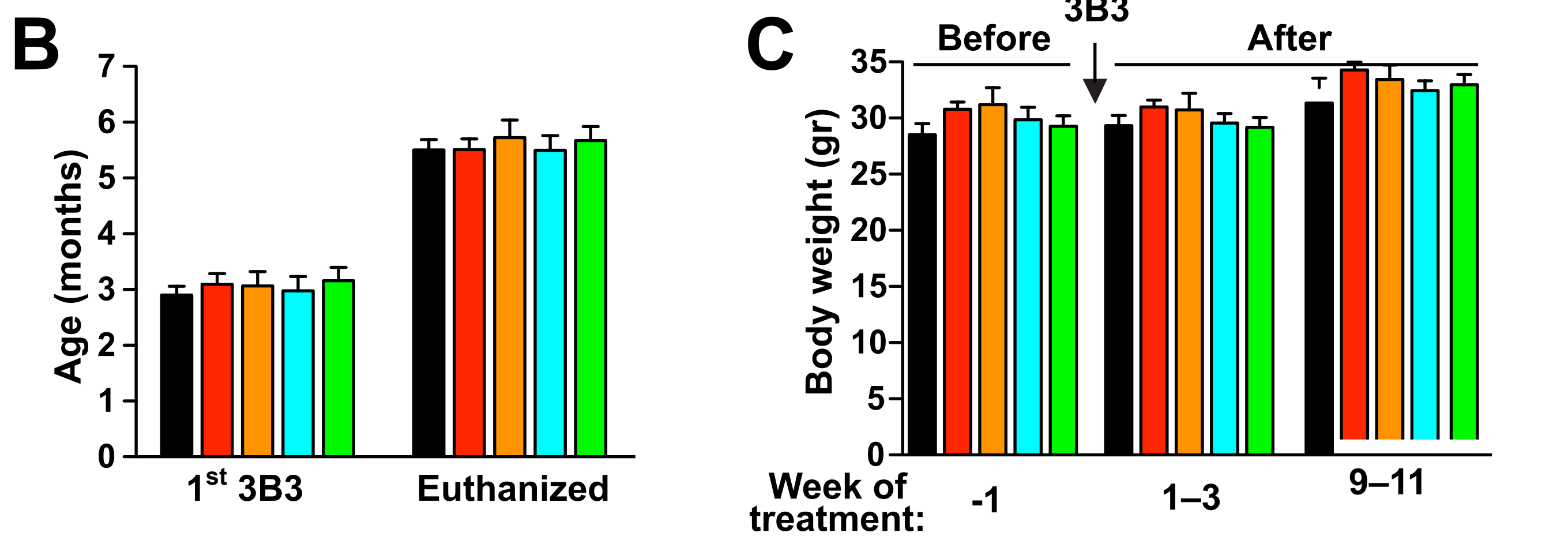
Method: hAPPJ20 mice received weekly intraperitoneal treatments with vehicle or ACU-3B3 at 5, 10, or 20 mg/kg. Treatment began at the earliest stages of amyloid deposition (3–5 months) and continued for 10 weeks until mice were 5–7 months old. During the treatment period, mice were assessed for locomotor activity, emotional behaviors, and cognitive functions.

Results: Across all experimental groups, mouse body weights remained stable during the 10-week period, indicating that ACU-3B3 was well tolerated. ACU-3B3 treatment reduced the hyperactivity phenotype of hAPPJ20 mice in the open field test and the Y-maze. Emotional alterations of hAPPJ20 mice in the elevated plus-maze were also normalized. Cognitive performance was assessed in the context-dependent habituation test and the Morris water maze. ACU-3B3 treatment enhanced cognitive performance in the novel environment habituation test (context learning) and during the visible platform water maze training (non-spatial learning), but not in the hidden version of the test (spatial learning). After treatment, brains were collected for pathological analyses. ACU-3B3 treatment reduced amyloid deposits in the hippocampus and cortex in a dose-dependent manner. In postmortem human brain from AD patients and hAPPJ20 mice, ACU-3B3 labelled neocortical diffuse and neuritic amyloid plaques with a distribution pattern and density similar but not identical, to those observed in adjacent sections stained with Aβ antibodies 6E10 and 82E1.

Conclusion: Our data indicate that ACU-3B3 targets an Aβ assembly state that is on path for in vivo amyloid formation and underlying behavioral deficits in hAPPJ20 mice, and clinical benefits may improve with second generation passive immunization approaches targeting soluble Aβ oligomers.

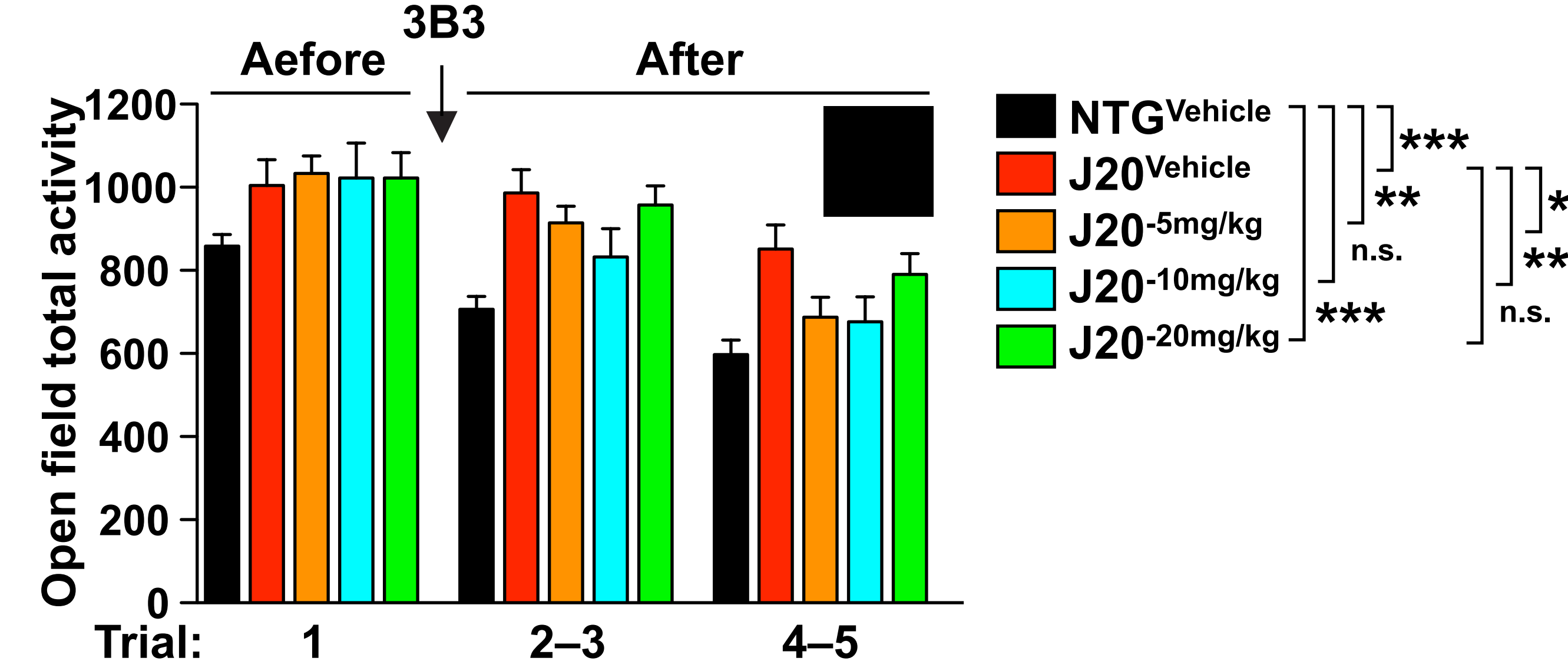
Experimental Design

A	Week of treatment:	-1	1–4	6	7	8	9	10
	Behavioral test:	Open field	Y-maze	Hidden – Visible water maze	Plus-maze	Euthanized		
	Mouse age (months):	2.9	4.1	4.5	4.8	5.1	5.3	5.6
Groups and n numbers:								
■ NTG-Vehicle		25	25	13	13	23		
■ J20-Vehicle		19	18	16	16	16 (-3)		
■ J20-3B3 5mg/kg		13	11	15	14	10 (-3)		
■ J20-3B3 10mg/kg		15	15	15	14	14 (-1)		
■ J20-3B3 20mg/kg		15	14			14 (-1)		



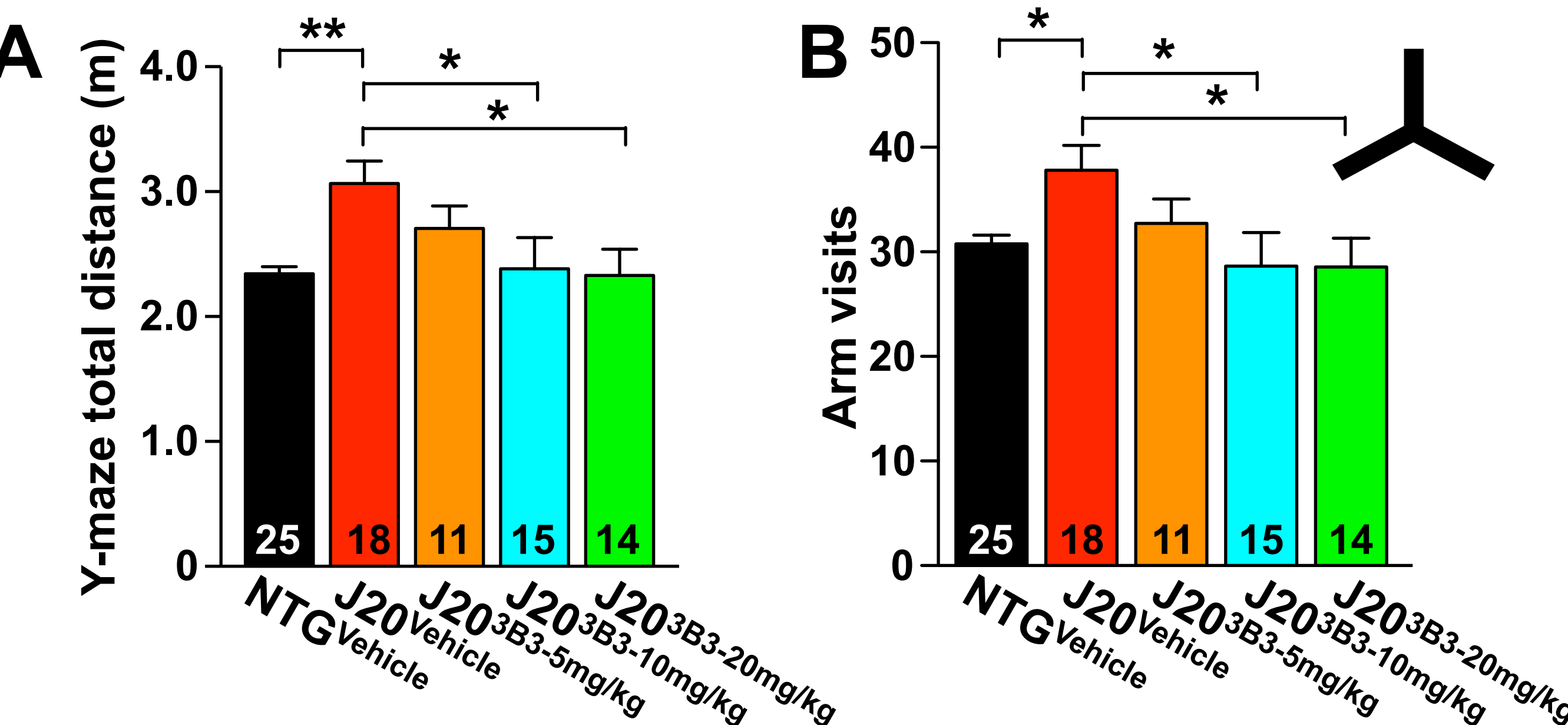
hAPPJ20 mice (J20) were weekly injected IP with 5, 10, and 20 mg/kg of mouse anti-Aβ-oligomer antibody (ACU-3B3) or vehicle (PBS) for 10 weeks. Non-transgenic (NTG) mice received vehicle injections. A) Locomotor activity, emotion-like behaviors and cognitive functions were assessed in the open field, y-maze, and elevated plus-maze tests during the 10-week treatment period. B) Age of the experimental groups at the first ACU-3B3 or vehicle injection and at completion of the study. C) ACU-3B3 treatment did not alter mouse body weight during the 10-week treatment period.

ACU-3B3 Reduces Habituation Deficits in J20 Mice after Four Weeks of Treatment



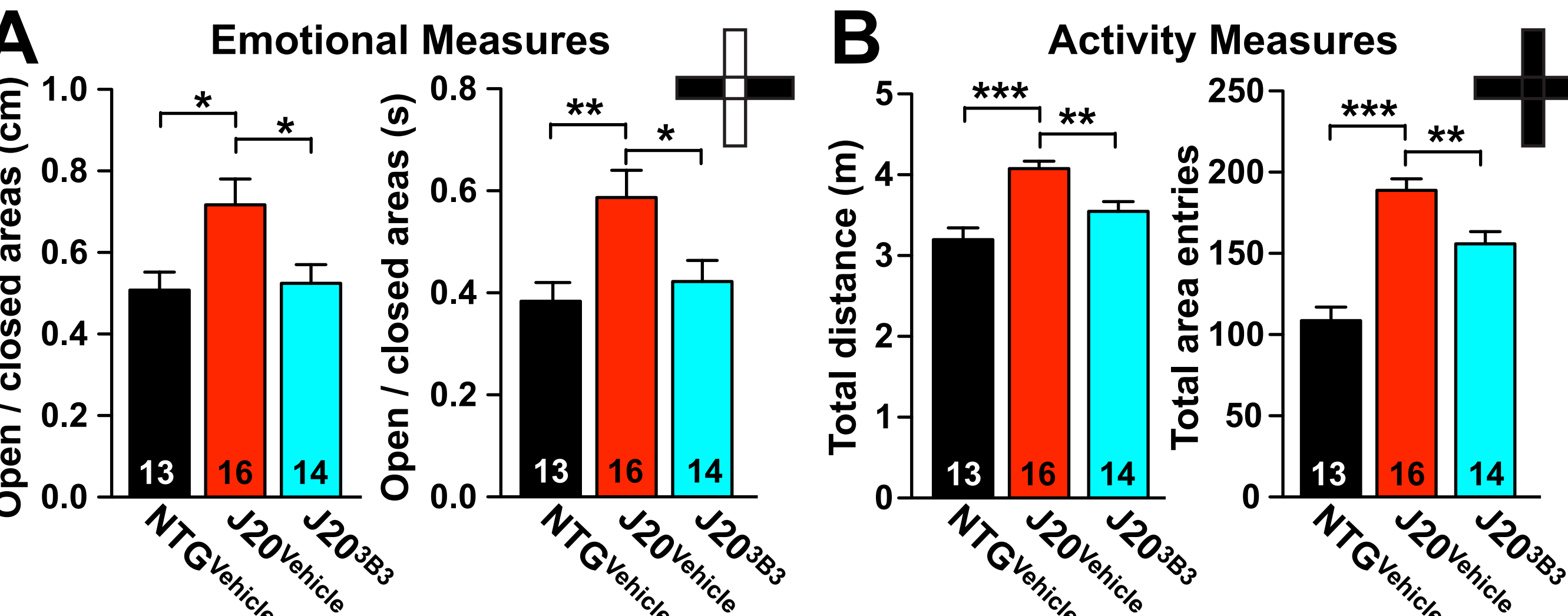
Untreated J20 mice (n = 62 naive J20 mice) were first tested in the open field (trial 1) and randomly assigned to vehicle or ACU-3B3 experimental groups. Thereafter mice were monitored for open field activity 24h after each weekly injection. Open field activity data are presented as average of trials 2-3 (middle bars) or 4-5 (right bars). One-way ANOVA and Bonferroni test for open field trials 2–5 (after ACU-3B3 treatment) confirmed that ACU-3B3 treatment reduced the hyperactivity phenotype in J20 mice at 5 and 10 mg/kg. *p<0.05, **p<0.01, and ***p<0.001 by one-way ANOVA and Bonferroni test.

ACU-3B3 Treatment Prevents the Hyperactivity Phenotype in J20 Mice



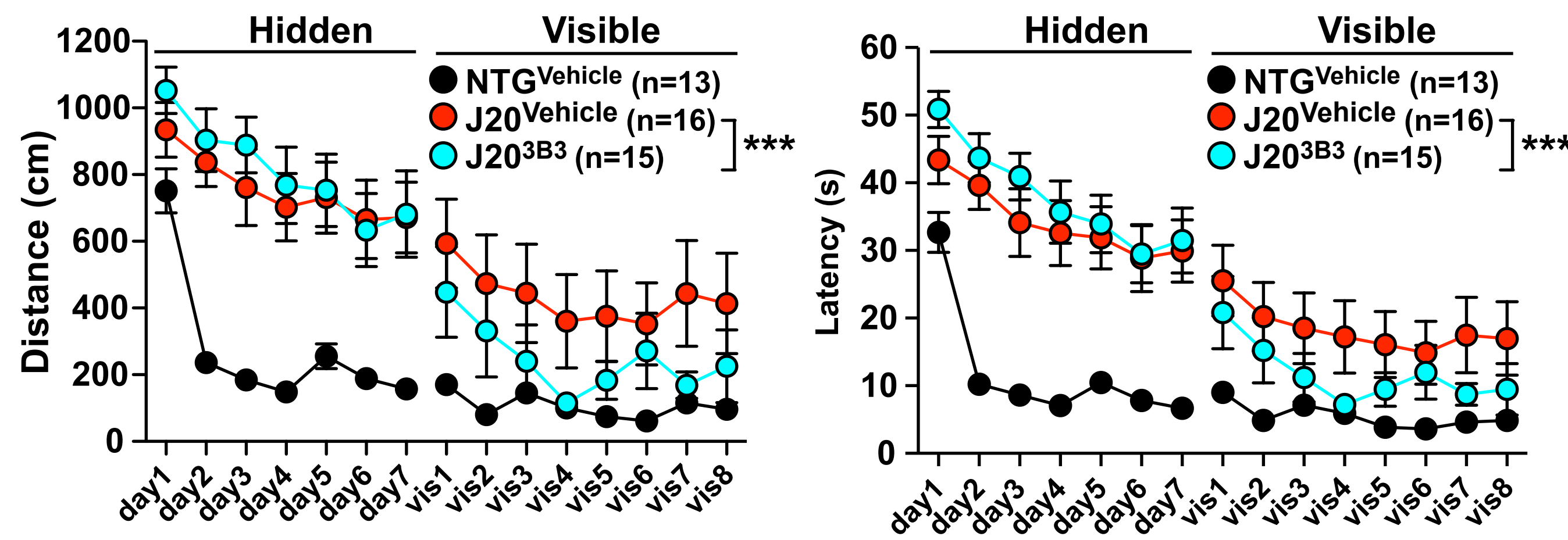
After 6 weeks of ACU-3B3 treatment, total locomotor activity (A) and arm visits (B) were assessed in the Y-maze. Spontaneous alternation was not altered in J20 mice (data not shown). However, ACU-3B3 treatment significantly prevented the hyperactive phenotype of J20 mice at 10 and 20 mg/kg. Bars represent mean + SEM. Numbers in bars are number of mice. *p<0.05, and **p<0.01 by one-way ANOVA and Bonferroni test.

ACU-3B3 Treatment Reduces Emotional Deficits in J20 Mice



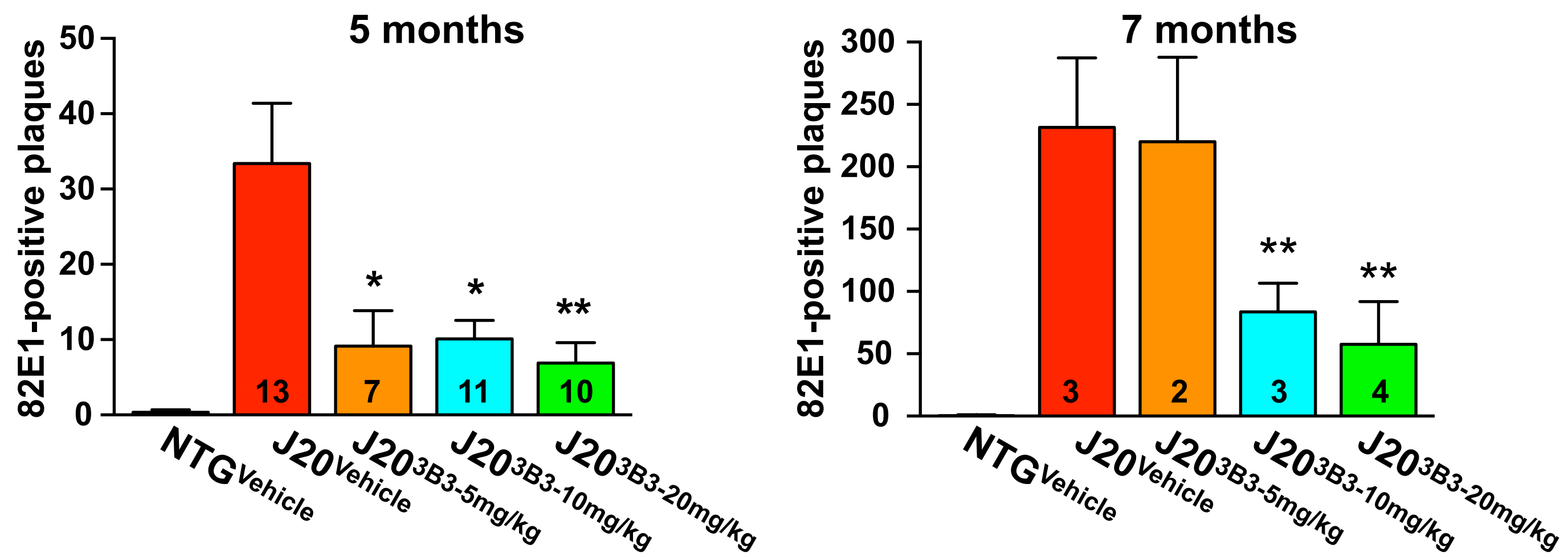
After 9 weeks of ACU-3B3-10mg/kg treatment, mice were tested in the elevated plus maze to assess their emotional response to an anxiogenic environment. A) The percent time spent in open arms was significantly higher in J20-Vehicle mice, a phenotype that was prevented by ACU-3B3-10mg/kg treatment (J20-3B3 mice) (left, distance; right, time) (B). ACU-3B3-10mg/kg treatment also reduced the hyperactive phenotype in the elevated plus maze. Bars represent mean + SEM. Numbers in bars are number of mice. *p<0.05, **p<0.01, and ***p<0.001 by one-way ANOVA and Bonferroni test.

ACU-3B3 Treatment Reduces Visible Platform Water Maze Training Deficits in J20 Mice



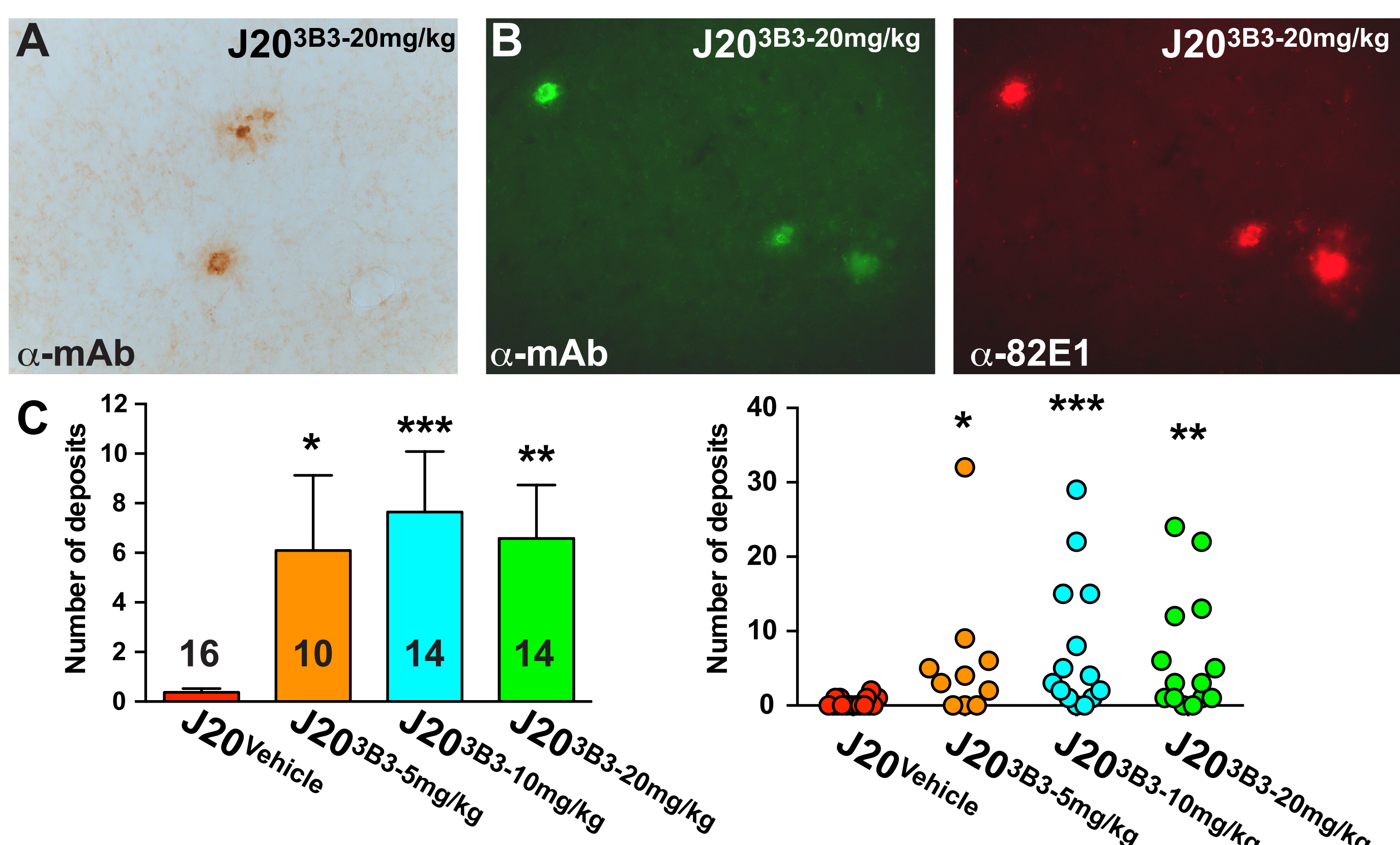
After 7 and 8 weeks of ACU-3B3-10mg/kg treatment, mice were tested in the hidden and visible versions of the water maze (left, distance; right, latency). ACU-3B3-10mg/kg treatment did not improve performance in the hidden version of the test (spatial learning), but improved performance in the visible version of the test (non-spatial learning). ***p<0.001 by repeated-measures one-way ANOVA and Bonferroni test.

ACU-3B3 Treatment Reduces Amyloid Plaques at Early and Moderate Stages of Amyloid Deposition in J20 Mice



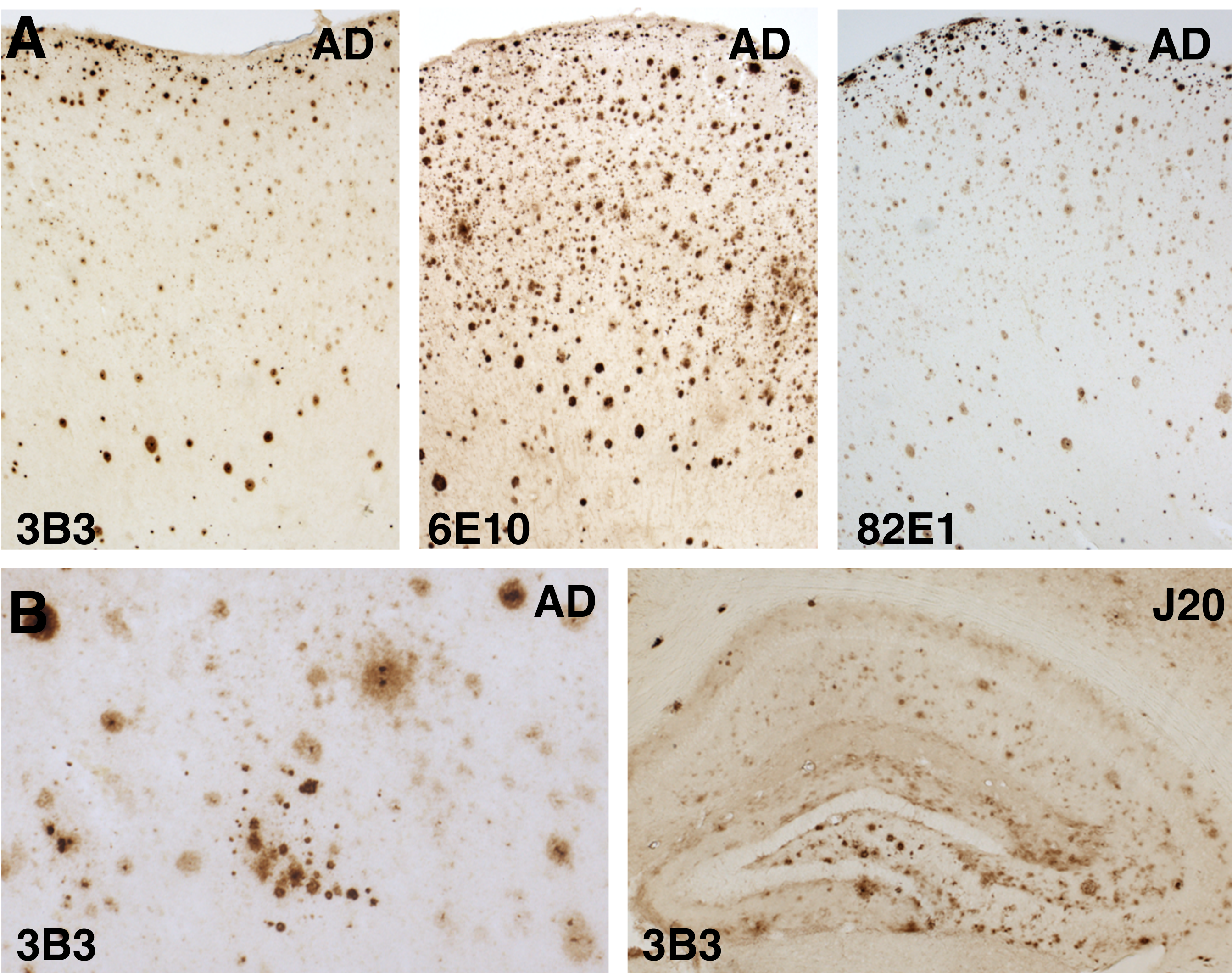
After 10 weeks of treatment, mouse brains were collected for pathological analyses. ACU-3B3 treatment reduced 82E1-positive amyloid deposits in the hippocampus and cortex in a dose-dependent manner at 5 (left) and 7 (right) months of age in J20 mice. Bars represent mean + SEM. Numbers in bars are number of mice. *p<0.05, and **p<0.01 by one-way ANOVA and Bonferroni test.

Target Engagement of ACU-3B3 in the Brain of J20 Mice



To evaluate brain penetration and target engagement of ACU-3B3, a secondary anti mouse antibody was used to detect ACU-3B3 in treated J20 mice after 10 weeks of treatment, taking advantage of the lack of IgG2 antibodies in the CNS. ACU-3B3 colocalized with 82E1-positive Aβ deposits showing positive brain penetration and target engagement in treated J20 mice. After 10 weeks of treatment, mouse brains were collected for pathological analyses. Bars represent mean + SEM. Numbers in bars are number of mice. *p<0.05, and **p<0.01 by one-way ANOVA and Bonferroni test.

ACU-3B3 Detects Aβ Accumulation in Brains of Humans with AD.



A) Human brain sections from the frontal cortex of an AD case were immunostained with the anti-Aβ antibodies 3B3, 6E10 and 82E1. Images show similar, but not identical, distribution of Aβ deposits by these three antibodies. B) Brain sections from the frontal cortex of an AD case (left) and a 22-month-old non-treated J20 mice (right) were immunostained with 3B3 antibody. 3B3 antibody detected predominantly diffuse Aβ deposits in human with AD and J20 mice.

Conclusions

- 1) ACU-3B3 is a novel soluble anti-Aβ-oligomer selective antibody with low affinity for monomeric and fibrillar Aβ.
- 2) ACU-3B3 treatment reduces behavioral alterations and improves cognitive functions at the earliest stages of disease manifestation in J20 mice.
- 3) ACU-3B3 treatment reduces amyloid deposition in the hippocampus and cortex of J20 mice.
- 4) ACU-3B3 antibody shows brain penetration and target engagement after subchronic treatment in J20 mice.
- 5) ACU-3B3 is readily detected in brain tissues of J20 mice after passive immunization and colocalizes with Aβ deposits.
- 6) Our data support that the potential clinical benefits in AD might be achievable using a new class of soluble oligomer selective antibodies in passive immunization approaches.

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