Soluble alpha-synuclein oligomers are associated with reduced synapsin expression and enhanced cognitive decline in Alzheimer's disease

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Abstract

We recently proposed that soluble, intraneuronal α-synuclein (αSyn) might modulate Alzheimer's disease (AD) pathophysiology in the absence of Lewy body (LB) pathology. With mounting evidence indicating that oligomeric forms of aggregation-prone proteins such as Aβ, tau and αSyn may be the major bioactive deleterious agents involved in AD, frontotemporal dementia and Parkinson's disease, we sought to identify the nature of the soluble αSyn species elevated in AD and to determine the relative contribution of soluble αSyn oligomers to AD-associated cognitive deficits. Using enzyme-linked immunosorbent assays designed to detect oligomeric αSyn in our well-characterized human cohort, we found elevated levels of soluble αSyn oligomers (o-αSyn) in AD brains compared to aged-matched controls in the absence of LB cytopathology. Upon finical measurements of soluble αSyn in subjects with AD, we not only detected 2 forms of monomeric αSyn but also apparent multimers of each monomer. Unexpectedly, only a subset of soluble o-αSyn species was elevated intracellularly while extracellular o-αSyn remained unchanged. Multivariate analyses revealed that the respective abundance of selective low molecular weight o-αSyn was associated with cognitive deficits in multiple domains. Finally, we found that elevating o-αSyn in an AD mouse model triggered a selective decrease in synapsins and exacerbated Aβ-induced cognitive deficits. Altogether, our data support differential roles for soluble, intraneuronal αSyn oligomers in Alzheimer's disease, which could extend to other synucleinopathies.

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Introduction

Neurodegenerative diseases including Alzheimer's, Parkinson's, Huntington's and frontotemporal dementia share the common feature of the misfolding and aggregation of proteins that are normally soluble under physiological conditions. In Alzheimer's disease (AD), the prototypical neuropathological lesions include extracellular amyloid/senile plaques composed of amyloid-β peptide (Aβ) fibrils and intraneuronal hyperphosphorylated tau aggregates known as neurofibrillary tangles (NFTs). However, 30-40% of AD cases present with additional signs of proteinopathies, including intracellular inclusions of α-synuclein (αSyn) known as Lewy bodies (LB) and Lewy neurites (Forst et al., 1993; Hamilton, 2000; Trojanowski, 2002). The presence of these additional lesions does not appear to be innocuous, as subjects presenting with the LB variant of AD generally display a more rapid rate of cognitive decline than subjects with AD alone (Olichney et al., 1998). While studies using experimental models have proposed that fibrillar Aβ, tau and αSyn may have synergistic adverse effects (Masliah et al., 2001; Giasson et al., 2003, Clinton et al., 2010), accumulating evidence has suggested that soluble, non-fibrillar Aβ (McLean et al., 1999; Walsh et al., 2002; Kayed et al., 2003; Cleary et al., 2005; Lesne et al., 2006; Shakar et al., 2008) and tau (Ramsden et al., 2005; SantaCruz et al., 2005; Roberson et al., 2007) assemblies may be the more neurotoxic species. Since a putative role for soluble αSyn in AD had not been studied before 2012, it raised the exciting possibility that soluble αSyn may play an important but previously neglected role in AD pathophysiology.

Alpha-synuclein (αSyn) is a 140 amino acid protein found primarily in the presynaptic terminals of neurons, although its name derives from both synaptic and nuclear localization. αSyn is an amphipathic protein prone to self-aggregation and contains a conserved alpha-helical lipid binding motif similar to that of apolipoprotein E (ApoE). While αSyn is associated with

Parkinson's disease (PD), Uéda and colleagues first identified it as the precursor to the non-amyloid beta component (NAC) of amyloid plaques found in AD brains (Uéda et al., 1993). Jakes and coworkers later determined that this non-amyloid beta component precursor (NACP) was homologous to the synuclein previously purified from the electric organ of the California torpedo ray by Maroteaux (Maroteaux, 1988; Jakes et al., 1994). Another synuclein, synelfin, was later found in the nuclei of songbirds associated with learning (George et al., 1995). αSyn was linked to PD when it was found that αSyn point mutations and gene multiplication lead to PD (Polymeropoulos et al., 1997).

Although αSyn is enriched in presynaptic terminals and associated with synaptic vesicles, it does not appear to be essential for synapse formation or function but rather appears to perform a regulatory Murphy et al., 2000). In addition, that same study suggested an interaction between αSyn and synapsins, due to their colocalization and common association with the recycling pool, as synapsin-I mediates the binding of recycling vesicles to the actin cytoskeleton. More recently, overexpression of wild-type human αSyn (h-αSyn) was shown to inhibit vesicle release presumably through a reduction in the synaptic vesicle recycling pool (Nemani et al., 2010). αSyn is also associated with the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex, where large multimeric molecular assemblies of αSyn were shown to inhibit exocytosis by preferentially binding to synaptobrevin, thereby preventing normal vesicle docking (Choi et al., 2013).

Beyond its physiological function, the native structure of αSyn is the subject of much debate. Up until 2011, the native state of αSyn was believed to be an unfolded ~14 kDa monomer, which only acquired an alpha-helical structure upon binding to lipids (Davidson et al., 1998). Dennis Selkoe’s group challenged this notion, claiming that the native state was primarily a folded tetramer of ~58 kDa (Bartels et al., 2011). While the bulk of the analyses was done with αSyn isolated from red blood cells, the authors found similar ~55-60 kDa bands immunoreactive to

αSyn antibodies in multiple cell lines using clear-native polyacrylamide gel electrophoresis (PAGE) and sodium dodecylsulfate (SDS)-PAGE of disuccinimidyl suberate (DSS)-crosslinked samples. Given that the native tetramer was not prone to aggregation, Bartels and colleagues concluded that the oligomeric and fibrillar forms likely result from destabilization of the apparent αSyn tetramer. As a result, they suggested that stabilizing this native conformation of αSyn could be a novel treatment approach. However, recent studies challenged the existence of a native tetrameric αSyn, concluding that αSyn aggregation results from the naturally disordered state of the protein (Fauvet et al., 2012; Burré et al., 2013).

Irrespective of the exact physiological conformation state of αSyn, our group sought to determine whether changes in soluble, non-fibrillar forms of αSyn occurred in AD independently of LB pathology (Larson et al., 2012). Using well-characterized human brain tissue from participants of the Religious Orders Study, we found that soluble intracellular (IC) monomeric αSyn was increased by ~2-fold in AD brain tissue in the absence of αSyn inclusions. This change was also observed at the mRNA level, indicative of an upregulation of the gene encoding for αSyn, *SNCA*. In addition, intracellular αSyn protein levels were inversely correlated with premortem cognitive measures and quantitatively stronger than any soluble Aβ or tau species measured. To determine that elevating soluble h-αSyn was associated with cognitive impairment in the absence of LB pathology, we compared the spatial reference memory performance of TgI2.2 mice overexpressing wild-type h-αSyn (Lee et al., 2002) with aged-matched non-transgenic animals in the Barnes circular maze (BCM). We also used the AD mouse model J20, which overexpresses the mutant human amyloid precursor protein (hAPP) $APP^{Swe/Ind}$ (Mucke et al., 2000) as an internal control of memory impairment. At seven months of age, TgI2.2 animals displayed a clear deficit in memory retention while memory learning appeared intact (Larson et al., 2012), suggesting that increasing wild-type h-αSyn may be sufficient to cause cognitive deficits. This

observation was also consistent with previous studies from the Chesselet group (Chesselet and Richter, 2011), although the mouse model used in these studies is known to present with αSyn pathology (Masliah et al., 2011).

Comparing brain tissue of subjects with clinical AD having elevated soluble αSyn levels to those with normal αSyn levels, we observed that the 2-fold increase in intracellular monomeric αSyn coincided with significant reductions in all but one isoform of synapsins and complexins (Larson et al., 2012). In addition, the protein content of presynaptic vesicles was also altered in AD brain tissue with elevated soluble αSyn (Larson et al., 2012). These findings were in agreement with previous reports indicating that experimental or pathological elevation of αSyn is associated with lowering of selective presynaptic proteins including synapsins and complexins (Nemani et al., 2010) and with an apparent dissociation of the composition of presynaptic vesicles (Scott et al., 2010). These observations also suggested a possible connection between dysregulation of αSyn gene expression and alterations in presynaptic vesicle composition and release.

Finally, we determined that the combined expression of hAPP and human Tau (hTau) in mice was required to induce an increase in soluble αSyn protein similar to those seen in AD brain. This finding suggested that human Aβ and human tau might exert a combinatory effect triggering the upregulation of αSyn *in vivo*.

With the overwhelming accumulation of evidence indicating that soluble, multimeric assemblies of amyloid proteins including Aβ, tau, and αSyn might be the most bioactive toxins at the origin of AD, tauopathies, and synucleinopathies (McLean et al., 1999; Walsh et al., 2002; Kayed et al., 2003; Cleary et al., 2005; Ramsden et al., 2005; SantaCruz et al., 2005; Lesne et al., 2006; Roberson et al., 2007; Shakar et al., 2008; Winner et al., 2011), we hypothesized that the apparent increase in soluble intracellular αSyn monomers was also accompanied by an elevation of oligomeric assemblies of αSyn (o-αSyn), contributing to a decrease in synapsins and enhanced

memory deficits. In the present study, we found that selective o-αSyn species accumulated in AD brain tissue in the absence of LB pathology. In particular, we observed that putative αSyn dimers migrating at 28-29 kDa were elevated by 2-fold, consistent with the 1.7-2.3-fold elevation previously documented (Larson et al., 2012). Using biochemical and immunological approaches, we confirmed the oligomeric nature of this αSyn assembly. Importantly, the levels of o-αSyn were inversely correlated with synapsins and cognitive function in our human cohort. Finally, we demonstrated that overexpressing wild-type h-αSyn (h-αSyn^{WT}) in the AD mouse line J20 induced the oligomerization of αSyn which was accompanied by a selective decrease of synapsins and exacerbated cognitive deficits.

Materials and Methods

Human brain tissue.

Biochemical analyses were performed on human brain tissue from the inferior temporal gyrus (ITG) (Brodman Area 20) of 84 subjects enrolled in the Religious Orders Study. The selection of the ITG for our analyses was guided by the following observations: (1) this region of the cerebral cortex shows reduced glucose utilization in AD and in asymptomatic individuals at risk genetically for AD (Small et al., 2000); (2) ITG gray matter thickness significantly predicts hippocampal volume loss in both amyloid-positive and hyperphosphorylated tau-positive individuals among Mild Cognitive Impairment (MCI) and AD individuals (Desikan et al., 2010); and (3) ITG amyloid loads and tangle density matched very well with average total brain amyloid burden (*Rho* = 0.946; p = 0.0001) and tangle density (*Rho* = 0.772; p = 0.0001). Cognitive status was assessed with the MMSE and 19 other tests summarized as a global measure of cognition and five cognitive domains (Boyle et al., 2006). Selected cases were chosen to ensure that the three groups (No Cognitive Impairment, MCI, and AD) would not differ significantly from the whole ROS cohort. Amyloid load and tangle density were quantified in six brain regions (Bennett et al., 2004) and subjects further characterized by Braak stage, CERAD and NIA-Reagan pathologic diagnoses (Bennett et al., 2005). The six brain regions included the hippocampus, entorhinal cortex, midfrontal gyrus, inferior temporal gyrus, inferior parietal gyrus, and calcarine cortex, with averages determined by pooling amyloid load and tangle density from each area. The characteristics of the three clinical diagnostic groups are summarized in **Table 1**. The pathological characteristics of the clinical diagnostic groups selected for this study were similar to those of the entire ROS cohort, whether assessed by amyloid load or tangle density (data not shown). Finally, the University of Minnesota Institutional Review Board has approved this study.

Table 1. Characteristics of the Religious Order Study Participants.

Group	NCI (*n* = 26)	MCI (*n* = 34)	AD (*n* = 24)	*P* values*
Age of death *(years)*, Mean ± SD	82.97 ± 7.53	86.33 ± 5.69	90.27 ± 7.20	0.108
No. of M/F (%)	12/14 (46.1%)	14/20 (41.2%)	9/15 (37.5%)	>0.99
Last MMSE score, Mean ± SD	28.35 ± 1.38	26.41 ± 2.96	12.33 ± 8.79	***<0.0001***
PMI *(hours)*, Mean ± SD (Range)	5.57 ± 2.25 (2-10)	4.90 ± 2.56 (1-9)	4.48 ± 1.66 (2-9)	0.191
Amyloid Burden *(% of area)*, Mean± SD	1.63 ± 0.40	1.57 ± 0.35	3.12 ± 0.42	***0.0099***
Tangle density *(#/mm^2)*, Mean ± SD	4.92 ± 3.93	5.81 ± 5.43	11.18 ± 10.08	***0.002***

Abbreviations: NCI, non cognitive impairment; MCI, mild-cognitive impairment; AD, Alzheimer's disease; SD, standard deviation; M/F, male/female ratio; MMSE, mini-mental status examination; PMI, post-mortem interval.

*Kruskal-Wallis test followed by Bonferroni-adjusted test for multiple comparisons. Italicized values indicate significance.

Transgenic animals.

Wild-type (wt) and heterozygous transgenic (Tg) mice were used in this study. Transgenic mice expressing the wild-type form of human α-synuclein under the control of the mouse prion promoter (Lee et al., 2002), moPrp-HuSyn line I2-2(WT), were used in conjunction with *SNCA*-null mice, which are deficient in endogenous mouse αSyn (Lee et al., 2002). In addition, transgenic mice expressing the human form of APP (hAPP) with the Swedish (K670N, M671L) and Indiana (V717F) familial AD mutations directed by the platelet-derived growth factor chain promoter (Mucke et al., 2000), APP line J20, were used. Bigenic J20xTgI2.2 mice were generated by crossing TgI2.2 and J20 mice. All lines used are in the C57BL6 background strain.

Both male and female animals were used in biochemical studies and Barnes Maze behavioral testing.

Protein extractions.

Soluble aggregation-prone protein levels in brain tissue were analyzed using the extraction protocol developed by Lesne (Lesne et al., 2006; Sherman and Lesne, 2011). All supernatants were ultra-centrifuged for 20 minutes at 100,000 x *g*. Before analysis, fractions were depleted of endogenous immunoglobulins by incubating lysates with 50 µL of Protein A-Sepharose, Fast Flow® beads for one hour at 4° C, followed by 50 µL of Protein G-Sepharose, Fast Flow® beads (GE Healthcare Life Sciences). Protein amounts were determined with the Bicinchoninic acid protein assay (BCA Protein Assay, Pierce).

Enzyme-linked immunosorbent assay (ELISA).

Intracellular-enriched protein fractions were used for the determination of o-αSyn levels in human brain tissue. Oligomeric forms of αSyn were identified using a custom sandwich ELISA assay, based on the principle documented earlier for Aβ (Klaver et al., 2010). This ELISA utilized a capture antibody (LB509) with 2 detection antibodies conjugated to different infrared molecules: LB509-IR800 and A11-IR680. High-binding clear bottom 96-well microplates (96 Well PS Microplate Flat Bottom w/o Lid, #655161, Greiner Bio-One) were treated with the capture antibody (1 µg/well) overnight, washed and incubated with PBS containing 1% BSA prior to incubation with the brain lysate (250 ng/well). Following application of the detection antibody, the plates were read with the Odyssey system (Li-Cor Biosciences).

Oligomer disassembly.

Hexafluoroisopropanol (HFIP) disassembly was performed on the extracellular and intracellular fractions of 4-, 7-, and 11-month-old TgI2.2 brain extracts, along with recombinant αSyn. Concentrations of HFIP ranging from 0% to 100% were incubated with 2mM edetate disodium salt dehydrate (EDTA) and 50 µg of the brain extract or 0.5 µg of recombinant αSyn for 1 hour at 37° C, while agitated at 800 rpm. The solutions were vacuum concentrated until dry, then

reconstituted with loading buffer for gel electrophoresis. The original samples without EDTA were included as controls in the analyses.

Size-exclusion chromatography (SEC).

Immunoaffinity purified protein extracts were loaded on Tricorn Superdex® 75 columns (GE Healthcare Bio-Sciences Corp.) and run at a flow rate of 0.5 ml/min. Fractions of 250 µL of eluate in PBS with .1% Triton X-100, were collected using a BioLogic DuoFlow QuadTec 40 system (Bio-Rad) coupled to a microplate-format fraction collector. A280 was determined live during the experiments and confirmed following each run on a DTX800 Multimode microplate reader (Beckman Coulter).

Western blotting and quantification.

Electrophoresis was done using *SDS-PAGE* on pre-cast 10-20% SDS-polyacrylamide Tris-Tricine gels, or 10.5-14% and 4-10.5% Tris-HCl gels (Bio-Rad). Protein levels were normalized by using 2-100 µg of protein per sample (depending on the targeted protein). The samples were resuspended with 4X Tricine loading buffer and boiled for 5 minutes prior to loading.

Transfer. Proteins were transferred to 0.2 µm nitrocellulose membrane (Bio-Rad) following electrophoresis.

Blotting. Membranes were blocked in TTBS (Tris-Buffered Saline-0.1%Tween®20) containing 5% bovine serum albumin (BSA) (Sigma) for 1-2 hours at room temperature, and probed with the appropriate antisera/antibodies diluted in 5%BSA-TTBS. Primary antibodies were probed with either anti-IgG immunoglobulins conjugated with biotin, HRP or InfraRed dyes (Li-Cor Biosciences, USA). When biotin-conjugated secondary antibodies were used, HRP- or IR-conjugated Neutravidin® (Pierce) or ExtrAvidin® (Sigma) was added to amplify the signal. Blots were revealed on an Odyssey platform (Li-Cor Biosciences).

Stripping. For reprobing, membranes were stripped using Restore™ Plus Stripping buffer (Pierce) for 5-180 min at room temperature, depending on the antibody affinity.

Quantification. Densitometry analyses were performed using the Odyssey software (Li-Cor Biosciences). Each protein of interest was probed in three individual experiments under the same conditions. Quantification by software analysis, expressed as Density Light Units (DLUs), followed determination of experimental conditions ascertaining linearity in the detection of the signal. This method allows for a dynamic range of ~100-fold above background ($0.01x10^6$ DLU). Respective averages were then determined across the triplicate Western blots. Normalization was performed against Actin or NeuN, which were also measured in triplicate (data not shown).

Antibodies.

The following primary antibodies were used in this study: LB509 [1:5,000-10,000], 4D6 [1:5,000], 4B12[1:5,000], 6E10 [1:2,500] (Covance, USA), anti-synapsin-I/II [1:1,000], anti-complexin-1/2 [1:5,000] (Synaptic Systems, Goettingen, Germany), anti-β-Syn (1:1,000), anti-Synaptophysin [1:25,000], anti-NeuN [1:5,000] (Millipore), αSyn C-20 [1:1,000] (Santa Cruz), rabbit-host anti-actin [1:10,000] (Sigma Aldrich), mouse-host anti-actin [1:10,000] (Pierce), Syn-33[1:500], F8H7 [1:500], A11 [1:2,000], OC [1:2,000], and Officer 1:2,000] (kind gifts of Dr. Rakez Kayed).

Barnes circular maze.

The Barnes circular maze (San Diego Instruments, USA) was used for behavioral testing of wild-type and TgI2.2 transgenic mice. The apparatus consisted of an elevated circular platform (0.91 m in diameter) with 20 holes (5 cm diameter) around the perimeter. One hole was connected to a dark escape recessed chamber, referred to as the target box. The maze was positioned in a room with large, simple visual cues attached on the surrounding walls. The protocol used was adapted from Sunyer et al., 2007 (Nature Protocol Exchange). Briefly, mice were habituated to the

training room prior to each training day for 30 minutes in their cage. In addition, on the first day mice were placed at the center of the maze in a bottomless opaque cylinder for 60 sec to familiarize the animals with the handling. Fifteen minutes later, training sessions started. Acquisition consisted of four trials per day for 4 days, separated by a 15-minute intertrial interval. Each mouse was positioned in the center of the maze in an opaque cylinder, which was gently lifted and removed to start the session. The mice were allowed 180 sec to find the target box on the first trial; all trials were 3 minutes long. At the end of the first 3 minutes, if the mouse failed to find the recessed escape box, it was gently guided to the chamber and allowed to stay in the target platform for 60 sec. The location of the escape box was kept constant with respect to the visual cues, but the hole location of the target platform was changed randomly. An animal was considered to have found the escape chamber when its back legs crossed the horizontal plane of the platform. An animal was considered to have entered the escape chamber when its entire body was in the chamber and no longer visible on the platform. Retention was tested 24 hours after the last training session (day 5) and 7 days after the initial probe (day 12). The same parameters were collected during the acquisition and retention phases using the ANY-maze software (San Diego Instruments, Stoelting Co.).

Statistical Analyses.

Nonparametric statistics (Spearman *rho* correlation coefficients, Kruskal-Wallis nonparametric analysis of variance followed by Bonferroni-corrected two-group *posthoc* Mann-Whitney U tests) were used when variables were not distributed normally. Univariate repeated measures ANOVA were performed to determine the effects of day, transgene and day*transgene interactions for behavioral experiments.

Analyses were performed using JMP 10 (SAS Institute, USA).

Results

Elevation of soluble αSyn oligomers in AD brain

To determine whether oligomeric αSyn might be elevated in parallel with the increase in monomeric αSyn previously reported (Larson et al., 2012), we created an in-house ELISA to detect soluble multimers of αSyn and soluble αSyn conformers immunoreactive to the A11 antibody (**Figure 1A**). In both detection sets used, oligomeric αSyn species were elevated in AD subjects compared to age-matched controls by 34% and 48%, respectively (**Figure 1B, C**). Of note, incremental amounts of freshly resuspended monomeric recombinant αSyn ranging from 1 pg to 10 ng were not detected by the assay (**Figure 1A**, dashed rectangle).

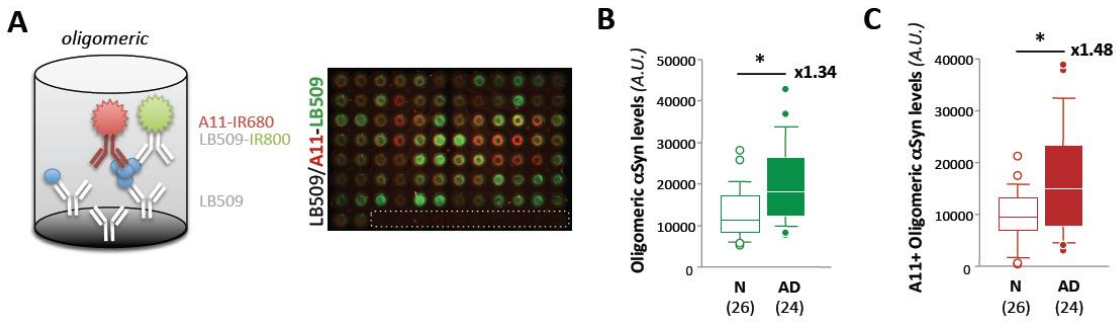

Figure 1. Identification of soluble αSyn assemblies present in human brain tissues. *(**A**) Infrared-based ELISA selective for oligomeric αSyn using LB509-IR800 or A11-IR680 as detecting antibodies revealed the presence of αSyn oligomers in human brain tissues. The dashed rectangle in the bottom right corner indicates that freshly resuspended recombinant monomeric αSyn (1 pg to 10 ng) is not detected in this assay. (**B, C**) Box plots for oligomeric αSyn species using either the dual LB509 sandwich (B) or the LB509-A11 sandwich (C). Statistical differences were observed between the AD group and the NCI group (Number of subjects used is listed between parentheses; n = 3 measurements/specimen; Mann-Whitney's U test, P < 0.05).*

To determine the possible nature of the soluble αSyn oligomers detected, intracellular-enriched fractions from AD individuals with normal or high levels of αSyn (Larson et al., 2012) were subjected to non-denaturing size exclusion chromatography (SEC). Isolated species were then

analyzed by Western blotting with 4D6 following SDS-PAGE (**Figure 2**). Consistent with numerous observations (Bartels et al., 2011; Fauvet et al., 2012; Burré et al., 2013), freshly resuspended monomeric recombinant αSyn did not behave as a globular protein during the fractionation process due to its disordered structure, resulting in its elution at fraction 49-53 (**Figure 2A**, *top insert*). Apparent brain-derived αSyn monomers eluted earlier at fractions 43-53, likely due to the presence of two distinct 17 kDa and 14 kDa monomeric forms (**Figure 2A**, *middle and bottom inserts*). In contrast, we noticed the presence of putative SDS-resistant αSyn that appeared to behave as globular proteins (**Figure 2A**, *middle and bottom inserts*). Several 4D6-immunoreactive bands of approximately 28, 35, 56, and 72 kDa were readily detected, consistent with potential dimers and tetramers of the 14 and 17 kDa αSyn monomers mentioned above. It is important to note that the putative 28 kDa dimer eluted at its predicted globular molecular weight and did not co-elute with any other detectable αSyn species. This observation argues against the possibility that this assembly is the result of a breakdown of a larger structure or of a self-aggregation of αSyn monomers. Interestingly, densitometry analysis revealed a significant increase in the 17kDa and 28kDa species (a 1.42 and 2.23-fold elevation respectively) in AD subjects previously identified as expressing high levels of αSyn compared with those expressing normal levels (**Figure 2B**).

To further characterize the nature of oligomeric forms of αSyn, we performed non-denaturing analyses of SEC fractions by dot blot (DB) assay using selected human and mouse brain tissues (**Figure 3**). Intracellular-enriched extracts from either AD brain tissue, TgI2.2, wild-type (WT), and *SNCA*-null mice were subjected to fractionation by SEC. Each fraction was subjected to a panel of commercially available antibodies detecting various epitopes within the αSyn molecule (LB509, 4B12, 4D6; **Figure 3A**) and to a panel of antibodies generated to detect oligomeric and aggregated amyloid proteins (A11, OC, Officer), including αSyn oligomers (Syn33, F8H7). We

also included analyses with the 6E10 antibody detecting $A\beta_{1-16}$ to determine whether the putative o- αSyn might be coupled to Aβ as a hybrid oligomer (Tsigelny et al., 2008).

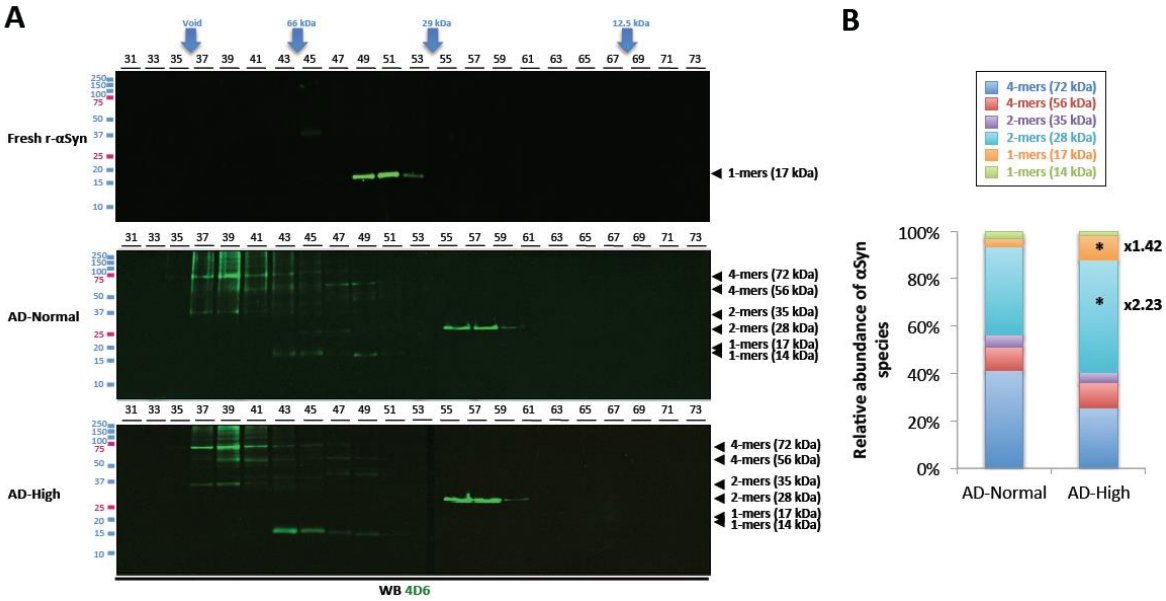

Figure 2. Identification of soluble αSyn assemblies present in human brain tissues. *(**A**) Comparison of SEC profiles of αSyn molecules from recombinant αSyn (top), AD brain tissue with normal levels of αSyn (middle) and AD brain tissue with high abundance of soluble αSyn (bottom). (**B**) Stacked plot histogram indicating statistical increases in 17 kDa and 28 kDa αSyn species (n = 5/group, Kruskal-Wallis ANOVA followed by Mann-Whitney's U test, P < 0.05).*

Human specific LB509 and 4B12 antibodies readily detected isolated monomeric αSyn in AD and TgI2.2 fractions but not in either WT or *SNCA*-null fractions (**Figure 3B**, *lane 2*). However, both proved quite poor at detecting αSyn molecules in SEC fractions containing apparent SDS-resistant αSyn oligomers (**Figure 3B**, *lanes 3-4*). Using 4D6 modestly improved detection (**Figure 3B**, *lanes 3-4*). We hypothesized that the 4D6 epitope was partly available due to the conformation of the putative o-αSyn species. To relax the folding of the protein, we boiled nitrocellulose membranes onto which samples had been previously pre-adsorbed. Under these conditions, the detection of αSyn with 4D6 was substantially increased revealing the presence of αSyn assemblies consistent with 28 kDa dimers and co-segregated 35 kDa/72 kDa multimers

(**Figure 3B**). To confirm that these species corresponded to αSyn oligomers, we used antibodies detecting various oligomeric forms of amyloid proteins, *i.e.* A11, OC, Officer (Kayed et al., 2003; Kayed et al., 2007), and antibodies specific to αSyn oligomers, Syn33 and F8H7 (Kayed et al., unpublished data). The antibodies OC and Officer detected the co-segregated 35 kDa/72 kDa αSyn species in both AD and TgI2.2 samples, suggesting that the αSyn forms detected are prefibrillar oligomeric αSyn assemblies. In contrast, the putative αSyn dimers were detected with A11 and F8H7 in AD brain tissue and to a lesser extent in TgI2.2, indicating that this αSyn species is indeed an oligomer. Of note, the same analysis performed with either WT or *SNCA*-null mouse tissue did not reveal any putative αSyn oligomers.

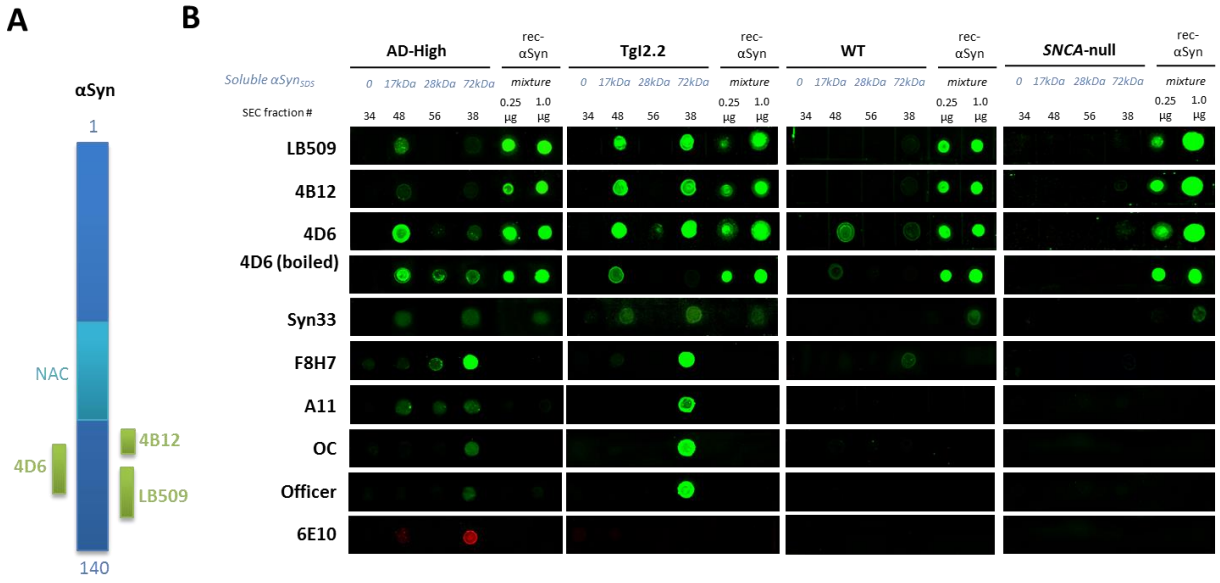

Figure 3. Dot blot analyses of soluble αSyn species isolated by liquid-phase chromatography. *Size exclusion chromatography (SEC) fractions containing segregated soluble αSyn species isolated from brain tissues of Alzheimer's disease subjects with high levels of αSyn (AD-High), or from mouse brain tissues (TgI2.2 overexpressing hαSyn^{WT}, wild-type, and SNCA-null mice) were analyzed under native conditions. The method chosen was dot blotting utilizing commercially-available antibodies against human αSyn (LB509 and 4B12), mouse/human αSyn (4D6), oligomeric αSyn (Syn33, F8H7), and aggregated amyloid proteins (A11, OC, and Officer, kind gifts of Dr. Rakez Kayed). Finally 6E10, a monoclonal antibody raised against human Aβ$_{1-16}$ was used as an internal control.* ***(A)*** *Schematic of known epitopes for αSyn monoclonal antibodies used in this study.* ***(B)*** *Representative dot blots of SEC-isolated soluble αSyn molecules (n = 3/group/antibody). TgI2.2 and WT mice: 11 months old, SNCA Null mice: 16.5 months old.*

Finally, we subjected TgI2.2 IC fractions to hexafluoroisopropanol (HFIP) to promote the disassembly of putative αSyn oligomers into soluble αSyn monomers (**Figure 4**). Low concentrations of HFIP (10-20%) appeared to trigger the oligomerization of low-molecular weight αSyn species, as evidenced by the detection of larger species immunoreactive to 4D6 creating the appearance of a smear in the upper parts of the SDS-PAGE gel and by the reduction in the abundance of low-*n* putative o-αSyn. Increasing HFIP concentration to 100% induced the depolymerization of o-αSyn multimers which included putative dimers (~35kDa), and tetramers (~56kD and ~72kDa), into monomeric αSyn molecules (**Figure 4A, B**). These results support the notion that these αSyn assemblies detected by 4D6 are composed of multiple αSyn monomers.

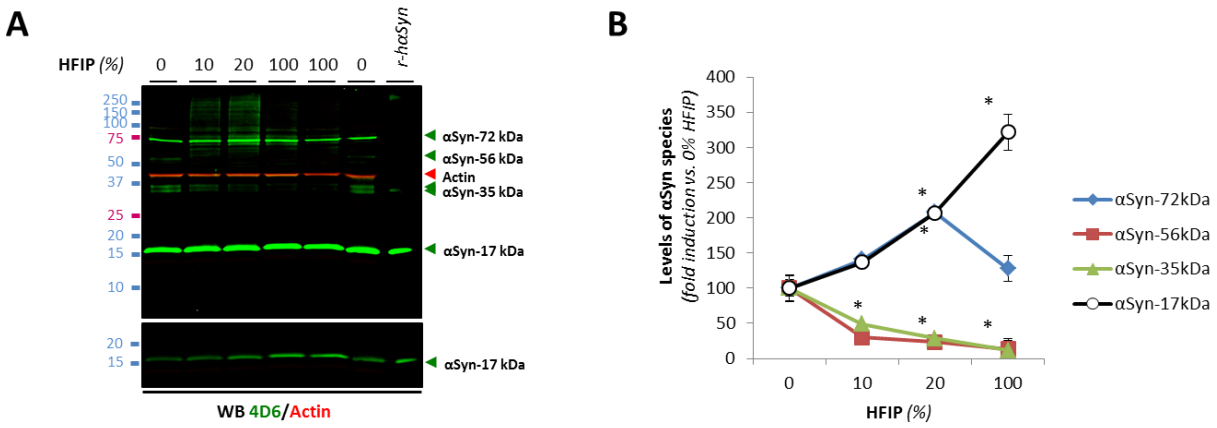

Figure 4. Solvent-induced disassembly of putative brain αSyn oligomers. *Modifying the physiological environment of αSyn oligomers by reducing the water content led to disaggregation of apparent αSyn assemblies and parallel increase in monomeric αSyn species.* ***(A)*** *Putative soluble αSyn oligomers depolymerize in >10% Hexafluoroisopropanol (HFIP), with concomitant enrichment of monomeric αSyn (lower exposure provided in the bottom insert for enhanced contrast).* ***(B)*** *The data for fold-change in αSyn species is the mean ± standard deviation of 3 experiments. *P < 0.05 compared to condition with 0% HFIP (ANOVA followed by Student t test).*

With the identification that 4D6 appeared more sensitive in detecting o- αSyn under denaturing conditions (**Figures 2, 3, 4**), we then re-analyzed the human brain specimens previously characterized using LB509 (Larson et al., 2012) by performing SDS-PAGE followed by Western Blot (WB). Extracellular- and intracellular (IC)-enriched fractions were used here in these new

measurements as both protein lysates were shown to contain high amounts of αSyn protein (Larson et al., 2012). As predicted, apparent SDS-resistant o-αSyn were readily detected by 4D6 in EC and IC fractions (**Figure 5A, B**). In agreement with our previous results, we found no differences in the brain levels of soluble extracellular αSyn monomers or oligomers between clinical groups (**Figure 5C**). In contrast, soluble αSyn species of ~17, 28, 56 kDa consistent with putative monomeric, dimeric and tetrameric αSyn molecules were respectively elevated by 1.64-, 1.75- and 1.64-fold in the IC fraction of AD subjects compared with non-cognitively impaired (NCI) individuals (**Figure 5D**). Of note, a rise of the ~28 kDa and ~56 kDa αSyn species was also detected in brain tissue from individuals diagnosed with mild cognitive impairment (MCI) compared to NCI. These results were consistent with the 1.67-fold upregulation of the *SNCA* mRNAs previously reported (Larson et al., 2012).

Altogether, our data suggest that specific low-molecular weight oligomeric αSyn species accumulate in AD in absence of LB pathology.

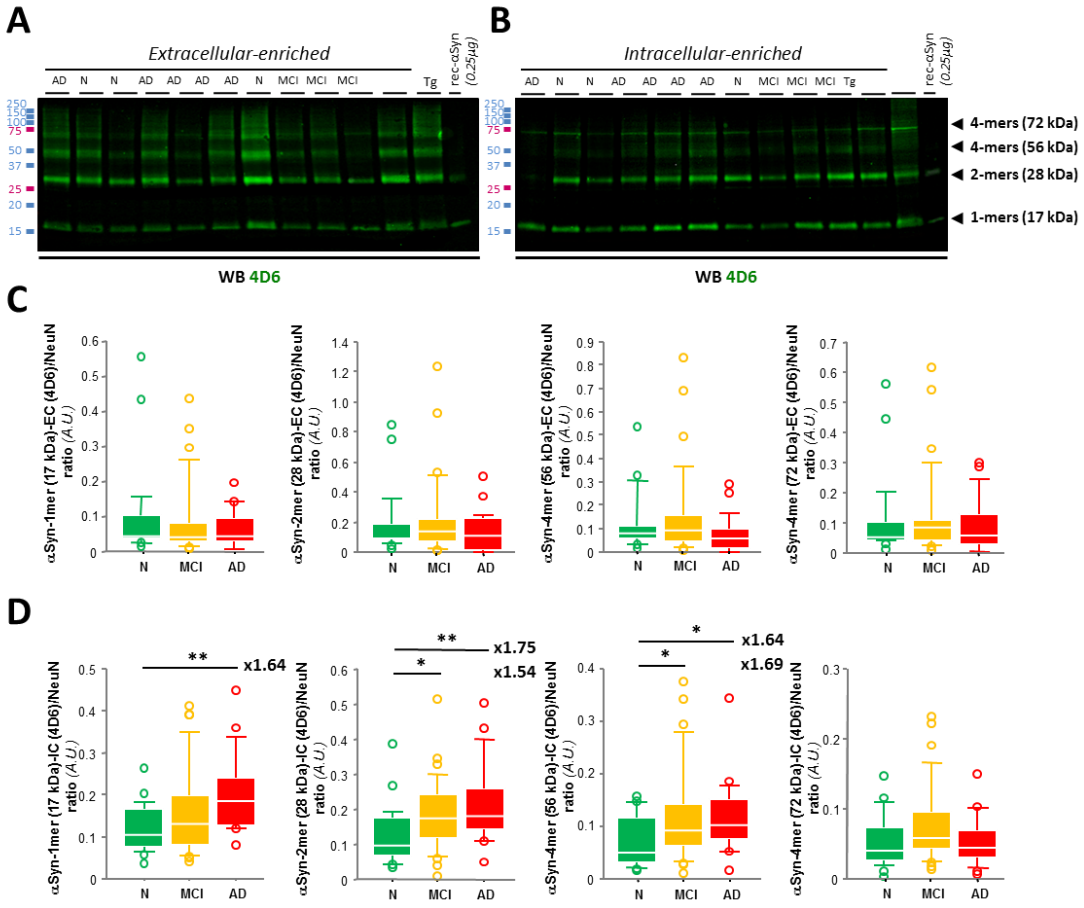

Figure 5. Increase in specific soluble αSyn oligomers in AD brain in absence of Lewy bodies/neurites. *(**A, B**) Western blot (WB) analyses of soluble αSyn species in extracellular (**A**), or intracellular (**B**)-enriched fractions using 4D6. Transgenic mice from the Tgl2.2 line were used as positive controls. Gel filtration combined with SDS-PAGE confirmed the presence of SDS-resistant αSyn soluble assemblies. (**C, D**) Quantification of monomeric and oligomeric αSyn species in the inferior temporal cortex of subjects with no cognitive impairment (NCI), MCI or AD. While monomeric and oligomeric αSyn remained unchanged across groups in EC fractions (**C**), intracellular monomeric and oligomeric αSyn levels were significantly higher in AD than in MCI and NCI brain tissues (**D**).*

O-αSyn negatively correlate with measures of cognitive performance

To determine whether the elevation in soluble o-αSyn species identified in the AD brain tissue might be associated with cognitive deficits, we performed multivariable regression analyses using all measurements of soluble forms of αSyn detected with either 4D6 or LB509 and measures of cognitive function (**Figure 6**). Cognitive modalities tested included episodic, semantic, working, and visuospatial memory, perceptual speed, and global cognition. Color maps for correlation

indexes revealed that neither extracellular nor intracellular o-αSyn were correlated to multiple cognitive domains in aged-matched controls (**Figure 6**, *left color map*). We observed that inferior temporal gyrus (ITG) levels of putative 28 kDa αSyn dimers were correlated with episodic memory deficits (*Rho* = -0.463, P = 0.0195). In contrast, there was a global trend towards inverse correlations between cognitive function and all intracellular αSyn species in our AD group (**Figure 6**, *right color map*). More specifically, while we confirmed that levels of intracellular monomeric αSyn were inversely correlated to semantic and visuospatial memory using a different antibody (*i.e.* 4D6 instead of LB509), we also found that the levels of the 28 kDa and 72 kDa αSyn species correlated with impairments in visuospatial memory and global cognition. However, the smallest αSyn assembly also correlated with greater deficits in semantic and most importantly episodic memory. These data indicate that specific o-αSyn assemblies might modulate AD dementia.

Brain levels of soluble αSyn oligomers correlate with the selective lowering in synapsins

In mice, the overexpression of h-αSyn^{WT} is associated with a selective reduction in synapsins and complexins (Nemani et al., 2010). We also previously reported a negative correlation between intracellular levels of monomeric αSyn and synapsins in postmortem AD brain tissue (Larson et al., 2012). Given that large o-αSyn species were recently proposed to inhibit the docking of presynaptic vesicles (Choi et al., 2013) and that synapsins regulate synaptic release, we hypothesized that the increase in o-αSyn measured in our human AD cohort could be related to the observed decrease in synapsins. We found that o-αSyn levels measured by ELISA using LB509 as the detection antibody inversely correlated with levels of synapsins in the ITG (R^2 = -0.346, P = 0.0241; **Figure 7A**). Similarly, A11-positive o-αSyn levels correlated with the lowering in synapsins (R^2 = -0.578, P = 0.0007; **Figure 7B**), although to a greater extent than LB509-positive αSyn. To assess whether these relationships were specific to synapsins, we

performed additional regression analyses using protein levels of other presynaptic markers, *i.e.* synaptophysin (SYP). No correlations were found between the levels of oligomeric αSyn and synaptophysin using either set (**Figure 7C, D**). These data suggest that the elevation of o-αSyn in AD might alter synapsin expression.

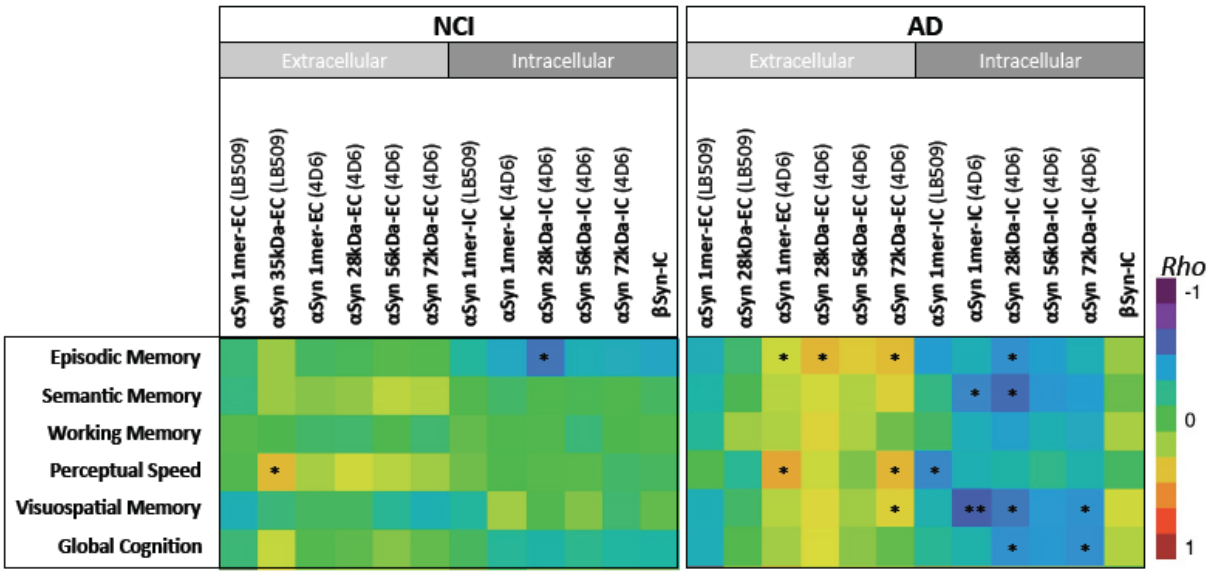

Figure 6. Color map of Spearman rho correlations of oligomeric forms of αSyn detected with either 4D6 or LB509, along with measures of cognitive function *Correlations were found between 4D6-detected oligomers and deficits in episodic and semantic memory, perceptual speed, visuospatial memory, and global cognition in the AD group. All measures of protein levels were performed identically, using SDS-PAGE followed by Western blot. Models were performed using the entire cohort.*

Oligomeric αSyn-associated lowering of synapsins correlates with episodic memory impairment

To determine if the reduction in synapsins identified in the AD brains might be associated with cognitive deficits, we performed regression analyses between the protein amounts of synapsins and episodic memory function in our ROS AD group, since this memory modality is specifically affected in AD (**Figure 8**). We found that greater deficits in episodic memory correlated with lower total synapsin levels (Spearman *Rho* = 0.4132; *P* = 0.0447), suggesting that synapsin might mediate the modulation of cognitive impairment enhanced by soluble αSyn.

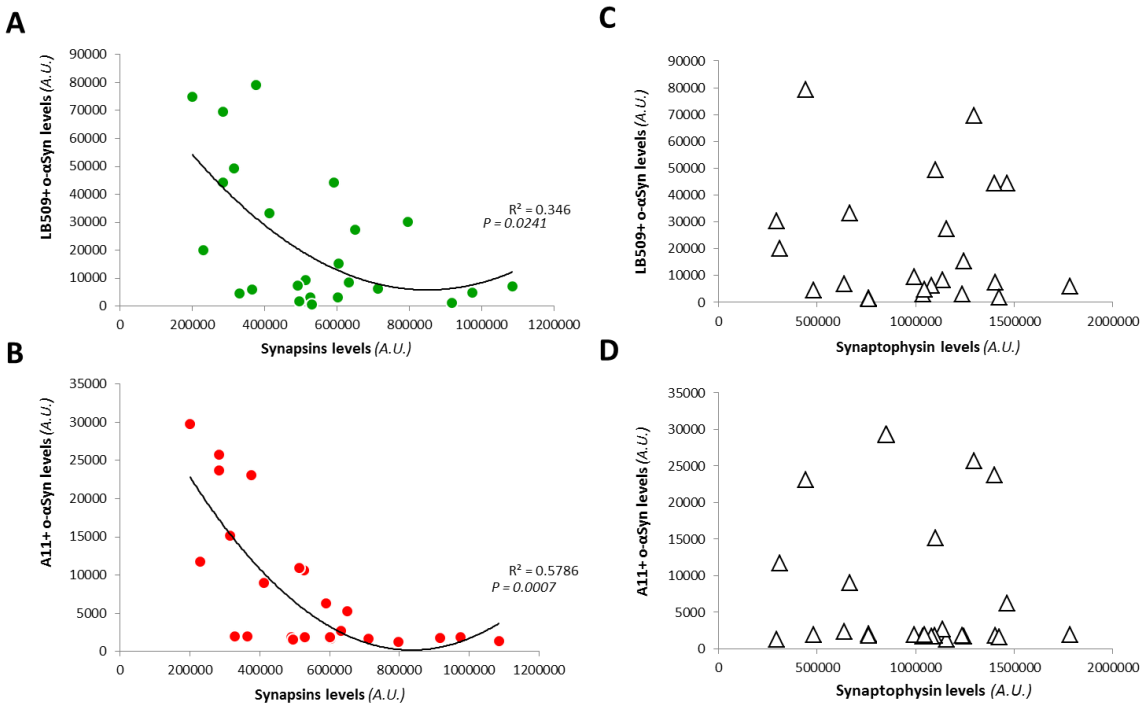

Figure 7. Brain levels of soluble αSyn oligomers measured by ELISA are inversely correlated with total synapsin levels in AD brain tissue. *(**A, B**) Regression analyses between total synapsin protein expression and oligomeric αSyn measured by ELISA using either LB509 (A) or A11 (B) as the detecting antibody in all AD cases tested (n = 24). Best fitting models indicated significant negative correlations for both o-αSyn measurements (Spearman Rho, P < 0.05, n = 24).* ***(C, D)*** *Regression analyses between synaptophysin protein expression and oligomeric αSyn measured by ELISA using either LB509 (C) or A11 (D) as the detecting antibody in all AD cases (n = 24). No correlations between o-αSyn and synaptophysin were found.*

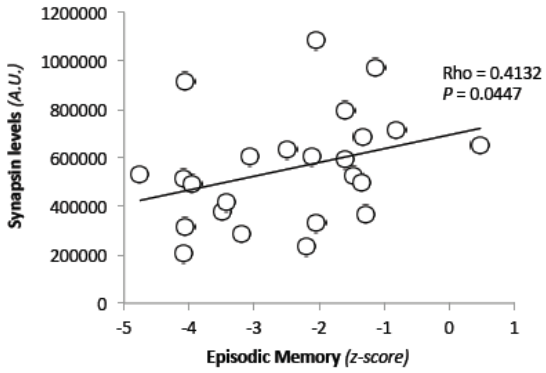

Figure 8. Synapsin levels correlate with episodic memory function in AD. *Regression analyses revealed a positive correlation between total synapsin levels and episodic memory performance in our AD cohort. All measures of protein levels were performed identically, using SDS-PAGE followed by Western blot.*

Overexpression of αSyn in the J20 AD mouse model is associated with reduced expression of synapsins

To test whether an elevation in soluble h-αSyn^{WT} oligomers is sufficient to induce a selective reduction in the synapsins vesicular proteins in a mouse model of AD, we created a bigenic mouse line by crossing J20 mice overexpressing the mutant form of hAPP$^{Swe/Ind}$ (Mucke et al., 2000) with TgI2.2 mice (Lee et al., 2002). Since Aβ and αSyn are known to promote the aggregation of each other *in vivo* (Masliah et al., 2001; Clinton et al., 2010), we expected to trigger the oligomerization of αSyn when Aβ is overexpressed. We first analyzed the protein levels of soluble αSyn in 3-month-old wild-type (WT), J20, TgI2.2, and J20xTgI2.2 mice by Western blotting (**Figure 9**). We observed that the expression of transgene-derived h-αSyn^{WT} monomers was not different between TgI2.2 and J20xTgI2.2 mice (**Figure 9A, B**). However, we noticed a 3.7-fold increase in low-*n* molecular weight o-αSyn in bigenic mice compared to TgI2.2 at 3 months of age (**Figure 9A, B**). This specific profile allowed us to test whether the ~4-fold elevation in o-αSyn was associated with a selective decrease in synapsins. We next measured the protein expression of multiple presynaptic markers including synapsins, synaptophysin and complexins by Western blotting, using actin as an internal control. While no overt changes in synaptophysin and complexins were observed across all mouse genotypes, a reduction in synapsins was obvious in our J20xTgI2.2 bigenic line compared to J20, TgI2.2 and WT animals (**Figure 9A**). Quantification of the band intensities revealed significant decreases of synapsin Ia, IIa, IIIa, IIb in the bigenic mice compared with WT, with no apparent changes in the levels of synaptophysin or complexins (**Figure 9C**).

Elevating o-αSyn potentiates memory deficits in the J20 AD mouse model

Finally, we wanted to determine whether the increase in o-αSyn and its associated decrease in synapsins exacerbated Aβ-induced cognitive deficits. We therefore subjected our four animal groups to behavioral testing using the Barnes circular maze (BCM) to assess spatial reference memory at 3 and 6 months of age (**Figure 10**). Already at 3 months, bigenic animals displayed an apparent delay in learning the task compared to WT and single transgenic J20 and TgI2.2 littermates (**Figure 10A**). Three months later, J20xTgI2.2 animals were unable to learn the task while age-matched J20, TgI2.2 and WT mice all learned equally well (**Figure 10A**). During the retention trial on day 5, 3-month-old J20xTgI2.2 mice did not show a search bias to the target hole whereas all other age-matched groups performed similarly (**Figure 10B**). At 6 months of age, both J20 and TgI2.2 displayed spatial memory deficits, which were enhanced in the J20xTgI2.2 bigenic mice (**Figure 10B**). These results suggest that learning and spatial memory recall were affected in young J20xTgI2.2 mice and that these deficits are accentuated with aging (**Figure 10B**). These genetic *in vivo* studies indicate that an increase in αSyn oligomers might modulate Aβ-induced cognitive deficits.

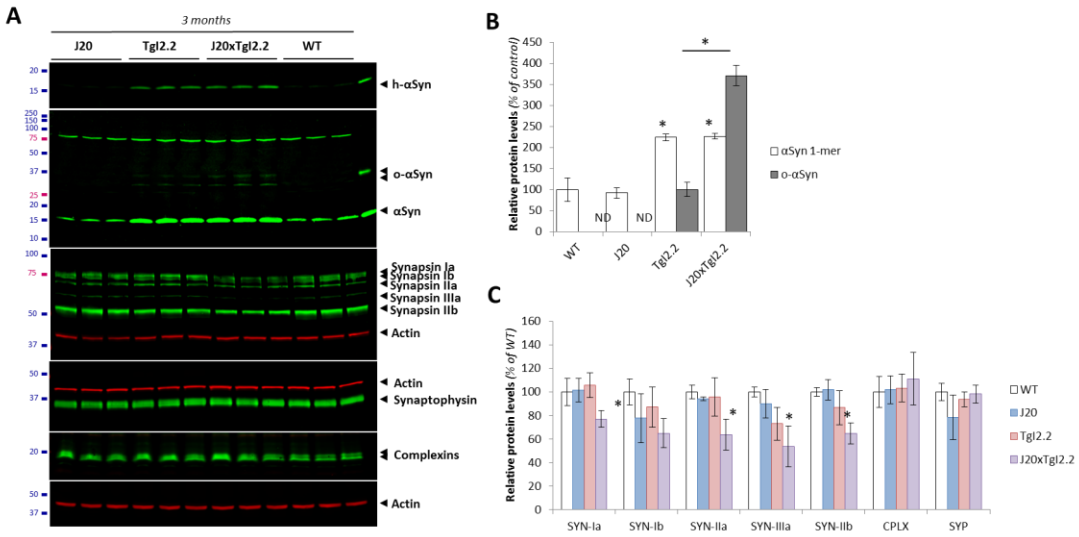

Figure 9. Elevation of oligomeric αSyn in the J20 mouse model of AD is associated with reduced expression of selective synapsins. *Three to six-month-old non-transgenic C57BL/6, J20, Tgl2.2 and J20xTgl2.2 mice were analyzed in the Barnes circular maze to assess their respective memory performance. Following behavioral testing, mice were euthanized for gene and protein analyses.* ***(A)*** *Representative Western blot images for αSyn, synapsins, synaptophysin, complexins and actin.* ***(B)*** *Quantification of αSyn species revealed a significant elevation of putative o-αSyn in J20xTgl2.2 mice at 3 months.* ***(C)*** *Densitometry analyses confirmed the apparent visual reduction in synapsins in bigenic J20xTgl2.2 mice compared to other mouse groups.* ***B & C****: ANOVA followed by Student t test with Bonferroni correction, P < 0.05, n = 3/age/genotype.*

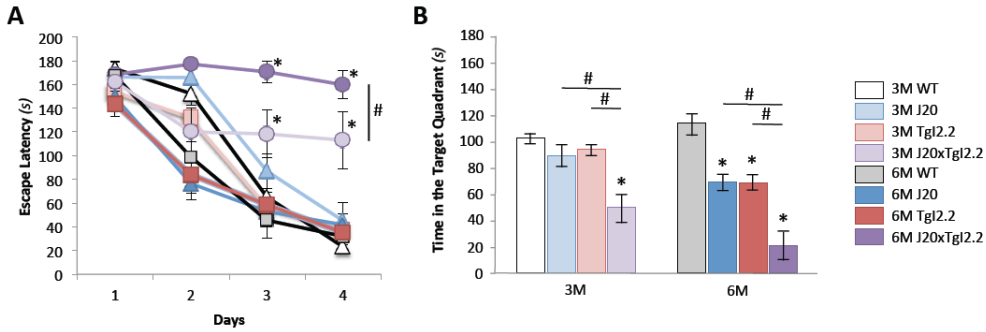

Figure 10. Overexpression of wild-type human αSyn in the mouse model of AD J20 causes impairments in memory acquisition in the Barnes circular maze. *3 and 6-month-old WT C57BL/6, J20, Tgl2.2 and J20xTgl2.2 mice were trained in the Barnes circular maze for 4 days. A probe trial (escape platform removed) was conducted 24h after the last training session.* ***(A)*** *During acquisition of the task, escape latency to complete the task was recorded. While J20 and Tgl2.2 groups learned this task comparably to WT, J20xTgl2.2 bigenic mice displayed a severe acquisition deficit at 3 and 6 months of age. In these mice, two-way repeated-measures ANOVA revealed an effect of transgene (F = 36.89, p = 0.0008) but no significant effect of training (F = 8.02, p = 0.8236). At 3 months of age, J20xTgl2.2 mice were partly able to learn the task while older bigenic mice failed to learn (#, p < 0.05).* ***(B)*** *During the probe trial on day 5, only WT mice displayed a search bias for the target quadrant. Bigenic J20xTgl2.2 mice consistently performed worse than age-matched single transgenic J20 and Tgl2.2 animals (Two-way ANOVA, p < 0.01). Data represent mean ± s.e.m. (n = 4-6 males/age/genotype; univariate RMANOVA test).*

Discussion

A key focus area in neurodegenerative diseases is the pathogenic contribution of misfolded proteins once aggregation occurs. In the past decade, however, there has been a paradigm shift towards studying the contribution of soluble, non-fibrillar forms of amyloid proteins such as Aβ, tau and αSyn (McLean et al., 1999; Walsh et al., 2002; Kayed et al., 2003; Cleary et al., 2005; Ramsden et al., 2005; SantaCruz et al., 2005; Lesne et al., 2006; Roberson et al., 2007; Shakar et al., 2008; Winner et al., 2011). In Alzheimer's disease, intense focus has been set on early aggregates of Aβ and tau, as the fibrillary forms of these proteins constitute the pathological hallmarks of the disease since the original description of the disorder by Dr. Alois Alzheimer in 1906. Last year however, we showed that the soluble form of another aggregation-prone amyloid protein, αSyn, might also be involved in AD pathophysiology (Larson et al., 2012). Since soluble multimeric assemblies of αSyn or αSyn oligomers have been reported to be neurotoxic (Danzer et al., 2007; Outeiro et al., 2008; Danzer et al., 2011; Winner et al., 2011; Colla et al., 2012), we hypothesized that the increase in apparent soluble αSyn monomers was accompanied with an elevation of αSyn oligomers, contributing to the observed decrease in synapsins and the exacerbation of memory deficits triggered by human Aβ and tau (**Figure 11**).

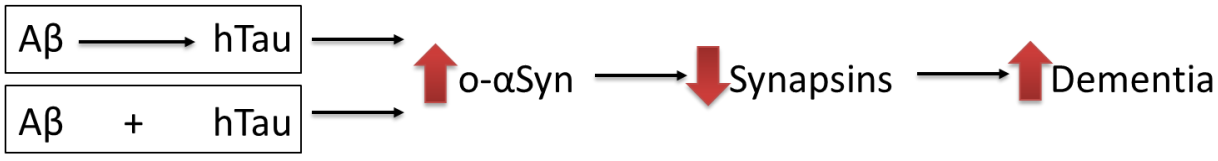

Figure 11. Hypothetical model illustrating the role of oligomeric αSyn in AD and AD-associated dementia.

Distinct soluble αSyn oligomers in AD

Our recent reports suggested that soluble αSyn might be responsible for the cognitive impairments seen in both TgI2.2 mice and subjects with AD. Previously, we found an abnormal elevation of soluble αSyn to be a better predictor of memory decline in AD patients than Aβ or tau (Larson et al., 2012). Here we found that oligomeric αSyn species were elevated in AD subjects compared with their age-matched controls. Using a combination of biochemical techniques including ELISA, dot blots, HFIP disassembly, SEC, SDS-PAGE, and Western blot analysis, we identified putative multimers of two monomeric αSyn species of 14 and 17 kDa that included putative dimers (~28 kDa and 35 kDa), and tetramers (~56 kDa and ~72 kDa). The recognition of this pattern suggests the possible existence of two likely pathways for the aggregation of αSyn *in vivo*, a principle first suggested by molecular modeling of αSyn aggregates (Tsigelny et al., 2007) and recently integrated into the proposed mechanisms of αSyn aggregation and propagation (Lashuel et al., 2013). However, we believe our identification in human and transgenic mouse brain tissue of both sets of these soluble αSyn assemblies might represent the first evidence of the smallest molecular bricks constituting these pathways. While other groups have provided evidence that SDS-resistant αSyn dimers can be detected in brain tissue (Kahle et al., 2001; Tsigelny et al., 2008), we speculate that the unique experimental biological specimens used, *i.e.*, brain tissue with elevated expression of αSyn combined with an absence of LB pathology, allowed us to detect multimers of 14 kDa and 17 kDa αSyn monomers.

We also believe that the detection of these various soluble forms was only possible through the use of the antibody 4D6, which we recognized to display enhanced sensitivity towards oligomeric forms of αSyn following relaxation or denaturation of αSyn molecules, even compared to antibodies specifically raised to detect o- αSyn such as Syn33 and F8H7 as shown in Figure 3. With these conditions, we were able to reliably identify and measure specific oligomeric forms of αSyn present in brain tissue. We found that o-αSyn appeared to accumulate intracellularly in AD brains compared to age-matched controls while soluble extracellular αSyn species remained

unaltered across clinical groups. Further, we reported that only a subset of intracellular αSyn oligomers inversely correlated with several premortem measures of cognition. These included putative 28 kDa αSyn dimers and 72 kDa αSyn tetramers. It is worth noting that while we observed an accumulation of a ~56 kDa αSyn species in IC fractions of AD brains, the levels of this possible tetramer of the 14 kDa monomeric αSyn were not correlated to deficits in memory modalities. While the exact structure and folding of endogenous αSyn remains controversial (Bartels et al., 2011; Fauvet et al., 2012; Burré et al., 2013), we posit that the ~56 kDa αSyn assembly detected could correspond to the ~55-60 kDa tetrameric αSyn identified by the Selkoe group (Bartels et al., 2011; Selkoe et al., 2013) and that this assembly might not be deleterious for neuronal function.

Synapsin lowering and cognitive deficits in AD

Supported by previous studies including our own (Nemani et al., 2010; Scott et al., 2010; Larson et al., 2012), it appeared that an elevation of soluble intracellular αSyn species is associated with a selective decrease in presynaptic proteins including synapsins. In this study, we further document that the amounts of o-αSyn detected by our ELISA assay using either LB509 or A11 antibodies under physiological conditions strongly correlated with reductions in synapsin levels in AD brains. This relationship appeared specific to o-αSyn/synapsins, as similar analyses with another presynaptic protein, synaptophysin, did not reveal any association with soluble o-αSyn levels. Moreover, we also found that total synapsin levels were correlated to the degree of episodic memory impairment within our ROS AD cohort, suggesting that decreases in synapsin isoform expression in AD might exacerbate Aβ- and tau-induced cognitive deficits. Our results are consistent with existing reports showing that reduction in synapsin-I gene expression induced by increased DNA methylation is linked to cognitive aging in rodents (Haberman et al., 2012) and showing that ablation of the synapsin-I gene, *SYN1*, causes age-dependent cognitive

impairment in mice (Corradi et al., 2008). In addition, 12- to 14-month-old *SYN1*-null mice display neuronal loss in the hippocampus and neocortex (Corradi et al., 2008) further highlighting the importance of putative changes in synapsin expression in AD. In humans, *SYN1* loss-of function mutations were recently shown to cause impaired synaptic function in autism and partial epilepsy (Fassio et al., 2011). Considering the results presented in this study and our previous results showing a 60-70% reduction in synapsins associated with the increase in soluble αSyn species in AD (Larson et al., 2012), we postulate that the lowering of synapsins observed in AD may mediate the enhancement of memory deficits triggered by o-αSyn.

Oligomeric αSyn-associated synapsin lowering and cognitive deficits in AD mice

To try to determine whether an elevation in soluble h-αSyn^{WT} oligomers could induce a selective reduction in the synapsin in a mouse model of AD, we generated a novel bigenic mouse line by crossing mice expressing human Aβ (J20 line) with animals expressing h-αSyn^{WT}. (TgI2.2 line). Based on earlier observations that Aβ and αSyn can potentiate the aggregation of each other *in vivo* (Masliah et al., 2001; Clinton et al., 2010), we anticipated that J20xTgI2.2 mice would display enhanced levels of o-αSyn species and would allow us to identify possible changes in synapsin expression and memory performance. At 3 months of age when pathological lesions are absent, o-αSyn levels were increased by ~4-fold in J20xTgI2.2 bigenic mice compared to TgI2.2 littermates while monomeric αSyn levels appeared similar. This marked rise of αSyn oligomers in brain tissues was associated with selective reductions in nearly all synapsin isoforms (Ia, IIa, IIb, IIIa) and with readily detectable deficits in learning and memory retention compared to single transgenic and wild-type animals. Although 6-month-old J20 and TgI2.2 animals displayed search bias deficits compared to WT mice, the deficit in memory retention observed in J20xTgI2.2 bigenic mice remained exacerbated. This observation contrasts with earlier results from the Masliah and Mucke groups showing that memory retention in 6-month-old bigenic hAPP/hSYN (J9 line x D line) and in hAPP mice were identical (Masliah et al., 2001). We

speculate that this apparent discrepancy between our two studies is likely due to the fact hSYN-line D elicits LB inclusions as early as 3 months of age and that motor function is compromised in bigenic hAPP/hSYN at 6 months (Masliah et al., 2000). Conversely, TgI2.2 mice do not develop pathological lesions (Lee et al., 2002) and did not display apparent motor deficits during our behavioral testing as assessed by animal speed (data not shown). Overall, our genetic experiment replicated the changes observed in AD brain tissue and supports the notion that an increase in o-αSyn is associated with synapsin lowering and potentiation of Aβ-induced cognitive impairment.

Altogether, we believe that soluble αSyn species are intrinsic component of the sequence of events leading to dementia in Alzheimer's disease thereby exacerbating the severity of cognitive impairment mediated by a selective lowering of synapsins. If confirmed by other groups and in other human cohorts, this αSyn/synapsin pathway might constitute a novel therapeutic target for limiting further cognitive decline and offer hope to patients at early stages of the disease.

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