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RESEARCH ARTICLE

Aβ oligomer induced cognitive impairment and evaluation of ACU193-MNS-based MRI in rabbit

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Abstract

Introduction: Amyloid-beta oligomers (AβOs) accumulate in Alzheimer's disease and may instigate neuronal pathology and cognitive impairment. We examined the ability of a new probe for molecular magnetic resonance imaging (MRI) to detect AβOs in vivo, and we tested the behavioral impact of AβOs injected in rabbits, a species with an amino acid sequence that is nearly identical to the human sequence.

Methods: Intracerebroventricular (ICV) injection with stabilized AβOs was performed. Rabbits were probed for AβO accumulation using ACUMNS (an AβO-selective antibody [ACU193] coupled to magnetic nanostructures). Immunohistochemistry was used to verify AβO presence. Cognitive impairment was evaluated using object location and object recognition memory tests and trace eyeblink conditioning.

Results: AβOs in the entorhinal cortex of ICV-injected animals were detected by MRI and confirmed by immunohistochemistry. Injections of AβOs also impaired hippocampal-dependent, but not hippocampal-independent, tasks and the area fraction of bound ACUMNs correlated with the behavioral impairment.

Discussion: Accumulation of AβOs can be visualized in vivo by MRI of ACUMNS and the cognitive impairment induced by the AβOs can be followed longitudinally with the novel location memory test.

KEYWORDS
Alzheimer's disease, amyloid-beta oligomers, biomarkers, dementia, diagnostics, eyeblink conditioning, magnetic resonance imaging

1 | INTRODUCTION

Worldwide, nearly 50 million individuals are living with Alzheimer's disease (AD). In the United States, AD is currently the leading cause of dementia accounting for between 50% and 70% of reported cases[1] and is the sixth leading cause of death according to the National Institute on Aging (2019). AD presents clinically as progressive cognitive decline that impacts memory, language, visuospatial processing, and executive function, with patients increasingly unable to function.[2] Histopathologically, AD displays two hallmark characteristics: insoluble plaques of amyloid β (Aβ) protein and neurofibrillary tangles of hyperphosphorylated tau (p-tau) protein.

The hypothesis that onset of AD might be triggered by toxic Aβ oligomers (AβOs) was first proposed in 1998 when it was shown that fibril-free synthetic preparations of Aβ were potent central nervous system neurotoxins.[3] Subsequent in vivo studies confirmed that both

synthetic and brain-derived AβOs disrupt both long-term potentiation (LTP) and long-term depression (LTD).[4–6] AβOs have been shown to instigate tau pathology[7–9] and are associated with deterioration of synapses,[10,11] oxidative stress,[12–15] neuroinflammation,[7,16–18] and secondary effects associated with the neuropathology of AD.[19]

Despite the fact that the disease was first described in 1907[20] and the Aβ peptide characterized in 1984,[21] a definitive diagnosis for AD remains possible only after death. However, positron emission tomography (PET) imaging with ligands for amyloid plaques coupled with clinical history, neuropsychological testing, and neurologic and psychiatric examinations have a high success rate of ante mortem diagnosis of AD, as confirmed with histopathology.[2,21–26] Imaging and fluid assays can be used to support a diagnosis of AD with the exclusion of other dementia causes, such as Parkinson's disease and frontotemporal dementia. Moreover, the pathogenic mechanisms leading to AD may begin as early as two decades before noticeable symptoms develop.[23–25] AβOs appear to be among the earliest manifestations of AD pathology[1] and offer appealing targets for diagnostics that could be used with individuals who should begin an approved therapy as one becomes available or for companion diagnostics in evaluation of investigational new drugs. However, because AβOs are much less abundant than monomeric Aβ, an assay faces challenges with respect to sensitivity and specificity.

The current study introduces a diagnostic imaging approach using ACU193, a humanized monoclonal antibody targeting AβOs, that was developed by Acumen Pharmaceuticals. ACU193 has 500-fold selectivity for AβOs over fibrils[26] and >2500-fold selectivity for AβOs over monomers.[27] In the current study, ACU193 was coupled to magnetic nanostructures (MNS) to create a probe (ACUMNS) that can detect AβOs by magnetic resonance imaging (MRI). MNS are superparamagnetic and cause localized decreases in signal intensity by shortening T2*. Due to their small diameter (under 20 nm), MNS lack permanent magnetization at room temperature, which affords them excellent stability in both localized and systemic delivery.[28,29] ACUMNS provides a powerful contrast probe with high affinity and specificity for AβOs.

Intracerebroventricular (ICV) injection of unstabilized, naturally produced human AβOs has previously been shown to disrupt cognitive function in rats.[30] Stabilized synthetic AβOs (as used in this study) administered through ICV injections have previously been shown to elicit cognitive impairment when examined with object recognition tasks in mice.[31] The goals of the present study were to evaluate in rabbits: (1) the potential of ACUMNS as a diagnostic tool for imaging AβOs by MRI and (2) the ability of stabilized AβOs to cause cognitive impairment in rabbits.

RESEARCH IN CONTEXT

1. **Systematic Review**: The authors reviewed relevant literature using PubMed sources and meeting abstracts and presentations. The pathophysiology of amyloid-beta oligomers (AβOs) and their usefulness as biomarkers of Alzheimer's disease (AD) progression have been a large focus of study since their initial discovery. These relevant citations are appropriately referenced.
2. **Interpretation**: Our findings demonstrate the usefulness of ACUMNS (an AβO-selective antibody [ACU193] coupled to magnetic nanostructures) and other antibody-based magnetic nanostructures as viable diagnostics for early AD pathology. Additionally, our results bolster the evidence of AβO-induced cognitive impairment.
3. **Future Directions**: The article proposes several future studies. For example, we did not examine the ability for our AβO preparations to elicit tau pathology, nor the relationship between p-tau and cognitive impairment. Also, future work should focus on understanding: (1) a better time course of study for ACUMNS, (2) if ACUMNS is cleared from the brain and how quickly, and (3) the potential of ACU193 as an immunotherapy.

2 | METHODS

2.1 | Animals

New Zealand White female rabbits (n = 16) were purchased from Envigo Global Services Inc. Rabbits were selected because the amino acid sequence for amyloid in the rabbit is nearly identical to the sequence for human amyloid.[32] Rabbits were 5 to 8 months of age at time of experiment. Rabbits were singly housed and kept on a 12:12 light:dark cycle. All rabbits were provided food and water *ad libitum*. All animal procedures were performed with approval of the Northwestern University Institutional Animal Care and Use Committee.

2.2 | Stereotaxic surgery

Rabbits were surgically implanted with a 22-gauge nylon cannulae (PlasticsOne) projecting to the left lateral ventricle (AP0.0, L2.0 from bregma, ~4.0 below dura) for ICV injections (Figure 1A), that is, the side contralateral to the eye that would be conditioned to blink. A premade acrylic piece with two nylon bolts was cemented to the top of the skull at the midline using Metabond and dental cement. The bolts were used to hold the animal's head in place during eyeblink conditioning (EBC) and to hold the animal's head and imaging coil in place during awake image acquisition. The procedures were as described previously[33] except that each headbolt had two instead of four bolts to accommodate a newer 3-channel receiver coil (RAPID MR International, Columbus, Ohio).

2.3 | Injections

The present study consisted of two cohorts of young New Zealand White rabbits. The first cohort of eight rabbits received daily ICV injections of stabilized preparations of AβOs (0.35 mg/mL; n = 4)

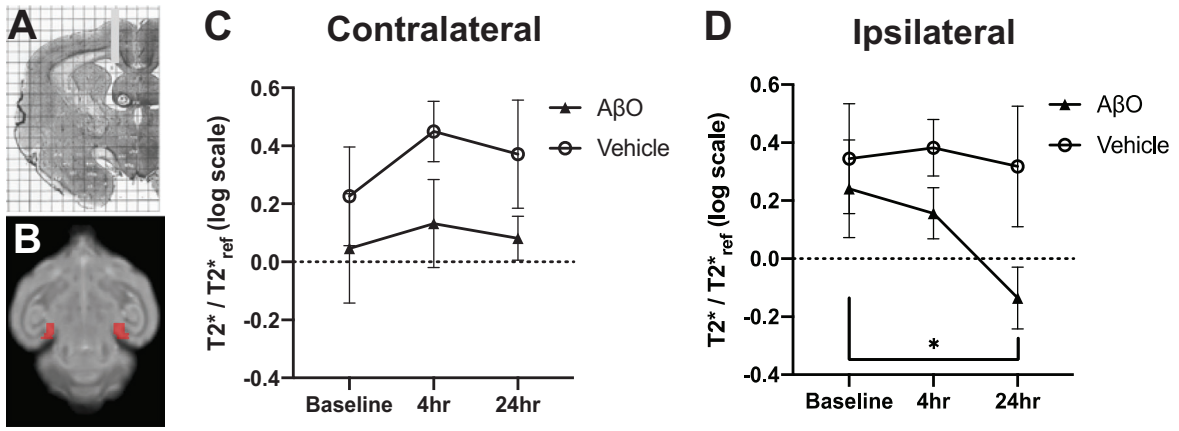

FIGURE 1 Regional differences in ACUMNS (an amyloid-beta oligomer [AβO]-selective antibody [ACU193] coupled to magnetic nanostructures)-induced changes in T2* over time. A, Approximate location of a guide tube inserted into the rabbit brain for injections into the lateral ventricle. Coordinates relative to Bregma: AP 0, L 2. The injector extended 0.5 mm beyond the end of the guide tube. B, Outline of the entorhinal cortex (red regions) used in T2* analysis displayed on T2* map from co-registered non-cannulated rabbits. C, The contralateral entorhinal cortex does not show significant changes in T2* for either AβO-injected (n = 5; $Q = 0.000$, $P = .999$) or vehicle-injected rabbits (n = 6; $Q = 2.333$, $P = .430$). However, in the ipsilateral entorhinal cortex (D), AβO-injected rabbits display a significant decrease in T2* over time (n = 5; $Q = 7.600$, $P = .0239$) while vehicle-injected rabbits do not display the same decrease (n = 6; $Q = 0.3333$, $P = .956$). Error bars ± 1 standard error of the mean. Statistical differences calculated using Friedman's test. Baseline measures were taken after all the daily injections of AβOs were completed, but prior to injection of ACUMNS; the 4 hours and 24 hours time points are relative to the injection of ACUMNS.

or vehicle (n = 4) for 16 days (prepared as previously described[31]). The vehicle used was a sodium borate buffer (25 mM Borax, pH 8.5). Total volume of each ICV injection was 100 µL administered at 5 µL/min using a programmable syringe injection pump (Cole-Parmer). Approximately 24 hours after the last injection of AβOs or vehicle, each rabbit was injected with 50 µL of ACUMNS probe (1 mg/mL in 10 mM Na_3PO_4, 0.9% NaCl, pH 7.4) at an injection rate of 5 µL/min. The same guide tube was used for injecting both ACUMNS and either AβOs or vehicle. Three of the rabbits from the first cohort that were injected with AβO exhibited pneumonia-like symptoms toward the end of the experiment and were euthanized prior to completing the entire sequence of tests (one rabbit injected with vehicle was excluded due to technical difficulties during EBC). To minimize health-related complications, the second cohort of eight rabbits received injections of stabilized AβOs (n = 4) or vehicle (n = 4) only three times a week at 2.5 µL/min and rabbits were allowed to rest on weekends.

2.4 | Assessment of cognition

EBC was performed after each daily injection (or at the same time of day on days when no injections occurred) with day 1 of injections corresponding to session 1 of conditioning. The conditioning stimulus (CS) was a vibration (250 ms, 62.5 Hz) of the B row of whiskers on the right side of the face. The unconditioned stimulus (US) was a 150 ms puff of nitrogen (150 ms, 3-5 psi) presented to the cornea of the right eye. Movement of the nictitating membrane (NM, third eyelid) was detected with a reflective infrared sensor. Extensions of the NM after CS onset and before US onset were considered conditioned responses (CRs) if the signal change was at least four standard deviation (SD) greater than the mean of the baseline signal for at least 15 ms.

Object location memory test (OLM) and novel object recognition test (NOR) were assessed after the 15 days of injections and EBC was completed. Rabbits were excluded if they did not meet a criterion of 8 total seconds of investigation time (two vehicle and two ABO injected rabbits were excluded from NOR results, and two ABO and one vehicle injected rabbit were excluded due to health concerns, see previous section). Final numbers for NOR/OLM testing after exclusions were n = 6 AβO and n = 7 vehicle during the OLM test, and n = 6 AβO and n = 5 vehicle during the NOR test. The complete protocol for each test can be found in the supporting information.

2.5 | Magnetic resonance imaging

Rabbits were placed in a loose cloth bag and then swaddled with a Lomir Bunny Snuggle.[34] While swaddled, rabbits were placed in a custom acrylic cradle and their heads and the imaging coil were secured to a cross bar across the cradle using the nylon bolts and hex nuts. Rabbits were then placed into a Bruker 7T ClinScan for image acquisition. The transmitter was a quadrature volume coil and the receiving coil was a 3-channel phase array coil. Respiration rate was monitored to ensure animal safety. Rabbits underwent three separate imaging sessions after all injections of AβOs were completed: baseline prior to ACUMNS injection, 4 hours after injection of the ACUMNS probe, and 24 hours after injection of the ACUMNS probe. Analysis parameters are outlined in the supporting information.

2.6 | Euthanasia and perfusion

Rabbits were euthanized using a ketamine/xylazine cocktail (100 mg/20 mg) administered through the marginal ear vein with subsequent bilateral thoracotomy and exsanguination of the body. Rabbits were perfused using 1X phosphate-buffered saline (PBS) followed by 10% formalin. After perfusion, rabbits were decapitated and the brain was removed and placed into 10% formalin for post-fixation.

2.7 | Histology

Free-floating 45μm thick sagittal sections were cut using a Leica SM2010 R sliding microtome and transferred to sterile PBS for storage. Sections were obtained from the hemisphere contralateral to the injection site to avoid the track mark resulting from the ICV injections. Five sequential sections were stored in each well. Sections for staining and mounting were randomly selected from each well. Free-floating sections were stained using the primary antibody NU2 (1.5 μg/mL)[35,36] and secondary antibody Alexa Fluor 488 goat anti-mouse immunoglobulin G (IgG; 1:2000, Invitrogen). Sections were mounted onto microscope slides, preserved using 1 to 2 drops of ProLong (Invitrogen), and a 0-thickness coverslip.

2.8 | Unbiased stereology

Labeled puncta of AβOs were visualized with a Zeiss Axio Imager M2 and analyzed using the area fraction fractionator probe (Stereo Investigator© Version 2019, Microbrightfield Biosciences). Slide scanning images were obtained at 62× magnification for analysis. For the hippocampus, four sections were analyzed for each rabbit with a section interval of 10. For the entorhinal cortex (EC), four sections were analyzed for each rabbit with a section interval of 5. EC sections from one vehicle rabbit were lost due to issues with long-term storage and were unable to be analyzed. The sampling grid size was 675 μm × 675 μm with a grid spacing of 5 μm and an area sampling fraction (asf) of 0.049.

2.9 | Antibodies and ACUMNS preparation

ACU193, a humanized monoclonal antibody selective for AβOs, was a gift from Acumen Pharmaceuticals.

2.9.1 | Synthesis of magnetic nanostructures

Eight nm ferrite (Fe_3O_4) and mixed metal ferrite ($ZnxMnyFe_3$-x-yO_4) nanoparticles were selected as MNS and synthesized by high temperature thermal decomposition of metal precursors as described in an earlier report,[37] that is, Fe(acac)3,1,2-hexadecanediol, oleic acid, oleylamine, and benzyl ether were charged in a flask and magnetically stirred under a flow of nitrogen. The mixture was refluxed for 1 hour before cooling to room temperature. The black-brown mixture was washed and then dispersed in hexane. Mixed metal ferrite nanoparticles were synthesized by replacing definite amount of Fe precursors with Mn and Zn precursors under identical conditions. The stoichiometry was controlled by tuning the initial molar ratio of the metal precursors. The size of the nanoparticles was controlled by tuning surfactant (oleic acid, oleylamine) to precursor ratio and solvent to precursor ratio.

2.9.2 | Preparation of dopamine-TEG-COOH and phase transfer

To make the organic phase synthesized MNS suitable for biological application we stabilized the MNS using carboxylate terminated ligand with nDOPA as an anchor. Synthesis of carboxylate terminated nDOPA ligand and functionalization of the MNS was carried out according to the following protocol: tetraethylene diacide, N-hydroxysuccinimide (NHS), N,N′-dicyclohexylcarbodiimide (DCC), nDOPA hydrochloride, and anhydrous sodium bicarbonate was dissolved in chloroform under argon atmosphere and stirred for 4 hours. Hexane-stabilized MNS was added and stirred for another 24 hours. The precipitate formed was separated by magnet, dispersed in water, and purified by dialysis.

2.9.3 | Preparation of ACUMNS

ACUMNS was prepared by covalently cross-linking mixed metal (Fe, Mn, and Zn) MNS with ACU193 antibody using a modification of the previously published method.[28] The carboxyl terminated MNS was activated by sulfo-N-hydroxy succinimide (sulfo-NHS) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) at pH 6.0 followed by incubation with ACU193 at pH 7.5 for 24 to 48 hours. Conjugated MNS were separated by magnet to remove excess reagent and antibody then re-dispersed in working media. Unconjugated carboxyl sites were regenerated by raising the pH > 8, then reactivating the free carboxyl groups as above and incubating with another aliquot of ACU193. Excess reagent and antibody was removed with the magnet. Conjugation efficiency was estimated using ultraviolet spectroscopy (absorbance at 280 nm) of the magnetically separated supernatant.

$$\text{Ab conc.} = (\text{total mg added Ab}) - (\text{mg Ab in supernatant})$$

ACU193 was stored at a concentration of 13.3 mg/mL and used at concentration of 1.5 μg/mL for immunofluorescence.

The working concentration of ACUMNS was 0.35 mg/mL of antibody.

2.10 | Statistical analysis

Statistical analysis was performed using GraphPad Prism version 8.2.0 for MacOS (GraphPad Software). EBC was analyzed using two-way repeated measures analysis of variance (ANOVA). T2* changes over time were analyzed for individual groups using the nonparametric

Friedman test. Correlational analysis was performed using Pearson's correlation and goodness of fit lines were generated using linear regression.

3 | RESULTS

3.1 | MRI detection of injected AβOs using ACUMNS

Left lateral ventricles were injected (Figure 1A) with AβOs or vehicle over a 3-week period after which rabbits were administered the ACUMNS MRI probe and scanned to acquire T2* maps using multiple gradient echo MR sequences. Differences in T2* values between AβO-injected and vehicle-injected rabbits are clear within the EC (Figure 1B), a region important for associative learning and memory[38,39] that shows early onset changes during AD.[40] Figures 1C and 1D show that AβO-injected rabbits, but not vehicle-injected rabbits, show a significant decrease over time in T2* values on the ipsilateral side according to the Friedman test (AβO $Q = 7.600$, $P = .0239$; vehicle $Q = 0.3333$, $P = .9563$). The contralateral EC does not exhibit observable differences for either the AβO-injected ($Q = 0.000$, $P > .9999$) or vehicle-injected groups ($Q = 2.333$, $P = .4297$). Decreases in T2* in the ipsilateral EC are due to the buildup of the MRI probe, whose MNS moiety shortens T2* and causes a local dimunition of signal. The ACU193 moiety of ACUMNS directs the probe to AβOs within the EC, where it accumulates with time. In the control rabbits, which lack AβOs, there is no accumulation of probe and hence no decrease in T2* values with time.

Across the whole brain (Figure S1 in supporting information), there was no statistically significant difference between groups for T2* values at baseline ($t[11] = 0.5417$, $P = .2994$). At 4 hours post-injection, quantitative analysis revealed decreases in the mean value for both groups, which is consistent with the spread of ACUMNS within the ventricular system. At 24 hours, there is a decrease in the mean T2* value in AβO-injected rabbits consistent with the migration of ACUMNS into the brain parenchyma to bind AβOs. The quantitative difference between groups at 24 hours is in the expected direction and approaches statistical significance ($t[9] = 1.594$, $P = .07$). The quantitative difference between groups is driven by the decrease in T2* values within the hemisphere ipsilateral to the injection site. T2* values from the hippocampus were not extracted due to more artifacts associated with its close proximity to the injection site and obstruction of the region by the injection tract of the implanted cannula.

3.2 | Immunofluorescent detection of AβOs using NU2

While MRI of ACUMNS is a translatable method, it lacks the sensitivity of *post mortem* immunohistochemical techniques. To verify that regional differences in T2* values was attributed to ACUMNS binding to oligomers within those regions, immunofluorescent staining and unbiased stereological counting was performed with the AβO-selective antibody NU2 within the hippocampus and the EC (Figure 2A). As shown in Figures 2B and 2C, AβO-injected rabbits showed a statistically significant increase in percentage of tissue volume occupied by NU2-labeled AβO puncta over vehicle-injected rabbits within the hippocampus ($t[11] = 3.381$, $P = .0031$) and within the EC ($t[10] = 8.138$, $P < .0001$). Immunofluorescent staining was performed in the hemisphere contralateral to the injection site to avoid the tissue damage caused by the cannula injection tract.

3.3 | AβO-induced effects on cognition

When evaluated using OLM and NOR tasks (Figure 3A), AβO injections resulted in a significant impairment of the spatial memory index score for the OLM test, as shown in Figure 3B (0.60 vs 0.06; $t[11] = 1.92$, $P = .041$). However, in the NOR test, as shown in Figure 3C, there was no significant difference observed between the mean memory index scores of AβO-injected and vehicle-injected rabbits (0.59 vs 0.66, respectively; $t[9] = 0.4076$, $P = .6931$), indicating that AβOs do not impair the ability of rabbits to recognize novel objects.

Trace EBC is a forebrain dependent associative memory[41–43] task that requires several sessions for acquisition. Time is also required for the AβOs to accumulate, which suggests that a learning deficit should not appear for several sessions. A plot of percent adaptive CRs (those present within 200 ms of US onset) for each daily 80 trial session indicates that AβO-injected rabbits exhibited fewer adaptive CRs than vehicle-injected rabbits as of session 6 (Figure 4) and the largest difference occurred during sessions 7 and 8. In addition to the percentage of trials with CRs, several measures of the CR were examined (onset latency of the CR, latency to the maximum amplitude of the CR, area under the curve of the CR). None of those measures were found to be significantly different between the two groups, and none exhibited any significant interactions of group and sessions.

The immunofluorescent results were used to determine the relationship between oligomer load and behavior. Within the hippocampus, oligomer load had a significant negative correlation with individual memory index scores in the OLM test (Figure 5A; $r = -0.4937$, $n = 13$, $P = .0432$). A similar result was found within the EC (Figure 5B; $r = -0.5699$, $n = 12$, $P = .0265$), as well as for CRs during session 7 of trace EBC, which exhibited the largest difference in percent CRs between groups (Figure 5C; $r = -0.6260$, $n = 12$, $P = .0147$).

4 | DISCUSSION

Injections of AβOs into the lateral ventricle impaired performance in hippocampally dependent associative learning tasks in rabbits. This impairment is in harmony with results using mice and non-human primates,[31,44] and supports the hypothesis that neural damage instigated by AβOs leads to AD-related dementia. The cognitive impairment caused by AβOs in multiple animal models underscores the goal of targeting these toxins for therapeutics and diagnostics. Translation of past animal model findings to the rabbit is particularly important

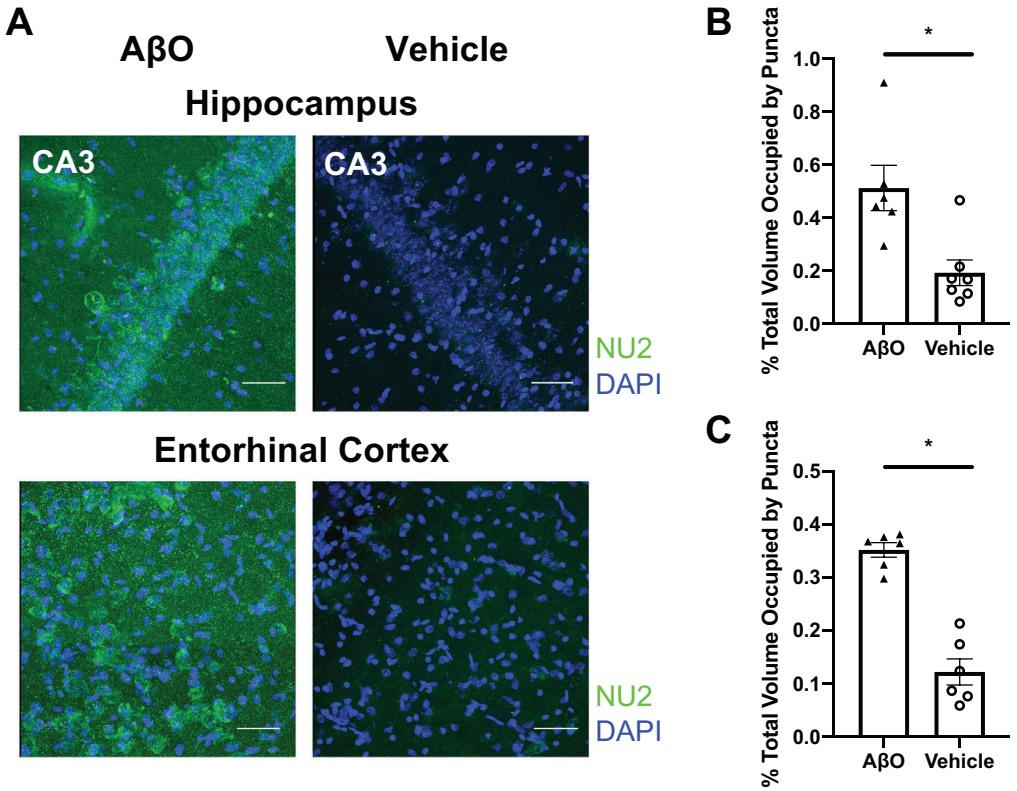

FIGURE 2 Amyloid-beta oligomer (AβO)-injected rabbits display a significantly greater volume of tissue with NU2-labeled puncta in both the hippocampus and entorhinal cortex. A, Representative images from the hippocampus and entorhinal cortex (from the side contralateral to the injection) showing diffuse puncta of NU2-labeled AβOs in the AβO-injected rabbit, but not in the vehicle-injected rabbit. Images were acquired at 40× magnification as z-stacks using a 40× objective lens and apotome and displayed as max projections. StereoInvestigator was used for data collection and analysis. Scale bars are 50 μm. B, AβO-injected rabbits (n = 6) display a statistically greater percent of the total volume of the hippocampus counted through systematic random sampling occupied by NU2-labeled AβOs than vehicle-injected rabbits (n = 7; $t[11] = 3.381$, $P = .0031$). C, AβO-injected rabbits (n = 6) display a statistically greater percent of the total volume of the entorhinal cortex counted through systematic random sampling occupied by NU2-labeled AβOs than vehicle-injected rabbits (n = 6; $t[10] = 8.138$, $P < .0001$). Statistical difference calculated using one-tailed independent samples *t* test.

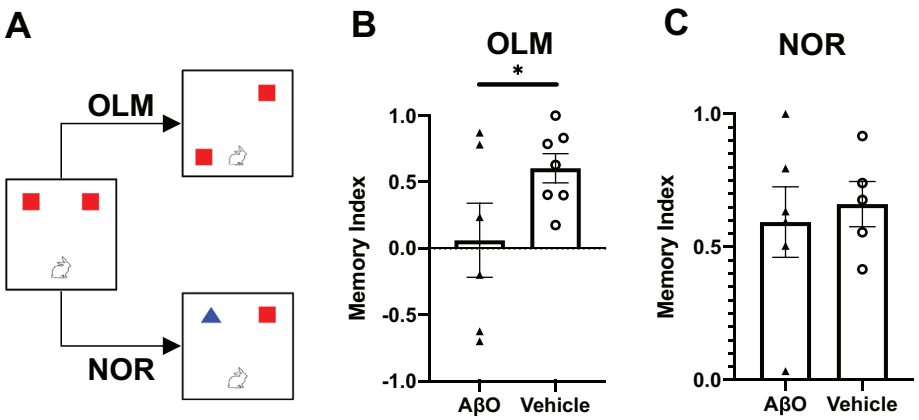

FIGURE 3 Amyloid-beta oligomer (AβO)-induced impairment of object location memory task. A, Schematic of novel object recognition and object location memory tests. B, Rabbits that received injections of AβOs (n = 6) showed an impairment of spatial memory as evaluated by the object location memory test, that is, they displayed a significantly lower mean memory index score than vehicle-injected (n = 7) rabbits ($t[11] = 1.921$, $P = .0405$). C, Rabbits that received injections of AβOs (n = 6) did not show an impairment in mean cognitive score during a novel object recognition test compared to vehicle-injected rabbits (n = 5) ($t[9] = 0.4076$, $P = .6931$). Statistical differences calculated by one-tailed independent samples *t* test. Error bars indicate ± 1 standard error of the mean.

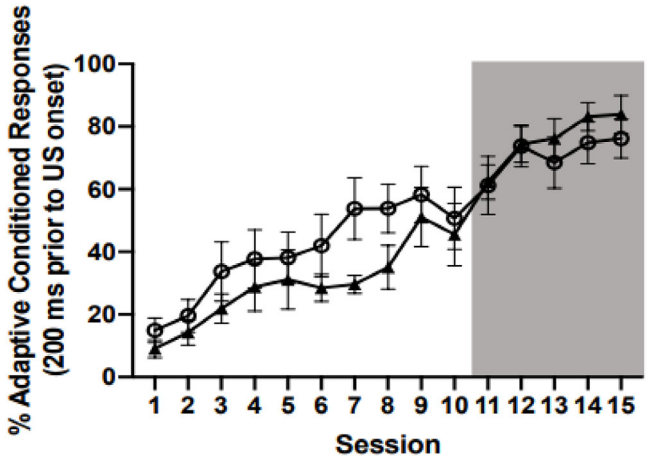

FIGURE 4 Daily injections of stabilized amyloid-beta oligomers (AβOs) impaired trace eyeblink conditioning. Adaptive conditioned responses (those occurring 200 ms or less prior to unconditioned stimulus onset) across 10 days of trace conditioning and 5 days of delay conditioning. The differences between groups were not statistically significant after correcting for the effect of multiple comparisons. There were also minimal differences between groups during non-hippocampal dependent delay conditioning (sessions 11–15).

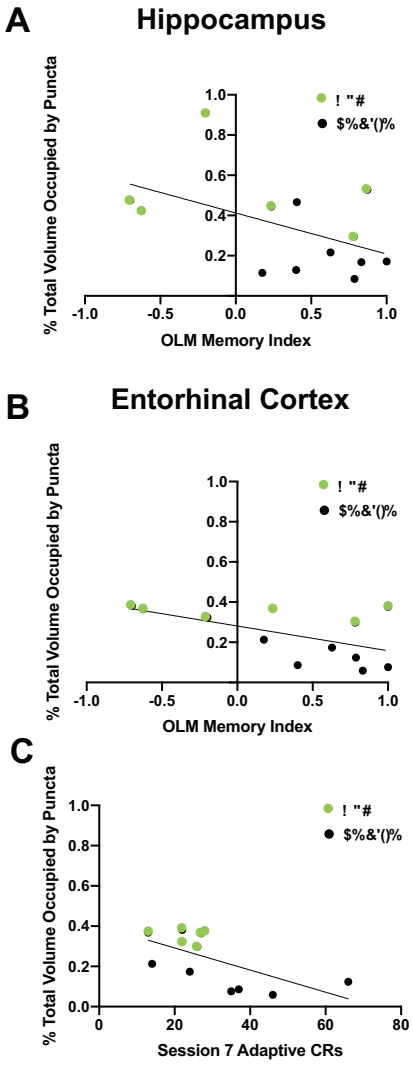

FIGURE 5 Memory impairment and immunohistochemical detection of NU2-labeled puncta in the hippocampus and entorhinal cortex are significantly correlated. A, Individual cognitive scores observed in the object location memory (OLM) test are significantly correlated with unbiased stereological estimates of NU2-labeled puncta in the hippocampus ($r = -0.4937$, n = 13, $P = 0.0432$). B, Individual cognitive scores observed in the OLM test are significantly correlated with unbiased stereological estimates of NU2-labeled puncta in the entorhinal cortex ($r = -0.5699$, n = 12, $P = .0265$). C, Estimates of NU2-labeled puncta in the entorhinal cortex significantly correlate with measures from session 7 of eyeblink conditioning adaptive conditioned responses ($r = -0.6260$, n = 12, $P = .0147$), that is, the conditioning session with the largest difference between groups. Correlations calculated through Pearson's correlation analysis and lines generated using linear regression analysis. Counts of NU2-labeled puncta from the side contralateral to the injections were used to derive the correlations with behavior.

because the amino acid sequence for amyloid in the rabbit is nearly identical to the human sequence[32] and rabbits are sensitive to pathological effects of a cholesterol enriched diet,[45] a risk factor for human AD, making the rabbit model useful for continued research on AD-related risk factors. For example, rabbits maintained on a diet enriched with 2% cholesterol (and 0.12 ppm copper) exhibit at least 12 pathological markers of AD.[46]

We show the feasibility of imaging AβOs in awake animals using ACUMNS as a probe for molecular MRI of AβOs. This is a step toward development of diagnostic agents for AβOs that will allow for earlier and more effective diagnosis of AD. We hypothesize that the rabbits injected with ABOs would have exhibited impairments in EBC if they had been injected for several days prior to the initiation of behavioral testing. Visuospatial memory (which was tested after many days of injections), however, was clearly affected. The high variability of OLM scores in the AβO-injected group is likely due to differences in oligomer load within sensitive circuits, while the correlation between oligomer load seen by immunohistochemistry between the hippocampus and OLM scores is consistent with the known toxicity of AβOs to hippocampal LTP and LTD.[4–6]

Importantly, the OLM behavioral test demonstrated that the AβO-induced cognitive impairment observed in this study was present in a hippocampally dependent learning task. No impairments were detected in the NOR test (Figure 3C), indicating that behavioral deficits associated with the AβO injections were related to deficits in the hippocampus and hippocampal-associated regions. This is consistent with other research demonstrating that these regions are particularly vulnerable in the earliest stages of AD.[47] The NOR test did not reveal an impairment as noted previously in mice.[31] This might reflect a species difference, methodological differences, that is, the novel object for the rabbit was placed at the same location as the replaced object, rather than at a novel location, or complex interactions of the insular cortex, perirhinal cortex, and ventromedial prefrontal cortex.[48]

The ability to quantify T2* in select regions of interest creates a highly sensitive tool for detection of the ACUMNS probe that binds to AβOs. Although whole brain quantitative analysis was less informative than immunohistochemical results, it may be useful for examining migration and clearance of ACUMNS, especially from the ventricular system. The large variability in vehicle-injected rabbits at 24 hours post-ACUMNS compared to the small amount of variability in AβO-injected rabbits (Figure 1/Figure S1) indicates that at 24 hours unbound ACUMNS within vehicle-injected rabbits had begun to clear from the ventricular system, while bound AβOs within the brain parenchyma of AβO-injected rabbits had successfully sequestered the probe and prevented its clearance from the brain. This finding suggests that with longer times for clearance within the vehicle group, perhaps 72 or 96 hours, T2* differences may have been increased and significant quantitative differences may have been detected. In the future, a push-pull system using bilateral cannula implantation would potentially alleviate pressure placed in the ventricle and prevent its expansion. Bilateral injections may also help to increase spread of the injected AβOs and avoid the observed differences between ipsilateral and contralateral T2* seen in this study. It is uncertain how quickly ACUMNS is cleared from the brain. In the present study, 24 hours was insufficient to allow for unbound ACUMNS to migrate through the ventricular system and be removed, which is consistent with MRI studies with transgenic mice.[28] Addition of multiple imaging days beyond 24 hours after administration of ACUMNS (to track the T2* values as they return to normal) would give valuable information for the timing of future imaging studies using ACUMNS.

Although the relation between injected AβOs and tau pathology was not examined here, other models have shown that tau pathology can be induced by AβOs. Whether AβOs or pathological tau correlate better with the observed cognitive impairments remains to be determined and would shed light on the relationship between Aβ and tau in the onset and progression of AD.[49,50]

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CONFLICTS OF INTEREST

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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