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Interactions between A β oligomers and presynaptic cholinergic signaling: age-dependent effects on attentional capacities

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Abstract

Substantial evidence suggests that cerebral deposition of the neurotoxic fibrillar form of amyloid precursor protein, β -amyloid (A β), plays a critical role in the pathogenesis of Alzheimer's disease (AD). Yet, many aspects of AD pathology including the cognitive symptoms and selective vulnerability of cortically-projecting basal forebrain (BF) cholinergic neurons are not well explained by this hypothesis. Specifically, it is not clear why cognitive decline appears early when the loss of BF cholinergic neurons and plaque deposition are manifested late in AD. Soluble oligomeric forms of A β are proposed to appear early in the pathology and to be better predictors of synaptic loss and cognitive deficits. The present study was designed to examine the impact of A β oligomers on attentional functions and presynaptic cholinergic transmission in young and aged rats. Chronic intracranial infusions of A β oligomers produced subtle decrements in the ability of rats to sustain attentional performance with time on task, irrespective of the age of the animals. However, A β oligomers produced robust detrimental effects on performance under conditions of enhanced attentional load in aged animals. *In vivo* electrochemical recordings show reduced depolarization-evoked cholinergic signals in A β -infused aged rats. Moreover, soluble A β disrupted the capacity of cholinergic synapses to clear exogenous choline from the extracellular space in both young and aged rats, reflecting impairments in the choline transport process that is critical for acetylcholine (ACh) synthesis and release. Although aging *per se* reduced the cross-sectional area of BF cholinergic neurons and presynaptic cholinergic proteins in the cortex, attentional performance and ACh release remained unaffected in aged rats infused with the control peptide. Taken together, these data suggest that soluble A β may marginally influence attentional functions at young ages primarily by interfering with the choline uptake processes. However, age-related weakening of the cholinergic system may synergistically interact with these disruptive presynaptic mechanisms to make this neurotransmitter system vulnerable to the toxic effects of oligomeric A β in robustly impeding attentional capacities.

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The supplementary information includes 3 figures and describes additional methods and results

Keywords

attention; cholinergic; presynaptic; soluble amyloid-beta; aging; Alzheimer's disease

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by irreversible cognitive deterioration. Although there is a widespread decline in various neurotransmitter-containing cell bodies and axonal terminals in end-stage AD, the most consistent losses are seen in the cortical projections of basal forebrain (BF) cholinergic neurons [1]. The activation of BF cholinergic neurons and consequent release of acetylcholine (ACh) in the cortex mediate attentional processes and capacities [2-5]. As attentional impairments constitute the core components of global cognitive decline in AD subjects [6], abnormally regulated cortical cholinergic transmission may underlie attentional dysfunction associated with this disorder.

The progressive accumulation of the extracellular neurotoxic fibrillar form of amyloid-beta (A β) protein is known to play a central role in the genesis of AD. Yet, many aspects of AD pathology including the cognitive symptoms and selective vulnerability of BF cholinergic neurons are not well explained by this hypothesis. Studies involving transgenic mice harboring mutations in AD-associated genes including amyloid precursor protein (APP) and presenilin-1, have provided insights into possible reciprocal interactions between cholinergic markers and A β [7-10]. However, the cause and effect relationship between A β accumulation and cholinergic dysfunction is not established. Specifically, it is not clear why cognitive decline appears early when the loss of cortically-projecting BF cholinergic neurons and plaque deposition are manifested late in AD pathology. Therefore, delineation of mechanisms that determine how neuropathological markers of AD disrupt cholinergic transmission is likely to provide gainful insights in understanding the neurobiological basis of cognitive decline in AD.

Substantial evidence suggests that synaptic loss predicts the degree of severity of cognitive deterioration in AD [11-13]. Moreover, the soluble oligomeric forms of A β 1-42 disrupted synaptic plasticity [14-15], and cognitive dysfunction in early AD correlated well with A β oligomers but not with plaques [16-17]. These findings supported the notion that intraneuronal or extracellular accumulation of soluble A β oligomers may produce synaptic and cognitive dysfunction in early stages of AD. A β oligomers disrupt synaptic plasticity and may produce cognitive dysfunction without producing neuronal cell death in early AD [18]. Moreover, attentional deficits appear early during the course of AD pathology [19-20] implicating dysregulation of cortical cholinergic transmission in early AD.

In BF neuronal cultures, the toxic effect of soluble A β appeared more rapidly in the cholinergic axon terminal than in cell bodies [21], exemplifying that disruption in synaptic cholinergic transmission and associated cognitive deficits may precede cholinergic cell loss in AD. A recent postmortem study showed a correlation between the levels of A β oligomers and reductions in choline acetyltransferase (ChAT) activity in the brains of AD subjects [22], substantiating detrimental effects of A β oligomers on the cholinergic system. How A β

oligomers modulate presynaptic cholinergic activity and influence attentional functions in the absence of cholinergic cell loss remains unknown.

The present study was designed to examine the impact of A β oligomers on presynaptic cholinergic transmission in the cortex and attentional functions that depend upon the integrity of these BF cholinergic projections. As aging is a well-recognized risk factor for AD, and the aging cholinergic system is more vulnerable to degeneration [1,23], we also explored how aging might influence the interactions between soluble A β and cortical cholinergic function.

Materials and methods

Animals

Male Wistar rats aged 2-3 months (young) or 10-12 months (middle aged; retired breeders) were acquired from Charles River Laboratories (Malvern, PA, USA). The animals were housed in a temperature- and humidity-controlled facility with a 12-hour light/dark cycle (lights “on”: 7:00AM) and had free access to food and water. Retired breeders were maintained until 22 months of age following which training in an operant attentional task was initiated (see behavioral procedures). Operant training took place 6 days/week. Rats were handled extensively prior to behavioral training and were partially water-deprived by restricting access to a 10-min period in the home cage following each behavioral session. On non-training days, water access was increased to a 30-min period. Rats were individually housed and food was available *ad-libitum* throughout the behavioral training and testing. All experiments were conducted in accordance with the National Institute of Health guidelines and were approved by the Institutional Animal Care and Use Committee at Temple University.

Behavioral training and testing

Apparatus—Rats were trained in operant chambers encased in sound-attenuating boxes, each containing a fan to provide ventilation and low-level background noise (Med Associates Inc., St. Albans, VT). Each chamber was equipped with two retractable levers, a central panel consisting of three panel lights (2.8 W each), a liquid receptacle attached to a water dispenser, and a house light (2.8 W) located on the rear wall. All events including the signal delivery, lever presentations, and water dispense were transmitted using programs written in Medstate notation via SmrtCtrl™ interface running through MED-PC software on a Dell Optiplex 960 computer.

Operant sustained attention task (SAT)—Young and aged rats were trained on an operant sustained attention task (SAT) as described previously [24-27]. Briefly, rats were initially autoshaped on a FR-1 schedule of reinforcement to attain the lever press response and subsequent reward (0.02 mL water). To deter a side bias, lever presses on the dominant lever (i.e. the lever with 5 presses) ceased to be reinforced until the discrepancy was reduced. Once the rats made 120 lever presses within a session, they were moved to the next phase of training, which required discrimination between signal (illumination of the central panel light for 1 s) and non-signal (no illumination) events. Each event was followed by the

presentation of two levers 2 s later; levers remained extended for 4 s or until a lever press occurred. If no response was made during the 4 s lever presentation, an omission was recorded and the intertrial interval (ITI; 12 ± 3 s) was reinstated. On signal trials, a left lever press was scored as a “hit” and rewarded; an incorrect response (depression of the right lever) was deemed a “miss”. During non-signal trials, a right lever press was scored as a “correct rejection” and reinforced, while a left lever press was considered a “false alarm.” The animals were not rewarded for incorrect responses. The presentation of signal and non-signal trials were pseudo-randomized. Half of the animals in a group were trained with the reverse set of rules.

After attaining 70% correct responses to signal and non-signal trials for three consecutive days, animals progressed to the *final* stage of training, during which the duration of signals was decreased to 25, 50, or 500 ms. Moreover, the ITI was reduced to 9 ± 3 s and the house light remained illuminated throughout the session. These events are known to constrain rats' behavior for continuous monitoring of the central panel [27]. Each behavioral session consisted of a pseudo-randomized sequence of 81 signal (27 per signal duration) and 81 non-signal trials (total 162 trials). Sessions were divided into three blocks of 54 trials (27 signal trials and 27 non-signal trials) with each signal type presented 9 times per block. Stable criterion performance was characterized by signal duration-dependent hit rates, 70% hits to 500 ms signals, 70% correct rejections, and a relatively low number of omissions (<10% of all trials; equal distribution among trial types) for at least 3 consecutive sessions. At this stage, animals were exposed to a distractor session (dSAT) that involved the presentation of distractors (flashing house light @ 0.5 Hz) in the second block [28]. This procedure was adopted to minimize the novelty effects of distractors while evaluating the effects of A β and age on performance under challenging conditions. Following the dSAT session, rats resumed training on SAT and were prepared for stereotaxic surgeries (see procedure below) after retaining criterion.

Behavioral measures—The total numbers of hits, misses, correct rejections, false alarms, and omissions were recorded for the entire behavioral session. Each session was analyzed in terms of the relative number of hits ($h = \text{hits}/(\text{hits} + \text{misses})$) for each signal length, and relative number of correct rejections ($cr = \text{correct rejections}/(\text{correct rejections} + \text{false alarms})$). The overall measure of attentional performance was calculated as performance score (SAT/dSAT score) using the formula: $(h - fa)/[2(h + fa) - (h + fa)^2]$ as described previously [25,28]. SAT/dSAT scores vary from +1.0 to -1.0; a score of +1.0 indicated that all responses on signal and non-signal trials were correct, 0 indicated the inability to discern signal from non-signal events, and -1.0 indicated that all responses were misses and false alarms. Performance scores were calculated for each signal duration and for the entire session. Performance measures were also calculated for each task block of 54 trials.

Preparation of A β oligomers—Synthetic A β oligomers were prepared for chronic *in vivo* administration using the hexafluoroisopropanol (HFIP) method [29]. Briefly, lyophilized A β_{1-42} (American Peptide Company, Sunnyvale, CA, USA) was solubilized in HFIP following which sterile water was added to minimize fibril formation. HFIP was evaporated under a gentle stream of nitrogen. The aqueous A β solution was stirred for 48 h to produce

aggregation of A β monomers. As the crosslinking of soluble A β with high density lipoprotein (HDL) prior to oligomer formation reduces the toxic effects of the preparation [30, 31], human HDL (Calbiochem/EMD Millipore, Darmstadt, Germany) was added during the aggregation step. Finally, 1 mM NaHCO₃ (pH 10) was added to the preparation to maintain the stability of oligomers during chronic infusions. The concentration of A β in the final preparation was 200ng/ μ L. A β oligomers were characterized by immunoblotting using a monoclonal 6E10 antibody (Covance, Princeton, NJ, USA) against A β residues 1–16. The blots revealed A β _{1–42} monomers, dimers, trimers, tetramers and large oligomeric assemblies ranging from 30 to > 100 kDa (Fig. 1A).

Stereotaxic surgeries and experimental design—All surgeries were performed under aseptic conditions. The animals were anesthetized with isoflurane (4–5% for induction and 1–2% maintenance dose) using an anesthesia machine (Surgivet, Dublin, OH, USA). Rats were mounted on a stereotaxic frame (Model 962; David Kopf Instruments, Tujunga, CA, USA) and the head was positioned into the head frame using ear bars. An isothermal deltaphase pad (Braintree Scientific, Braintree, MA, USA) was used to consistently maintain animals' body temperature at 37°C throughout the surgical procedure. Ophthalmic ointment was applied to lubricate animals' eyes. Animals' heads were shaved using clippers, and iodine tincture was applied to clean the skin. A 3 cm incision was made in the midline of the scalp. For chronic i.c.v. infusions, osmotic minipumps (Alzet model 1004; DURECT Corporation, Cupertino, CA, USA) were filled with A β oligomers and inserted into the subcutaneous pocket of the midscapular area created toward the animals' back. The brain infusion cannula (DURECT Corporation) connected to the pumps via a catheter was inserted into the right lateral ventricle (AP: –0.3 mm, ML: –1.5 mm, and DV: –3.5 mm). Dental cement was applied on and around the cannula and secured with bone screws. The skin was closed with sterile sutures, and triple antibiotic cream was applied to the surrounding area. Moreover, animals were given injections of an antibiotic (Baytril) and an analgesic (Buprenorphine) to combat any infection and minimize pain. Soluble A β solution was infused at a rate of 0.11 μ L/hr (50 ng/hr) for 4 weeks. For control animals, A β peptide with reverse sequence (A β _{42–1}; control peptide) was infused at a similar concentration.

A schematic illustration of the experimental design is shown in Fig. 1B. Young and aged rats trained to criterion at SAT were randomly assigned into two groups (control peptide or A β oligomer; N = 7–8/manipulation/age). Following surgery, rats were allowed to recover for a period of 72 h and then placed back on task for SAT/dSAT testing. As the major objective of the study was to determine the impact of chronic intracranial administration of soluble A β on attentional capacities and cholinergic signaling, osmotic minipumps remained implanted for four weeks and performance was monitored during this duration. Animals were subjected to a dSAT session on the last day of testing and then either prepared for electrochemical recordings of cholinergic transmission or immunoblot analysis of terminal marker proteins as described below. For final behavioral analysis, SAT data from the last 3 sessions prior to the dSAT testing procedure were averaged. Behavioral data for the dSAT testing session were analyzed separately.

In vivo amperometric recordings—Following the completion of behavioral testing, rats (N = 4/manipulation/age) were prepared for amperometric recordings of depolarization-evoked ACh release using choline-sensitive microelectrodes as described earlier [26, 32, 33]. Ceramic-based platinum microelectrode arrays were enzyme-coated and calibrated for choline sensitivity (see Supplementary Methods and Results). *In vivo* recordings were conducted from the prelimbic region of the medial prefrontal cortex (PFC) from urethane-anesthetized animals placed in a stereotaxic apparatus. Dental cement and the infusion cannula were carefully removed and the skull was thoroughly cleaned with sterile saline prior to microelectrode implantation. Single barrel glass capillaries were pulled using a micropipette puller to attain a tip diameter of ~15 μm and attached to the microelectrodes so that the tip of the capillaries were positioned between the pairs of enzyme-coated and sentinel channels. The spacing between the capillaries and microelectrodes were kept at 70-100 μm . Glass capillaries were filled with KCl (70 mM) that passed through a sterile syringe filter (0.22 μm). The microelectrode/capillary structure was lowered into the right medial PFC (AP: +3.0 mm, ML: -0.7 mm, DV: -2.7-3.0 mm) using a microdrive (MO-10, Narishige International, East Meadow, NY, USA). An Ag/AgCl reference electrode prepared from a miniature silver wire was implanted in the rostral cortical region of the contralateral hemisphere. Amperometric recordings were conducted at 2 Hz by applying a fixed potential of +0.7 V and data was digitized using a FAST-16 potentiostat (Quanteon). Background currents were stabilized for 60 min following which brief pulses of potassium (200 nL) were locally applied to produce rapid depolarization of prefrontal cholinergic terminals. These pulses were applied at 2-10 psi every 2 min through the capillaries via a PTFE tubing connected to a picospritzer (ALA Scientific Instruments, Farmingdale, NY, USA). The *in vivo* capacity of high affinity choline transporters (CHTs) to clear extracellular choline was evaluated by assessing the clearance of choline signals generated by exogenously applied choline (1 mM; 80-100 nL). At the end of recording sessions, animals were perfused and brains were isolated for ChAT immunohistochemistry (below) and for Nissl-staining to verify the placement of infusion cannula into the right ventricle and microelectrode into the right PFC.

Amplitudes of choline signals were calculated by recording peak change in current over baseline values and dividing the difference by choline sensitivity obtained from *in vitro* calibration. Choline uptake rate was used as a measure of *in vivo* CHT capacity and calculated from the slope of the linear regression of data points on the clearance curve corresponding to the decline between 40 and 80% of the peak amplitude. Signals of similar amplitudes (10-15 μM) were compared in all animals [33]. Currents recorded via enzyme-coated sites were self-referenced by subtracting currents recorded on sentinel sites [26, 34, 35] in cases when background noise levels on enzyme-coated channels exceeded 10 pA or pressure-ejection artifacts occurred. The averages of two responses/drug manipulation/animal were used for statistical analysis.

ChAT Immunohistochemistry—The animals were transcardially perfused using 100 mL of ice-cold 0.1 M phosphate-buffered saline (PBS) followed by 300 mL of 4% paraformaldehyde (PFA; pH 7.4-7.6). Isolated brains were post-fixed overnight in PFA and then transferred to 30% sucrose (in 0.1 M PBS) for 72 h. Coronal sections (50 μm) were

taken on a freezing microtome (SM2000R, Leica, Wetzlar, Germany) and the slices were stored in cryoprotectant solution (15% glucose, 30% ethylene glycol, and 0.04% sodium azide in 0.05 M PBS) at -20°C until further processing.

Brain slices from the BF (nBM/SI region) were processed for ChAT immunostaining to examine morphological changes in BF cholinergic neurons based on the previous protocol [26]. Briefly, three serial sections collected every 500 μm from the rostral-caudal axis spanning 1 mm (0.9 mm – 1.9 mm posterior to bregma) were thawed and rinsed in 0.05 M Tris-buffered saline (TBS). The sections were blocked in 10% donkey serum for 1 h and then incubated with goat anti-ChAT antibody (1:100 dilution; EMD Millipore) overnight. Following washes in TBS with 0.1% Triton, sections were incubated with biotinylated donkey anti-goat IgG (1:1000) for 2 hrs. The staining was developed by incubating the sections in streptavidin-HRP followed by 3-3'-diaminobenzidine (DAB).

ChAT expression in the medial PFC was also examined to determine the impact of age and manipulations on cortical cholinergic fiber density. For this analysis, serial coronal sections (AP: +3.2 – +2.8 mm) that alternate with those taken for the placement of the microelectrode were used to sample cholinergic fibers from the prelimbic region. The staining protocol remained similar to what is described above for cholinergic neurons except that the secondary antibody dilution was 1:200. Stained sections were mounted on gelatin-coated slides, air dried, dehydrated and coverslipped with DPX.

All sections were visualized using a Leica brightfield/fluorescent microscope (DM4000B) equipped with DFC 425C digital camera and Leica Application Suite software (Leica Microsystems Inc.). Images were acquired at 400x magnification in the brightfield mode and processed for semi-quantitative analysis. Morphometric analysis of BF cholinergic neurons was conducted by measuring the cross-sectional area of soma using NIH *Image J*. ChAT-immunopositive cells from nBM/SI region labeled with DAB were randomly selected from both hemispheres. Cell borders were marked and the area of the cell was automatically calculated by the program based on the length (in pixels) and area (pixel^2) of the user-defined border. The cross-sectional area was converted from pixel^2 to μm^2 based on the scaling at 400x magnification (12.09 $\text{pixels}/\mu\text{m}$). Fifty cells were analyzed from three sections per animal. Prefrontal ChAT density was analyzed from cortical layers III/V using a grid counting procedure [25, 26, 28]. The threshold level of images was adjusted to maximize visualization of ChAT-immunopositive fibers using a macro created in Adobe Photoshop CS4. Fiber counts were made in the area of 40,000 μm^2 using 2500 μm^2 grids. ChAT-positive counts were based on the average counts from three sections per animal.

Western blotting—The changes in the expression of presynaptic cholinergic markers, CHT and vesicular ACh transporter (VAChT), in the PFC were examined using immunoblotting. Animals ($N = 3\text{--}4/\text{group/manipulation}$) were decapitated under urethane anesthesia and prefrontal cortices were isolated. The caudal region was snap frozen and utilized for histological verification of i.c.v. cannula placement. Samples containing pooled cortical tissues from both hemispheres were homogenized in a glass tissue grinder using 1 mL of ice-cold extraction buffer (50 mM HEPES NaOH, pH 7.4, 0.32 M sucrose, 5mM MgCl_2 , 0.2 mM EDTA, 0.2 mM EGTA, 1% Triton X-100 and protease inhibitor cocktail).

The homogenates were kept on ice for 30 min following which they were centrifuged at $13000 \times g$ for 15 min to obtain tissue lysates. Supernatants were stored at -80°C until analyzed. Protein concentrations were determined by using a modified Lowry Protein Assay (Pierce, Rockford, IL). Equal quantities (25 μg) of protein were subjected to immunoblot analysis and each sample was assayed in duplicate. Proteins were separated on 4-15% Tris HCl polyacrylamide gels and transferred to PVDF membranes. Following blocking in 5% non-fat dry milk, membranes were incubated with primary antibodies (EMD Millipore Co.: mouse anti-CHT monoclonal antibody clone 62-2E8, Santa Cruz Biotechnology Inc.: rabbit anti-VACHT H-160) overnight on an orbital shaker at 4°C . Membranes were washed with 0.01M PBS containing 0.1% Tween and incubated with HRP-conjugated sheep anti-rabbit antibody (GE Healthcare). Blots were visualized for chemiluminescence detection using ECL Advance system (GE Healthcare Biosciences, Pittsburgh, PA) and images were captured using Molecular Imager Chemidoc EQ system (Bio-Rad, Hercules, CA). All membranes were stripped in Restore Plus buffer (Pierce) and reprobed with mouse anti- β -tubulin antibody to detect β -tubulin that served as a gel-loading control. Densitometric analysis was performed by calculating the integrated pixel densities using NIH ImageJ software. Blot densities of CHT and VACHT were normalized to the levels of β -tubulin-immunoreactive bands for each sample analyzed.

Statistics—Statistical analyses were performed using SPSS/PC+ version 21.0 (IBM-SPSS, Armonk, NY, USA). Behavioral data for hits, correct rejections and SAT/dSAT scores were analyzed using mixed factor repeated-measure ANOVAs with block (3 levels) and/or signal duration (3 levels) as within-subject variables, and A β manipulation (2 levels) and age (2 levels) as between-subject variables. Response latencies and omissions were analyzed using 2x2 between-subject design ANOVAs. When appropriate, one-way ANOVAs and Fisher's least significant difference (*LSD*) tests were used for *post hoc* comparisons to determine the source of interactions. The effects of age and manipulation on cortical ACh release, choline clearance capacity, cholinergic cell cross-sectional area, cortical ChAT fiber density, CHT and VACHT expression were analyzed using 2x2 ANOVAs. All *post hoc* comparisons were conducted using Fisher's *LSD* test corrected for multiple comparisons. A cut-off *p* value of 0.05 was considered statistically significant. Pearson's *r* was calculated for all correlational analysis. Exact *p* values were reported as recommended by [36].

Results

Pre-surgery performance of young and aged rats

Aged rats required more training sessions to reach criterion in SAT as compared to the young animals (aged: 70.15 ± 5.93 sessions; young: 38.63 ± 2.57 sessions). However, SAT performance after the attainment of criterion and prior to surgeries, remained comparable between young and aged rats (average SAT scores: young = 0.49 ± 0.05 , aged = 0.43 ± 0.04 ; $F_{1,28} = 1.01$; $p = 0.32$). To ensure that no differences in baseline performance exists between the treatment groups, the behavioral measures were also compared for animals allocated to the control peptide and soluble A β_{1-42} groups. Performance on both signal and non-signal trials did not differ between the two groups (hits: $F_{1,28} = 0.84$; $p = 0.37$; correct rejections: $F_{1,28} = 1.18$; $p = 0.29$). No significant group differences were observed in the SAT scores

($F_{1,28} = 1.18$; $p = 0.29$). Additionally, omissions remained low and similar in both groups (control: $0.84 \pm 0.07\%$; A β_{1-42} : $2.17 \pm 0.92\%$; $F_{1,28} = 2.07$; $p = 0.16$). These data confirm that attentional performance prior to the implantation of the osmotic pump for chronic i.c.v. infusions remained similar in all groups.

A β oligomers produced subtle decrements in SAT performance irrespective of age

The main results on the effects of chronic intracranial infusions of soluble A β in young and aged rats on SAT performance are summarized in Fig. 2. The overall performance measure expressed as SAT scores was signal-duration dependent, indicating that animals detected longest duration signals more reliably than the shorter duration signals (main effect of signal: $F_{2,52} = 133.02$; $p < 0.001$; see multiple comparisons in Fig. 2A). Mixed-factor ANOVAs yielded a significant manipulation $\times$ signal $\times$ block interaction ($F_{4,104} = 3.55$; $p = 0.009$). To determine the source of these interactions, one-way ANOVAs for each signal and block were conducted. This analysis indicated that the ability to discern 500 ms and 25 ms signal from non-signal events marginally but significantly declined in the A β -infused rats as compared to the control animals in the second and third block of trials respectively (block 2, 500 ms: $F_{1,28} = 4.49$; $p = 0.04$; block 3, 25 ms: $F_{1,28} = 4.37$; $p = 0.05$; Fig. 2C, D). Absence of any differences in the SAT scores in the first block of trials (all signals; $p > 0.45$; Fig. 2B) may indicate that A β oligomers interfered with the ability to sustain performance over longer periods of time. Although performance of aged animals appeared to be more variable, statistical analysis neither revealed a main effect of age ($F_{1,26} = 3.66$; $p = 0.08$) nor a 4-way interaction (age $\times$ manipulation $\times$ signal $\times$ block: $F_{4,104} = 0.91$; $p = 0.46$) for SAT scores. These data illustrate that aging *per se* did not robustly affect SAT performance. As the reduction in the index of overall attentional performance could either reflect decreases in response accuracy for signal trials (hits) or increases in claims for signal in non-signal trials (false alarms), we further analyzed performance of animals in signal and non-signal trials.

As expected, the proportion of hits varied significantly between each signal duration ($F_{2,52} = 138.20$; $p < 0.001$; Fig. 2E) but this measure did not differ across blocks ($F_{2,52} = 1.38$; $p = 0.26$). However, the effects of manipulation on response accuracies in signal trials interacted with signal duration and block (manipulation $\times$ signal $\times$ block: $F_{4,104} = 3.40$; $p = 0.01$). Infusions of A β oligomers marginally affected hit rates in the second and third block of the 54-trial sessions and these effects were observed on the highest and lowest signal durations (500 ms: $F_{1,28} = 5.42$; $p = 0.03$; 25 ms: $F_{1,28} = 4.82$; $p = 0.04$; both control peptide vs. A β oligomers; Fig. 2G,H). Hit rates did not differ between young and aged rats as evidenced by lack of age $\times$ manipulation $\times$ signal $\times$ block interaction ($F_{4,104} = 0.87$; $p = 0.48$). Behavioral data was further examined to determine whether manipulation-induced changes in hit rates on longest vs. shortest signals in the second and third block occurred due to an altered behavioral strategy. We observed a positive correlation between 500 ms hits in block 2 and 25 ms hits in block 3 for aged A β but not young A β rats (aged A β : Pearson's $r = 0.86$; $p = 0.01$; young A β : Pearson's $r = 0.14$; $p = 0.75$). This may indicate that the detrimental performance effects observed in block 2 of aged A β animals translated to block 3 presumably due to higher response bias towards long duration signals. However, reduced hit rates in block 2 vs. 3 for different signal durations remained totally random in young A β animals. To derive additional information on the time course of A β -induced attentional

deficits, behavioral data from the third block was consolidated into bins of 3-day averages and performance from the first and fourth weeks after manipulation was assessed. We did not find any age-related differences or the effects of manipulation at the earlier time point (age: $F_{1,26} = 0.57$; $p = 0.45$; manipulation: $F_{1,26} = 0.05$; $p = 0.83$). However, a significant effect of A β oligomers on hits to 25ms signals at a later time point emerged ($F_{1,26} = 5.16$; $p = 0.03$) indicating that A β -induced performance decrements presumably occurred as consequence of sustained interactions of this toxic protein with the presynaptic cholinergic signaling (see Discussion). Again, the main effect of age on hits at this time point were not observed ($F_{1,26} = 0.79$; $p = 0.38$) further suggesting that these impairments were not age-sensitive.

The performance on non-signal trials measured as correct rejections remained stable over blocks of trials ($F_{2,52} = 1.74$; $p = 0.19$; Fig. 2I). Moreover, this measure was neither affected by A β oligomers nor age (main effect of manipulation: $F_{1,26} = 0.04$; $p = 0.85$; main effect of age: $F_{1,26} = 2.09$; $p = 0.16$; manipulation $\times$ age $\times$ block interaction: $F_{2,52} = 0.30$; $p = 0.59$). In conjunction with data on SAT scores, these results indicate that chronic infusions of A β oligomers produce marginal decrements in attentional performance irrespective of the age of animals, and these impairments primarily reflect deficits in signal processing during the later stages of the task. The response latencies for performance on signal and non-signal trials in SAT did not differ between the two manipulations (signal: $F_{1,26} = 2.97$; $p = 0.10$; non-signal: $F_{1,26} = 0.59$; $p = 0.45$) and age groups (signal: $F_{1,26} = 0.20$; $p = 0.66$; non-signal: $F_{1,26} = 0.67$; $p = 0.42$; Fig. 2J, K). Moreover, the two factors did not interact (both $p > 0.45$). The rate of omissions remained substantially low and comparable in all groups (manipulation: $F_{1,26} = 0.29$; $p = 0.60$; age: $F_{1,26} = 3.05$; $p = 0.09$; manipulation $\times$ age: $F_{1,26} = 0.001$; $p = 0.98$; Fig. 2L). These data reflect that motivation to perform and attain goals in SAT was neither affected by A β oligomers nor age.

Performance under conditions of high attentional load reveals interactions between aging and soluble A β

Attentional performance under distracting conditions (dSAT) was assessed by presenting visual distractors through a flashing houselight (see Methods) in the second block of trials. This procedure recruits top-down prefrontal cholinergic mechanisms to maintain goal-directed behavior and performance under conditions of high attentional load [37, 38]. The dSAT scores differed significantly across blocks ($F_{2,52} = 11.12$; $p < 0.001$). Overall dSAT performance declined in the distractor block as compared to pre- and post-distractor blocks (*LSD*: $p < 0.001$ block 2 vs. block 1; $p = 0.01$ block 2 vs. block 3; Fig. 3A) indicating that the presentation of distractors did indeed place a toll on rats' attentional capacities. Moreover, the performance following distractors recovered in the post-distractor block but did not reach the levels as observed in the pre-distractor block ($p = 0.03$ block 3 vs. block 1). Repeated-measure mixed-model ANOVAs on dSAT scores did not show a main effect of signal ($F_{2,52} = 0.19$; $p = 0.82$) or manipulation ($F_{1,26} = 1.91$; $p = 0.17$). However, dSAT scores differed significantly between the young and aged rats, and there was a 4-way interaction (main effect of age: $F_{1,26} = 8.27$; $p = 0.008$; signal $\times$ block $\times$ manipulation $\times$ age: $F_{4,104} = 2.56$; $p = 0.04$). Attentional performance in the pre-distractor block followed a pattern similar to SAT scores with marginal decrements in both young and aged A β groups

(Fig. 3B); however one-way ANOVAs on dSAT scores conducted for all signals did not reveal any significant group differences (all $p > 0.05$). dSAT scores declined in a parallel fashion in all groups during the distractor presentation, indicative of a generalized decline in performance under taxing conditions (all $p > 0.16$; Fig. 3C). However, recovery performance differed significantly between groups for higher signal durations in the post-distractor block (dSAT 500 ms: $F_{3,26} = 5.99$; $p = 0.003$; dSAT 50 ms: $F_{3,26} = 3.58$; $p = 0.02$). dSAT scores remained substantially lower in the aged A β group (see multiple comparisons in Fig. 3D). A β infusions did not impact performance of young rats in block 3 ($p > 0.23$ vs. young control for all signal durations). However, dSAT scores for 500 ms signal were significantly lower in aged rats infused with the control peptide than young controls indicative of moderate age-related impairments under conditions of extreme attentional load ($p = 0.006$). dSAT scores for the lowest signal duration (25 ms) remained similar and near chance levels for all groups (Fig. 3D).

The proportion of hits differed significantly across blocks ($F_{2,56} = 23.97$; $p < 0.001$; Fig. 3E); correct responses on signal trials decreased dramatically in the distractor block ($p = 0.007$ vs. pre-distractor block). However, unlike dSAT scores, hit rates did not recover and even worsened in the post-distractor block (block 3 vs. block 2 / block 1, both $p < 0.001$). Similar to the analysis of SAT performance, the proportion of hits remained signal-dependent on the dSAT phase of the task ($F_{2,52} = 80.06$; $p < 0.001$). Moreover, there was a significant 4-way interaction on this measure (signal $\times$ block $\times$ age $\times$ manipulation: $F_{4,104} = 2.52$; $p = 0.04$). To determine the source of these interactions, separate one-way ANOVAs were conducted for signal durations within each block. Hit rates remained comparable for all signal lengths in the pre-distractor block (all $p > 0.05$; Fig. 3F). However, A β infusions in aged rats interfered with accuracies on 500 ms signal duration trials during the presentation of the flashing house light (one-way ANOVA: $F_{3,26} = 3.51$; $p = 0.02$; *LSD*: $p = 0.004$ vs. young control; $p = 0.03$ vs. aged control; Fig. 3G). Moreover, significant group differences in hit rates in the post-distractor block primarily occurred due to poor recovery in these animals (500 ms: $F_{3,26} = 4.84$; $p = 0.008$; 50 ms: $F_{3,26} = 3.52$; $p = 0.03$; refer Fig. 3H for *post hoc* comparisons). The performance on non-signal trials was also affected significantly by blocks, with reduced correct rejections in block 2 (main effect: $F_{2,52} = 29.74$; $p < 0.001$; *LSD*: $p = 0.001$ vs. block 1; Fig. 3I). These data indicate that, in general, animals made more claims for signals in non-signal trials during the distractor presentation. Correct rejections recovered completely and in fact, showed a rebound increase in block 3 ($p < 0.001$ vs. block 2 and block 1). These observations explain the partial recovery of dSAT scores despite worsening of hit rates in the post-distractor block (above). The performance on non-signal trials remained unaffected by age ($F_{1,26} = 3.65$; $p = 0.07$) or manipulation ($F_{1,26} = 1.39$; $p = 0.25$), and the 3-way interaction remained insignificant (block $\times$ age $\times$ manipulation: $F_{2,52} = 0.17$; $p = 0.85$; Fig. 3J-L). Collectively, our dSAT data reflect that soluble A β produced more pronounced attentional deficits in aged rats under conditions of an elevated cognitive load, and that these deficits are primarily associated with the inability to detect signals.

Cortical ACh release and choline clearance capacity

Phasic (transient; second-based) increases in ACh release in the PFC mediate the detection of attention-demanding stimuli [39-41]. The capacity of prefrontal cholinergic synapses to

produce phasic increases in cholinergic activity was probed by locally applying rapid potassium pulses [26, 28, 32]. As illustrated in Fig. 4A-C, the amplitudes of depolarization-evoked cholinergic transients were significantly reduced by age (main effect: $F_{1,12} = 5.99$; $p = 0.03$) and by manipulation (main effect: $F_{1,12} = 13.43$; $p = 0.003$). However, age-related attenuation of cholinergic signal amplitudes did not interact with the manipulation (age $\times$ manipulation interaction: $F_{1,12} = 0.69$; $p = 0.42$).

The ability of cholinergic synapses to clear choline from the extracellular space *in vivo* was calculated as uptake rate by analyzing the hemicholinium-sensitive component of the clearance curve as described earlier [33, 42]. Exogenous choline was applied locally in the PFC to produce similar amplitudes (see Methods) for comparison of clearance kinetics, as uptake rates may vary as a function of amplitudes [33, 42]. As expected, choline signal amplitudes did not differ by age ($F_{1,12} = 0.01$; $p = 0.90$) or manipulation ($F_{1,12} = 2.44$; $p = 0.14$), and there was no interaction between the two factors ($F_{1,12} = 0.10$; $p = 0.75$). The kinetics of choline signals following exogenous application of choline in young and aged animals that received either control peptide or A β infusions are exemplified in Figures 4D and E. A two-way ANOVA on choline uptake rates yielded a main effect of age ($F_{1,12} = 5.39$; $p = 0.03$; Fig. 4F). Moreover, A β oligomers significantly reduced choline uptake rate ($F_{1,12} = 11.31$; $p = 0.006$); however, the effects of age and manipulation did not interact ($F_{1,12} = 0.34$; $p = 0.57$). Taken together, these data indicate that A β infusions reduced clearance of exogenously-applied choline and this effect may possibly involve a disruption of CHT function (see below).

Considering the low sample size in these experiments, it was hard to conclude whether the effects of age and A β manipulations on depolarization-evoked ACh release and choline uptake rates were either potentially additive or synergistic. Although we did not find significant interactions between age and A β on the indices of presynaptic cholinergic function, these data should be treated cautiously as neurochemical changes appeared to be unparalleled between young and aged animals. Notably, a steeper reduction in these cholinergic measures was observed in A β -infused aged rats as compared to young rats (ACh: 50% reduction in aged vs. 30% in young; choline uptake rates: 40% reduction in aged vs. 24% in young; Fig. 4C and F). Therefore, the possibility of a synergistic interaction occurring between chronic A β infusions and age, and its relation to some of the behavioral effects observed in the present study could not be entirely dismissed. Importantly, we found positive correlations between cholinergic measures and post-distractor performance on signal trials (ACh: Pearson's $r = 0.51$; $p = 0.04$; choline uptake rate: Pearson's $r = 0.57$; $p = 0.02$) which led us to speculate that the interaction effects between soluble A β and age on performance deficits under conditions of higher attentional load (above) were possibly linked to disruption in presynaptic cholinergic mechanisms.

Age-related reduction in cholinergic soma size and presynaptic cholinergic markers

Figure 5A illustrates ChAT-immunoreactive neurons from the nBM/SI region of young and aged rats that received chronic i.c.v. infusions of the control peptide and soluble A β oligomers. Morphometric analysis indicated marked reduction in the cross-sectional area of BF cholinergic neurons in aged animals (main effect: $F_{1,12} = 9.52$; $p = 0.009$; Figure 5A).

However, A β infusions did not impact cholinergic cell size ($F_{1,12} = 0.83$; $p = 0.38$) and the two factors did not interact ($F_{1,12} = 0.02$; $p = 0.88$). The integrity of cortical cholinergic inputs was assessed by determining the density of ChAT-positive fibers in the PFC. Likewise, aging produced a robust reduction in prefrontal cholinergic fibers ($F_{1,12} = 26.37$; $p < 0.001$; Figure 5B) and the interaction between age and manipulation remained insignificant ($F_{1,12} = 0.56$; $p = 0.46$). However, there was a trend for reduced cholinergic fibers by manipulation ($F_{1,12} = 4.16$; $p = 0.06$) that might have stemmed from lower ChAT-fiber densities in the aged rats infused with A β oligomers (86.5 ± 11.74) as compared to young rats (143.5 ± 7.64).

The effects of age and A β oligomers on the integrity of cortical cholinergic terminals were also assessed by immunoblot analysis of presynaptic proteins CHT and VACHT. There was a trend for reduced expression of cortical CHTs with age (Fig. 5C); however, this effect did not reach significance (CHT: $F_{1,10} = 4.62$; $p = 0.06$). Moreover, chronic A β did not exert any effect on CHT expression ($F_{1,10} = 0.58$; $p = 0.46$) and the effects of age and A β on CHT did not interact ($F_{1,10} = 0.05$; $p = 0.83$). These findings were surprising as we noted reduced choline uptake rates in rats infused with chronic A β (see amperometry data above). Because the mobilization of intracellular pools of CHTs to the presynaptic membrane maintains sustained increases in cholinergic transmission [43, 44], we explored the effects of A β oligomers on subcellular CHT distribution in potassium-stimulated synaptosomes (Supplementary Methods). Incubation of cortical synaptosomes with soluble A β reduced potassium-stimulated increases in surface CHT densities (Supplementary Results), indicating that reduced choline clearance observed in A β -infused rats might result from reduced surface trafficking of CHTs. The expression of VACHT examined in the homogenates prepared from the PFC show a reduction with age ($F_{1,10} = 5.71$; $p = 0.03$) but no effect of the manipulation ($F_{1,10} = 1.33$; $p = 0.27$; Fig. 5D). Moreover, age-related reduction in VACHT did not interact with soluble A β infusions ($F_{1,10} = 0.77$; $p = 0.40$).

Discussion

Atrophy of the medial temporal lobe and mnemonic deficits are prominent features of AD. However, brain regions mostly associated with attentional information processing are known to be affected at the earlier stages in this pathology [19, 45], and are proposed to contribute to progressive impairments in episodic memory and executive functions [6, 46]. Therefore, it is important to delineate neuronal mechanisms for attentional dysfunction in AD. The prefibrillar, soluble oligomeric forms of A β are hypothesized to be the underlying cause of synaptic dysfunction and cognitive decline during the earlier course of AD pathology [18, 47]. Extensive evidence from *in vitro* and *in vivo* studies indicate that natural and synthetic forms of A β oligomers disrupt synaptic plasticity, produce loss of synapses and interfere with learning and memory [14, 15, 48-50]. Moreover studies involving the transgenic animal models of AD and post-mortem studies from human subjects diagnosed with AD pathology indicated correlations between soluble A β and cognitive impairments [51-55]. These studies supported the notion that accumulation of A β oligomers triggers cognitive decline by producing detrimental effects on synaptic function. However, to our knowledge there is no study that systematically assessed the effects of A β oligomers on attentional functions and cortical cholinergic inputs that are critical for maintaining attentional capacities.

In the present study, chronic intracranial infusions of synthetic A β ₁₋₄₂ oligomers produced subtle decrements in SAT performance. These attentional deficits were mostly associated with impaired ability of rats to detect signals over time on task. However, SAT performance did not differ between young and aged animals indicating that aging *per se* did not influence the ability to sustain attention in a well-practiced task under normal conditions. These data are consistent with the previous studies that did not find robust deficits in SAT performance or steeper vigilance decrement in aged rats as compared to the young rats [26, 56, 57]. We did observe a slower task acquisition in aged rats that might either reflect reduced learning abilities or exploring different strategies to perform the task during the initial phases of training. The interactions between age and A β oligomers became prominent as the attentional load increased on task via presentation of visual distractors (dSAT). Chronic i.c.v. infusions of oligomeric A β produced profound attentional deficits under distracting conditions in aged rats as compared to young animals. Moreover, post-distractor performance recovery in these animals remained extremely low primarily due to reduced hit rates but not correct rejections. As cortical cholinergic inputs are critical for the optimization of the signal-driven cognitive performance, our findings indicate that detrimental effects of A β oligomers on attentional capacities, which are more pronounced in aged rats, may reflect dysregulation of presynaptic cholinergic transmission (as discussed below). Surprisingly, the performance of young A β rats did not differ from age-matched controls in the second and third block of the dSAT session. It is possible that distractor presentation taxed cognitive resources in a parallel fashion in both groups overriding any detrimental effects that might have been related to marginal decrement in bottom-up processing in A β young animals, as previously noted in SAT performance (see Results).

It is well established that attentional performance is associated with increases in cortical cholinergic transmission [4, 58-60]. Recent evidence from electrochemical studies suggests that transient (phasic; over the time scale of seconds) increases in ACh release in the PFC foster attentional performance by switching from a default response mode to the detection mode [39-41]. Moreover, prefrontal cholinergic inputs also recruit efferent circuitry to mediate top-down effects under conditions of higher attentional load and the magnitude of ACh release varies as a function of demands on attention [25, 37, 38]. Therefore, reduced depolarization-evoked cholinergic transients in the PFC of aged rats exposed to A β oligomers may suggest that the ability of presynaptic cholinergic synapses to sustain phasic cholinergic transmission under attention-demanding conditions was compromised in these animals. These data are consistent with previous studies that show that the aging cholinergic system is more vulnerable to insults produced either by partial deafferentation of the cortical cholinergic inputs [56] or reduced nerve growth factor (NGF) signaling via tropomyosin-related kinase A (trkA) receptor [26].

Chronic A β did not seem to strongly impact the amplitudes of phasic cholinergic signals in young rats as opposed to the aged rats that show a robust decline in signal amplitudes. These findings were intriguing as we did observe a main effect of manipulation but not age on SAT performance. Tonic increases in cortical ACh release occurring on the time scale of minutes is known to influence attentional performance by regulating arousal states [39, 40]. Although the effects of A β oligomers on tonic cortical cholinergic transmission were not

evaluated in this study, it remains a possibility that subtle decrements in baseline attentional performance occurred due to lower tonic ACh in both young and aged rats.

The transport of choline into the presynaptic cholinergic terminals via CHT dictates the rate of ACh synthesis and release [5, 43, 61, 62]. In the current study, the capacity of cholinergic synapses to clear exogenously applied choline, assessed by estimating the uptake rate from the CHT-mediated component of the choline clearance curve, declined in both soluble A β -exposed young and aged rats. The detrimental effects of soluble A β on choline uptake rates were more pronounced in aged rats indicating that severely compromised CHT function in these animals might have put a toll on cortical cholinergic transmission resulting in attentional impairments under distracting conditions. Hit rates remained unaffected during the dSAT task in aged control and young A β animals presumably because phasic cholinergic signals in the PFC were maintained and did not alter in these animals. Although it remains unclear how CHT-mediated choline uptake influences phasic and tonic components of cholinergic transmission, it is possible that a moderate decline in CHT capacity only influences the slowly occurring tonic cholinergic transmission but not phasic cholinergic signaling. These alterations may be sufficient to produce subtle performance decrements in SAT by reducing cortical arousal as discussed earlier. However, generalized weakening of the cholinergic system in aging (see below) makes presynaptic terminals more vulnerable to the toxic effects of A β oligomers, thereby dramatically impacting choline uptake to the extent that these terminals cannot produce the sustained increases in phasic ACh release required to maintain performance under distracting conditions.

Cholinergic transmission and choline uptake is regulated by the trafficking of CHTs [43, 62]. Attentional performance increases the translocation of prefrontal CHTs from intracellular presynaptic compartments to the synaptosomal plasma membrane [63]. Furthermore, CHT hemizygous mice display attentional impairments and an inability to maintain cholinergic transmission due to reduced intracellular pools available for trafficking [44]. Although, we did not study how chronic A β oligomers would influence CHT trafficking under *in vivo* conditions, soluble A β exposure of isolated cortical synaptosomes reduced the mobilization of intracellular CHTs to the membrane surface under stimulating conditions (supplementary information). Therefore, it is plausible that SAT performance decrements in A β oligomer-infused young and aged rats occurred primarily due to detrimental effects of these synaptotoxic proteins on performance-associated changes in subcellular CHT distribution. A β oligomers are shown to interact with the extracellular domain of p75, the low-affinity NGF receptor, and A β -induced neuritic degeneration was reversed in BF cholinergic neuronal cultures prepared from p75 knockout mice [64]. As the over-activation of p75 signaling is linked to cholinergic dysregulation in AD and A β -reduced choline uptake [65], it is conceivable that extracellular oligomeric A β may produce aberrant CHT trafficking by activating p75-mediated downstream signaling pathways and disrupt cortical cholinergic transmission and attentional capacities. Recent evidence suggests that the A β peptide could be internalized into the BF cholinergic neurons via a p75 receptor-dependent mechanism [66]. Thus, we could also not rule out that soluble A β -mediated disruption of choline transport and CHT function result from intracellular accumulation of these synaptotoxic oligomers. Future research is warranted to further explore oligomeric A β

and CHT interactions in the *in vivo* system and how they impact presynaptic cholinergic function.

We observed shrinkage of cholinergic cell soma and lower levels of presynaptic cholinergic proteins (ChAT, VAcHT and CHT) in aged rats. It is important to note that normal aging is not associated with cholinergic cell loss [26]; however, variable age-related changes in the morphological appearance of cholinergic neurons have been reported [67]. Therefore, reduced expression of presynaptic cholinergic markers might actually represent lower expression of these proteins rather than terminal loss. Surprisingly, chronic intracranial delivery of soluble A β did not seem to affect cholinergic cell size and presynaptic proteins in both young and aged rats. These data, in conjunction with our findings from behavioral and electrochemical recording experiments, suggest that excessive slowing of CHT-mediated choline uptake and reduced depolarization-evoked cholinergic transients in soluble A β -infused aged rats may possibly be linked to a synergism between interference in CHT function triggered by A β oligomers and a generalized age-related reduction in the expression of presynaptic cholinergic proteins required for ACh synthesis and release. Lack of effect on phasic ACh release in the presence of moderate reduction in CHT function in young A β -infused rats further support our interpretation. These data are in alignment with our behavioral findings that show dissociation between age-related attentional performance effects of chronic A β infusions under conditions of higher cognitive load. On the other hand, stable attentional performance in normal aged rats with a weakened cholinergic system is indicative of the presence of plastic presynaptic cholinergic mechanisms that maintain cholinergic transmission. These mechanisms may be disrupted by soluble A β , consequently producing attentional deficits as observed in AD patients.

One of the limitations of this study could be the use of soluble A β preparations generated from the synthetic peptide and not the pathological form of A β oligomers known to exist in AD. Moreover, our A β preparation was also found to contain monomers that may possess some neuroprotective activity [68]. Therefore, it is possible that accumulation of A β oligomers in AD pathology may exert more detrimental effects on presynaptic cholinergic function than what is reported in this study. Future studies are warranted to determine the consequences of A β oligomers that trigger AD pathogenesis on the cholinergic system and attention.

In conclusion, the presented evidence suggests that accumulation of soluble oligomeric forms of A β interfere with presynaptic cholinergic mechanisms in the cortex by primarily disrupting choline uptake mechanisms, thus impeding rats' ability to sustain attention with time on task. Moreover, age-related weakening of the corticopetal cholinergic neurons and reduction in presynaptic proteins required for ACh synthesis, vesicle loading, and choline uptake produce synergistic detrimental effects in interaction with soluble A β to robustly affect performance under attention-demanding conditions. These data have major implications for understanding the neurobiology of attentional impairments in early AD. Therapeutic strategies aimed at reducing the levels of insoluble fibrillar forms of A β have proven ineffective in ameliorating cognitive decline in clinical trials [69]. Therefore, development of drugs targeting A β oligomers may hold promise to restore presynaptic cholinergic transmission and decline in attentional capacities in early AD. As abnormal tau

phosphorylation is also induced by A β oligomers [70-72], and this pathological feature is also linked to cholinergic atrophy during the later stages of AD [73], therapeutic approaches that detoxify oligomeric A β may also offer tremendous potential to slow down the disease progression and cholinergic dysfunction with age.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Soluble A β produced subtle decrements in SAT performance irrespective of age
- Performance under distracting conditions robustly declined in A β -infused aged rats
- Soluble A β disrupted the capacity of cholinergic synapses to clear choline
- Depolarization-evoked ACh release declined in aged rats infused with A β oligomers.
- Aging but not soluble A β reduced the expression of presynaptic cholinergic proteins

Figure 1.

Composition of synthetic Aβ oligomers and the experimental design. (A) The soluble Aβ1-42 preparation used for chronic infusions consisted of monomers, dimers, trimers, tetramers, and a series of large oligomeric peptides as detected by 6E10 antibody using western blotting. (B) Rats trained to criterion in the sustained attention task were prepared for chronic administration of Aβ oligomers. Oligomers or control peptide (Aβ42-1) was chronically administered into the ventricles using an implanted cannula connected to a mini-osmotic Alzet pump that released oligomeric Aβ solution (50 ng/hr) for 28 days. Attentional performance was assessed throughout this period following which the animals were prepared either for amperometric recordings or immunoblotting studies.

Figure 2.

Effects of chronic intracranial infusions of Aβ oligomers on SAT performance in young and aged rats. Average SAT scores (A) and hits (E) were signal duration-dependent across all groups. Segmenting average SAT scores by block demonstrated no difference between groups in the initial block (B). However, a significant decrease in SAT scores of the Aβ groups compared to the controls was noted specifically on the 500 ms signal in block 2 (C) and the 25 ms signal in block 3 (D). A similar pattern was observed when analyzing only the performance on signal trials (%hits; F-H). Overall SAT scores and hit rates did not differ between young and aged rats. Performance on non-signal trials, as demonstrated by correct rejections, did not vary across block or groups (I). Response latencies did not differ between groups for either signal (J) or non-signal trials (K). Omissions remained relatively low (< 10%) and were similar between the two age groups and manipulations (L). All data are mean ± SEM. (main effect of signal: ***, $p < 0.001$; *post hoc* comparisons: +, $p < 0.05$)

Figure 3.

Attentional performance under distracting conditions. dSAT sessions consisted of the presentation of visual distractors (flashing house light @ 0.5 Hz) during block 2 of the session, as indicated by green shading, while block 1 and block 3 were similar to normal SAT conditions. (A) dSAT scores declined significantly during the distractor block and rebounded slightly during block 3. (B) As observed in SAT, the experimental groups did not differ by signal duration in block 1. (C) During the distractor block, decreases in dSAT scores were observed across all groups and all signal durations. (D) However, in the post-distractor block, interactions between signal duration, A β treatment, and age were observed. dSAT scores were significantly lower in aged rats infused with the control peptide on 500 ms signal as compared to young animals. Chronic infusions of soluble A β produced robust impairments in aged rats on both 500 ms and 50 ms signals in block 3. Conversely, no significant effect of A β was observed in young rats. dSAT performance for the 25 ms signal approached chance levels for all groups. (E) The proportion of hits, on the other hand, displayed a steady decline from block 1 to block 3, with no differences seen between groups during the first block (F) but significant interactions emerging during the distractor and post-distractor blocks (G and H). Aged rats chronically treated with A β displayed reduced hits in the 500ms signal trials in block 2 and 3, as well as a significant decrease in hits in 50 ms signal trials in block 3 in comparison to aged controls and young A β -infused rats. Hit rates did not differ between the young and aged control groups indicating that minor decrements in dSAT performance in the latter might have occurred due to variations in non-signal trial performance. (I) Correct rejections decreased during the distractor block but recovered in the post-distractor block. (J-L) No differences in correct rejections were observed between the groups during the blocks. All data are mean $\pm$ SEM. (main effect of signal: *, **, *** $p < 0.05, 0.01, 0.001$; *post hoc* comparisons: +, ++ $p < 0.05, 0.01$)

Figure 4.

Prefrontal ACh release and choline clearance capacity assessed using choline-sensitive microelectrodes and fixed-potential amperometry *in vivo*. Representative traces depicting choline spikes following brief depolarizing pulses of potassium in the medial PFC of young (A) and aged (B) rats that received chronic i.c.v. infusions of either oligomeric A β or control peptide. These signals reflect ACh release and occur as a consequence of rapid hydrolysis of ACh by acetylcholinesterase. The hydrolyzed choline is oxidized by the enzyme choline oxidase present on the platinum recording site at a fixed potential to generate increases in current. (C) Depolarization-evoked cholinergic signal amplitudes were lower in aged as well as A β -infused rats. Examples of current traces illustrate the effects of either A β oligomers or control peptide on clearance kinetics of exogenously applied choline in young (D) and aged (E) rats. Choline uptake rates were calculated from the hemicholinium-sensitive component of the clearance curve that accounts for the decrease in choline concentration from 40% to 80% of the peak amplitudes. (F) The capacity to clear choline from the extracellular space declined in soluble A β -infused rats and this effect was more prominent in aged animals (refer statistical analysis from 2×2 ANOVA in results). Data are Mean $\pm$ SEM. A β (-) depict chronic infusions with the control peptide while A β (+) indicate animals infused with soluble A β in (C) and (F), respectively.

Figure 5.

Effects of age and chronic A β infusions on BF cholinergic neurons and cortical presynaptic cholinergic proteins. (A; upper panel) Representative coronal sections depicting ChAT-immunopositive neurons (marked by black arrowheads) from the nucleus basalis of young and aged rats infused with either A β or control peptide. (A; lower panel) The cross-sectional area of cholinergic neurons robustly declined with age but not by chronic A β infusions. (B; upper panel) Photographs illustrate sampled ChAT-immunostained fibers from the prelimbic region of the PFC. (B; lower panel) The density of cortical cholinergic processes was significantly lower in aged rats. However, prefrontal ChAT fiber counts in soluble A β -infused rats did not differ from the control animals. Immunoblot analyses of cortical CHT (C) and VAcHT (D). These presynaptic protein markers for cholinergic terminals were estimated in homogenates prepared from prefrontal cortices. Representative immunoblots (top) show CHT- and VAcHT-immunoreactive bands at 55 kDa and 68 kDa respectively. Bar charts (bottom) show CHT and VAcHT densities normalized to β -tubulin. A downward trend in prefrontal CHT density was observed in aged animals ($p = 0.06$ vs. young). Likewise, VAcHT expression also significantly reduced with age. However, neither CHT nor VAcHT protein expression was affected by soluble A β . Data are Mean $\pm$ SEM. (main effects: *, **, *** $p < 0.05, 0.01, 0.001$).