α-synuclein induced synapse damage is enhanced by amyloid-β1-42

Clive Bate1*, Steve Gentleman2, Alun Williams1,3

Abstract

Background: The pathogenesis of Parkinson’s disease (PD) and dementia with Lewy bodies (DLB) is associated with the accumulation of aggregated forms of the α-synuclein (αSN) protein. An early event in the neuropathology of PD and DLB is the loss of synapses and a corresponding reduction in the level of synaptic proteins. However, the molecular mechanisms involved in synapse damage in these diseases are poorly understood. In this study the process of synapse damage was investigated by measuring the amount of synaptophysin, a pre-synaptic membrane protein essential for neurotransmission, in cultured neurons incubated with αSN, or with amyloid-β (Aβ) peptides that are thought to trigger synapse degeneration in Alzheimer’s disease.

Results: We report that the addition of recombinant human αSN reduced the amount of synaptophysin in cultured cortical and hippocampal neurons indicative of synapse damage. αSN also reduced synaptic vesicle recycling, as measured by the uptake of the fluorescent dye FM1-43. These effects of αSN on synapses were modified by interactions with other proteins. Thus, the addition of βSN reduced the effects of αSN on synapses. In contrast, the addition of amyloid-β (Aβ)1-42 exacerbated the effects of αSN on synaptic vesicle recycling and synapse damage. Similarly, the addition of αSN increased synapse damage induced by Aβ1-42. However, this effect of αSN was selective as it did not affect synapse damage induced by the prion-derived peptide PrP82-146.

Conclusions: These results are consistent with the hypothesis that oligomers of αSN trigger synapse damage in the brains of Parkinson’s disease patients. Moreover, they suggest that the effect of αSN on synapses may be influenced by interactions with other peptides produced within the brain.

Background

Parkinson’s disease (PD) is a neurodegenerative motor disorder affecting up to 2% of the population over the age of 65. Although it is characterised by the presence of bradykinesia, resting tremor and rigidity, up to 88% of patients also show significant psychiatric and autonomic symptoms [1]. The most common of these non-motor symptoms are Parkinson’s disease dementia (PDD), with a cumulative prevalence ranging between 50 and 75% of cases [2] and dementia with Lewy Bodies (DLB), a similar condition to PDD except that dementia rather than motor symptoms are primary. DLB is the second most common cause of dementia after Alzheimer’s disease (AD) and is characterised by progressive cognitive decline and parkinsonism [3]. Currently, there is no long-term cure for PD, PDD or DLB.

The major histopathological hallmark of PD, PDD and DLB is the alpha-synuclein (αSN) positive intraneuronal inclusion known as a Lewy body (LB). Although the presence of LBs in the substantia nigra is diagnostic for PD, αSN pathology is also seen in multiple extranigral regions and may account for the wide range of non-motor symptoms observed. The detailed mechanisms underlying the pathological changes in PD are not known but αSN is thought to play a central role. αSN is predominantly expressed in central nervous system neurons where it is localised to pre-synaptic terminals, regulates synaptic vesicle formation and neurotransmitter release [4,5] and can affect synaptic plasticity during learning [6]. However, recent evidence suggests that small oligomer aggregates of αSN accumulate at the pre-synaptic membrane and trigger synapse degeneration in PD and DLB [7-9]. The transfer of αSN to neighbouring neurons...
may account for the stereotypical progression of αSN pathology through the brain similar to the staging of tau pathology in AD [12]. The loss of synapses in the hippocampus is characteristic of the PD patients that develop dementia [13] and in a rat model of α-synucleinopathy, synaptic degeneration preceded neuronal loss [14]. Thus, synapse degeneration is a common feature observed in PD, PDD and DLB.

There has been little study of the molecular mechanisms underpinning αSN induced synapse degeneration in these disorders. To investigate these mechanisms the effect of αSN on synapses in cultured cortical or hippocampal neurons was determined by quantifying the amount of synaptophysin using an enzyme-linked immunoassay (ELISA) [15]. Synaptophysin is a pre-synaptic membrane protein associated with recycling vesicles that are essential for neurotransmission [16,17] and the amount of synaptophysin has been used to access synaptic density in the brain [18-20] and cultured neurons [15]. An understanding of the molecular mechanisms that underlie αSN-induced synapse damage may help identify drugs that reduce this process.

**Results**

**αSN causes synapse damage**

The synapse degeneration in PD and DLB that is associated with oligomers of αSN [7-9] was modelled in vitro. The addition of recombinant human αSN reduced the synaptophysin content of cortical neurons in a dose-dependent manner (Figure 1A). The synaptophysin content was reduced to 50% of control neurons (EC50) following the addition of 500 nM αSN. This effect of αSN on synapses occurred at concentrations that did not kill neurons; for example, the addition of 10 μM αSN reduced the synaptophysin content of cortical neurons by greater than 80% without affecting their viability as measured by thiazolyl blue tetrazolium (100% cell survival ± 8 compared with 96% ± 6, n = 9, P = 0.22). Similarly, immunoblots showed that αSN reduced the amount of synaptophysin in neuronal extracts without affecting the amount of β-actin (Figure 1B). The addition of βSN, another member of the synuclein family of proteins [6], did not affect the synaptophysin content of cortical neurons. We found that the addition of αSN, but not βSN, also reduced the amount of synaptophysin in hippocampal neurons (Figure 1C), an observation consistent with a report that the loss of synapses in the hippocampus is characteristic of the PD patients that develop dementia [13].

**αSN reduced synaptic vesicle recycling**

The uptake of FM1-43, a fluorescent dye that is taken up into synaptic vesicles, was used as a measure of synaptic vesicle recycling and hence neurotransmission [21]. Here we report that the uptake of FM1-43 by cortical neurons was reduced following the addition of αSN, but not after the addition of βSN (Figure 2). This effect of αSN was observed at lower concentrations than that required to reduce the synaptophysin content of neurons and the concentration of αSN required to reduce synaptic vesicle recycling by 50% was approximately 30 nM.

**βSN reduced αSN-induced synapse damage**

Although transgenic mice studies showed that the expression of βSN reduced neurodegeneration in mice expressing human αSN [22,23], the molecular mechanisms underlying the interactions between αSN and βSN are unknown. We found that pre-mixing αSN with an excess of βSN (1:10) reduced the αSN-induced loss of synaptophysin in cortical neurons, whereas pre-mixing αSN with human serum albumin (1:10) had no affect (Figure 3). As this result may have been caused by a
direct effect of βSN on neurons, cortical neurons were pre-treated with 10 μM βSN, washed and then incubated with αSN. Pre-treatment of neurons with βSN did not affect the loss of synaptophysin induced by αSN (data not shown).

**Aβ1-42 enhanced αSN-induced synapse damage**

The amyloid hypothesis of Alzheimer’s disease (AD) pathogenesis maintains that the primary event is the production of neurotoxic amyloid-β (Aβ) peptides following the proteolytic cleavage of the amyloid precursor protein into different sized fragments [24,25]. These fragments include Aβ1-42 which is widely regarded as a major pathogenic species in AD [26]. Since recent reports showed that αSN and Aβ1-42 co-exist in heterologous oligomers [27,28] the effect of Aβ1-42 on αSN-induced loss of synaptophysin was examined by pre-mixing the two peptides. The addition of Aβ1-42 in the ratio (1:50) increased αSN-induced synapse damage (Figure 4A). Thus, while the EC50 of αSN alone was 500 nM, the EC50 of Aβ1-42:αSN (1:50) was 25 nM. These concentrations of Aβ1-42 did not affect synapses when added on their own. In contrast, pre-mixing αSN with the control peptide Aβ42-1 (1:50) did not affect αSN-induced loss of synaptophysin. Since the predominant Aβ species found within the brain is Aβ1-40 [29] the effect of Aβ1-40 on αSN was...
also tested. However, there was no significant difference in the synaptophysin content of cortical neurons incubated with αSN and neurons incubated with Aβ1-42/αSN (1:50) (data not shown). These results may have been caused by a direct effect of Aβ1-42 on the neurons. Our observation that pre-treatment of cortical neurons with 1 nM Aβ1-42 did not affect αSN-induced loss of synaptophysin (Figure 4B) suggested that Aβ1-42 did not sensitise neurons to the effects of αSN.

The ability of synthetic Aβ peptides to self associate results in a mixture of physical complexes ranging from small soluble oligomers to large fibrils. Since the dynamic nature of Aβ aggregation means that it is difficult to ascribe biological function to specific Aβ assemblies using synthetic peptides, the activity of naturally derived, stable Aβ oligomers was also examined. We found that pre-mixing Aβ with 7PA2-conditioned medium (7PA2-CM), which contained naturally secreted stable Aβ oligomers [30,31], increased the αSN-induced loss of synaptophysin in cortical neurons (Figure 4C). In contrast, pre-mixing αSN with CHO-CM had no effect.

Aβ enhanced αSN-induced inhibition of synaptic vesicle recycling
The addition of Aβ oligomers also affected αSN-induced inhibition of synaptic vesicle recycling. Thus, pre-mixing αSN with 7PA2-CM enhanced αSN-induced inhibition of FM1-43 uptake into synapses. The concentration of αSN alone required to reduce synaptic vesicle recycling by 50% was 30 nM, while the concentration of αSN that had been mixed with 7PA2-CM to have a similar level of effect was 1 nM (Figure 5).

αSN enhanced Aβ1-42-induced synapse damage
Next we examined whether non-toxic concentrations of αSN affected Aβ1-42-induced loss of synaptophysin. Here we show that pre-mixing Aβ1-42 with αSN increased the Aβ1-42-induced loss of synaptophysin from neurons (Figure 6). Thus, while the EC50 of Aβ1-42 alone was 50 nM, the EC50 of αSN:Aβ1-42 (2:1) was 5 nM. In contrast, the addition of βSN (2:1) did not affect Aβ1-42-induced loss of synaptophysin. Pre-treatment of cortical neurons with 10 nM αSN did not affect Aβ1-42-induced loss of synaptophysin (data not shown). We also sought to determine if αSN affected another peptide that caused synapse damage. Synapse degeneration is a feature of human and experimental prion diseases [32,33] which was modelled by the addition of the prion-derived peptide PrP82-146 to cortical neurons [15]. As shown in Figure 6B, there was no difference in the synaptophysin content of cortical neurons incubated with PrP82-146 and those incubated with a combination of αSN/PrP82-146 (2:1).

αSN did not affect the accumulation of Aβ1-42 in synapses
We explored the possibility that αSN increased the binding of Aβ1-42 to synapses as an explanation of the effect of αSN on Aβ1-42-induced loss of synaptophysin. Time course studies showed that Aβ1-42 accumulated in synaptosomes isolated from cortical neurons after 1 hour. Therefore cortical neurons were incubated with 100 nM biotinylated Aβ1-42, or 100 nM biotinylated-Aβ1-42 that had been pre-mixed with 500 nM αSN for 1 hour. The amount of biotinylated Aβ1-42 found in synaptosomes isolated from these neurons was not altered by pre-mixing with αSN (Figure 7A), indicating that αSN did not alter the binding, or trafficking of Aβ1-42 to synapses. The effect of Aβ1-42 on the accumulation of αSN in synapses was also examined. Cortical neurons were incubated with 200 nM αSN, or a combination of 4 nM Aβ1-42 and 200 nM αSN (1:50), for 1 hour and synaptosomes prepared. Immunoblots showed that the amount of αSN found within synapses was not affected by presence of Aβ1-42 (Figure 7B).

Discussion
The loss of synapses is a prominent feature of many neurodegenerative diseases including AD, PDD and LBD. The main mediators of neuropathology in PDD

![Figure 5 Aβ1-42 enhanced the αSN-induced inhibition of synaptic vesicle recycling](image-url)
and LBD are thought to be oligomers of αSN [7,9] and in this study the addition of αSN impaired synapse function and triggered a loss of synaptophysin from cortical neurons. These effects occurred at concentrations of αSN that did not affect neuronal survival; an observation consistent with reports that synapse degeneration preceded neuronal loss in a rat model of α-synucleinopathy [14]. A reduction in the synaptophysin content of hippocampal neurons was observed after incubation with αSN, consistent with reports that a loss of synapses in the hippocampus is characteristic of the PD patients that develop dementia [13].

The addition of βSN did not affect synapses indicating that synaptic defects were dependent upon the specific amino acid sequence of αSN. Recent reports from a transgenic mouse model of PD showed that the expression of βSN reduced the accumulation of αSN and neurodegeneration in mice expressing human αSN [22,23]. Another study showed that βSN formed mixed oligomers with αSN [34]. In this study we showed that mixing with βSN reduced the loss of synaptophysin induced by αSN; results consistent with the idea that molecular interactions between αSN and βSN affect the toxicity of αSN.

Approximately 25% of AD patients develop parkinsonism and 50% of PD patients develop AD-type dementia after 65 years of age [35]. In addition, 70% of patients with sporadic AD display αSN-positive, LB-like inclusions in the amygdala and limbic structures [36-38]. The loss of synapses that occurs in AD is associated with the production of Aβ oligomers [39-41]. Both Aβ and αSN accumulate in the brain in DLB [3] and levels of αSN are increased in AD [42], observations which suggest that interactions between αSN and Aβ affect the pathogenesis of AD, PDD and DLB [43].

The addition of small amounts of Aβ1-42, which had no effect on synapses on their own, increased the effects of αSN on synapses. Critically pre-treatment with Aβ1-42...
did not sensitize cortical neurons to the synaptic effects of αSN and while we cannot exclude the possibility of a transient sensitizing effect of Aβ1-42, our results suggest that direct interactions between Aβ1-42 and αSN increased the toxicity of αSN. The studies using synthetic Aβ1-42 peptides were complemented by studies using 7PA2-CM containing naturally secreted, stable Aβ oligomers [30,31]. Pre-mixing with 7PA2-CM also increased the effects of αSN upon synaptic vesicle recycling and synapse damage. Relatively small amounts of Aβ1-42 (1:50) were required to facilitate the αSN-induced loss of synaptophysin suggesting that Aβ1-42 seeded the formation of toxic αSN oligomers; an observation consistent with reports that Aβ promotes the aggregation of αSN in transgenic mice [44]. Conversely, the addition of non-toxic concentrations of αSN increased Aβ1-42-induced loss of synaptophysin.

Aβ1-42 exists in multiple forms from small soluble toxic oligomers to large insoluble amyloid fibrils. As the toxicity of Aβ1-42 is affected by its state of aggregation [45,46] the addition of αSN may stabilize Aβ1-42 oligomers in a toxic configuration. Mixing αSN with Aβ1-42 did not increase the amount of αSN or Aβ1-42 found within synapses showing that the increased toxicity of hetero-oligomers was not due to increased binding of Aβ1-42 or αSN to synapses.

Although Aβ1-42 is considered to be the major neurotoxin generated in AD [47-50] other Aβ fragments are produced [24,25,51] and since Aβ1-40 is the predominant Aβ species formed in AD [29,52] the effect of Aβ1-40 on αSN-induced loss of synaptophysin was also tested. We found that αSN did not affect the reduction in synaptophysin in response to Aβ1-40, nor did Aβ1-40 affect the reduction in synaptophysin induced by αSN. Synapse degeneration is also a feature of human and experimental prion diseases [32,33] and the prion-derived peptide PrP82-146 triggered a reduction in synaptophysin in cortical neurons [15]. Although PrP82-146 has similar biophysical properties to Aβ1-42 in that it adopts a β-helix-rich conformation, forms oligomers and fibrils which are protease resistant [53], αSN did not affect PrP82-146-induced loss of synaptophysin.

Conclusions
We conclude that the addition of αSN reduced the synaptophysin content of cultured cortical and hippocampal neurons, a model that mimics the synapse damage observed in PDD and DLB. The effect of αSN was modified by other proteins found in the central nervous system including βSN which reduced the effects of αSN, and Aβ1-42 which increased the effects of αSN on synapses. Conversely, αSN increased the effects of Aβ1-42 on synapses. Our results suggest that interactions between the synucleins and Aβ peptides may affect synapses in AD, PDD and Lewy body disorders.

Methods
Primary neuronal cultures
Cortical neurons were prepared from the brains of mouse embryos (day 15.5) as described [15]. Neurons were plated at 2 x 10^5 cells/well in pre-coated 48 well plates (5 μg/ml poly-L-lysine) in Ham’s F12 (PAA) containing 5% foetal calf serum (FCS) for 2 hours. Cultures were shaken (600 r.p.m for 5 minutes) and non-adherent cells removed by 3 washes in PBS. Neurons were grown in neurobasal medium (NBM) containing B27 components (PAA) for 10 days. Immunohistochemistry showed that the cells were greater than 97% neurofilament positive. Fewer than 3% of cells stained for glial fibrillary acidic protein (astrocytes) or for F4/80 (microglial cells). Hippocampal neurons were prepared from the brains of adult mice as described [54]. Hippocampi were dissected from brains and triturated in Ham’s F12 containing 5% FCS, 0.35% glucose, 0.025% trypsin, and 0.1% type IV collagenase. After 30 minutes at 37°C, the cells were triturated and the cell suspension was passed through a 100 μM cell strainer. Cells were collected, washed twice and plated at 2 x 10^5 cells/well in 48 well plates (pre-coated with poly-L-lysine). After 24 hours cultures were shaken (600 r.p.m for 5 minutes) to remove non-adherent cells, washed twice and the remaining neurons were cultured in NBM/B27 and 10 ng/ml glial-derived neurotrophic factor (Sigma) for 7 days. Neurons were incubated with peptides for 24 hours and the amount of synaptophysin in cell extracts measured.

Cell extracts
Neurons were washed 3 times with PBS and homogenised in a buffer containing 150 mM NaCl, 10 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.5% Nonidet P-40, 0.5% sodium deoxycholate, 0.2% sodium dodecyl sulphate (SDS) and mixed protease inhibitors (4-(2-Aminothyl) benzenesulfonyl fluoride hydrochloride (AEBSF), Aprotinin, Leupeptin, Bestatin, Pepstatin A and E-64) (Sigma) at 10^6 cells/ml. For immunobLOTS cells were homogenised in extraction buffer (as above) at 10^7 cells/ml and digested with DNase (Sigma) for 1 hour at 37°C. Cell debris was removed by low speed centrifugation (300 x g for 5 minutes).

Synaptophysin ELISA
The amount of synaptophysin in neuronal extracts was measured by ELISA [15]. Briefly, the capture mAb was anti-synaptophysin MAB368 (Millipore). Samples were added for 1 hour and bound synaptophysin was detected using rabbit polyclonal anti-synaptophysin (Abcam)
followed by a biotinylated anti-rabbit IgG (Dako), extravidin-alkaline phosphatase and 1 mg/ml 4-nitrophenol phosphate. Absorbance was measured on a microplate reader at 405 nm and the synaptophysin content of samples was expressed as units where 100 units was defined as the amount of synaptophysin in untreated neurons.

Synaptic vesicle recycling
The fluorescent styryl dye FM1-43 (Biotium) that is readily taken up into synaptic recycling vesicles [55] was used to determine synaptic activity as described [21]. Treated neurons were incubated with 1 μg/ml FM1-43 and 1 mM acetylcholine (ACh) for 10 minutes, washed 5 times in ice cold PBS and solubilised in methanol at 1 x 10⁶ neurons/ml. Soluble extracts were transferred into Sterlin 96 well black microplates and fluorescence was measured using excitation at 480 nm and emission at 525 nm. Background fluorescence was subtracted and samples were expressed as “% fluorescence” where 100% fluorescence was defined as the amount of fluorescence in untreated neurons incubated with FM1-43 and ACh.

Synaptosome preparations
Synaptosomes were prepared on a discontinuous Percoll gradient [56]. Briefly, 10⁶ cortical neurons were homogenized at 4°C in 1 ml of SED solution (0.32 M sucrose, 50 mM Tris-HCl, pH 7.2, 1 mM EDTA, and 1 mM dithiothreitol) and centrifuged at 1000 x g for 10 minutes. The supernatant was transferred to a gradient of 3, 7, 15, and 23% Percoll in SED solution and centrifuged at 16,000 x g for 30 minutes at 4°C. The synaptosomes were collected from the interface of the 15% and 23% Percoll steps. The fraction was washed twice (16,000 x g for 30 minutes at 4°C) and suspended in extraction buffer containing 150 mM NaCl, 10 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.2% sodium dodecyl sulphate and mixed protease inhibitors.

Biotinylated Aβ1-42 ELISA
The amounts of biotinylated Aβ1-42 in extracts were determined by ELISA. Nunc Maxisorb Immunoplates were coated with 1 μg/ml protein A (Innova) followed by 0.1 μg/ml mAb reactive to amino acids 1 to 16 of β-amyloid (clone 6E10 - Signet) and blocked with 5% milk powder. Samples were boiled in 0.2% SDS, cooled and incubated for 1 hour. Biotinylated Aβ1-42 was detected with extravidin-alkaline phosphatase and 1 mg/ml 4-nitrophenol phosphate (Sigma). Absorbance was measured on a microplate reader at 405 nm and results were calculated by reference to a standard curve generated form serial dilutions of biotinylated Aβ1-42.

Western analysis
Samples were mixed with an equal volume of Laemmli buffer, boiled, and subjected to electrophoresis on a 15% polyacrylamide gel (Invitrogen). Proteins were transferred onto a Hybond-P PVDF membrane (Amersham Biotech) by semi-dry blotting. Membranes were blocked using 10% milk powder; synaptophysin was detected using a mouse monoclonal antibody (mAb) anti-synaptophysin SY38 (Abcam), β-actin was detected by incubation with a mouse mAb (clone AC-74, Sigma) and human αSN was detected by incubation with mAb 211 raised against amino acids 121 to 125 of human αSN (Santa Cruz Biotech). These were visualised using a combination of biotinylated secondary antibodies (Dako), extravidin-peroxidase and an enhanced chemiluminescence kit.

Peptides
Recombinant human αSN and βSN were purchased from Sigma. Synthetic peptides containing the amino acids 1 to 42 (Aβ1-42) or 1 to 40 (Aβ1-40) of the Aβ protein, biotinylated-Aβ1-42 and a control peptide consisting of amino acids 1 to 42 in reverse order (Aβ1-42c) were obtained from Bachem. Peptides containing amino acids 82 to 146 of the human PrP protein (PrP82-146) and was a gift from Professor Salmona (Mario Negri, Milan). Aβ peptides were first dissolved in hexafluoroisopropanol, lyophilised and subsequently solubilised and stored at 1 mM in DMSO. Stock solutions of peptides were stored at 1 mM, thawed on the day of use and diluted/mixed in NBM for 1 hour at 37°C. Dilutions/mixtures were subjected to vigorous shaking (Disruptor Genie, full power for 10 minutes) before they were added to neurons. Chinese hamster ovary (CHO) cells stably transfected with a cDNA encoding APP751 containing the Val717Phe familial AD mutation (referred to as 7PA2 cells) were cultured in DMEM for 10% FCS [30,31]. Conditioned medium from these cells contains stable Aβ oligomers (7PA2-CM). Conditioned medium from non-transfected CHO cells (CHO-CM) was used as controls. These were mixed with peptides and subjected to vigorous shaking (as above), diluted in NBM and added to neurons.

Statistical Analysis
Differences between treatment groups were determined by 2 sample, paired T-tests. For all statistical tests significance was set at the 1% level.

Abbreviations
(ACh): Acetylcholine; (AD): Alzheimer’s disease; (αSN): alpha-synuclein; (βSN): beta-synuclein; (Aβ): amyloid β; (CHO): Chinese hamster ovary; (CM): conditioned medium; (DLB): dementia with Lewy bodies; (DMSO): di-methyl sulphoxide; (ELISA): Enzyme linked immunoassay; (NBM): neurobasal
medium, (LB): Lewy body; (PD): Parkinson’s disease; (PDD): Parkinson’s diseases dementia. (PBS): phosphate buffered saline.

Acknowledgements
This work was supported by a grant from the European Commission FP6 - NeuroFron - Network of Excellence and NIH grant AG 12411. We thank Professor Mario Salmona (Mario Negri, Milan) for supplying the PrPβ2-146 peptide.

Author details
1Department of Pathology and Infectious Diseases, Royal Veterinary College, Hawkshede Lane, North Mymms, Herts, AL9 7TA, UK. 2Neuropathology Unit, Department of Medicine, Imperial College London, Charing Cross Campus, St Dunstan’s Road, London, W6 8RP, UK. 3Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge, CB3 0ES, UK.

Authors’ contributions
CB was responsible for the conception, planning and performance of experiments and for writing the manuscript. AW and SG contributed to the planning of experiments, interpretation of results and the writing of the manuscript. All authors approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Received: 5 August 2010 Accepted: 7 December 2010

References
1. Poewe W: Non-motor symptoms in Parkinson’s disease. Eur J Neurol 2008, 15(Suppl 1):13-14.
2. Aarsland D, Andersen K, Larsen JP, Lolk A, Kragh-Sorensen P: Synaptic pathology in the preclinical stage of Parkinson disease. J Neurol Neurosurg Psychiatry 2006, 77(5):522-527.
3. McKee IG, Dickson DW, Lowe J, Emre M, O’Brien JT, Feldman H, Cummings J, Duda E, Lippa EK, et al: Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. Neurology 2005, 65(12):1863-1872.
4. Bonini NM, Giasson BI: Synaptic vesicle proteins and regulated exocytosis. J Cell Sci 1993, 77:15-79.
5. Cabin DE, Shimazu K, Murphy D, Cole NB, Gottschalk W, McIlwain KL, Orrison B, Chen A, Ellis CE, Paylor R, et al: Synaptic vesicle protein B3 (SV2B): Synaptic vesicle proteins and regulated exocytosis. J Cell Sci 1993, 77:15-79.
6. Clayton DF, George JM: Mechanisms of Parkinson’s disease dementia. Dementia with Lewy bodies: a clinical and pathologic entity. Arch Neurol 2000, 57(10):13010-13015.
7. Brundin P, Melki R, Kostopoulou M, Prion-like transmission of protein aggregates in neurodegenerative diseases. Nat Rev Mol Cell Biol 2010, 11(4):301-307.
8. Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E: Staging of brain pathology related to sporadic Parkinson’s disease. Neurobiol Aging 2003, 24(2):197-211.
9. Galvin JE, Uryu K, Lee VM, Trojanowski JQ: Axon pathology in Parkinson’s disease and Lewy body dementia hippocampus contains alpha-, beta-, and gamma-synuclein. Proc Natl Acad Sci USA 1999, 96(23):13450-13455.
10. Chung CY, Koprich JB, Siddiqi H, Iascon A: Dynamic changes in presynaptic and axonal transport proteins combined with striatal neuroinflammation precede dopaminergic neuronal loss in a rat model of AAV alpha-synucleinopathy. J Neurosci 2009, 29(11):3365-3373.
11. Brundin P, Melki R, Kopito R: Mechanisms of Parkinson’s disease. NeuroRx 2010, 7(3):203-214.
12. Efendik LA, Scheller RH: Synaptic vesicle proteins and regulated exocytosis. J Cell Sci 1993, 17:75-79.
13. Eby G, Brown GM, Park BS, Jacques J,Jude R, Whetzel WJ Jr, Kaye J, Manczik M: Differential loss of synaptic proteins in Alzheimer’s disease: implications for synaptic dysfunction. J Alzheimers Dis 2005, 7(2):103-117.
14. Counts SE, Nadeem M, Lad SP, Wuu J, Mufson EJ: Differential expression of synaptic proteins in the frontal and temporal cortex of elderly subjects with mild cognitive impairment. J Neuropath Exp Neurol 2006, 65(6):592-601.
15. Modi E, Terry RD, Alford M, DeTeresa R, Hansen LA: Cortical and subcortical patterns of synaptophysinlike immunoactivity in Alzheimer’s disease. Am J Path 1991, 138(1):235-246.
16. Bate C, Williams A: Amyloid-β 1-40 induces Activation of Cytoplasmic Phospholipase A2 and Synapse Degeneration. J Alzheimers Dis 2010, 21(3):985-993.
17. Hashimoto M, Rockenstein E, Mante M, Mallory M, Masliah E: β-Synuclein inhibits alpha-synuclein aggregation: a possible role as an anti-parkinsonian factor. Neuron 2001, 32(2):213-223.
18. Park JY, Lansbury PT Jr: Beta-synuclein inhibits formation of alpha-synuclein protofibrils: a possible therapeutic strategy against Parkinson’s disease. Biochemistry 2003, 42(13):3696-3700.
19. Vassar R, Citron M: Aβ-Generating Enzymes: Recent Advances in β- and γ-Secretase Research. Neuron 2000, 27(3):419-422.
20. Hardy J, Selkoe DJ: The amyloid hypothesis of Alzheimer’s disease: progress and problems on the road to therapeutics. Science 2002, 297(5580):353-356.
21. Parkin BA, Lu T: Amyloid β peptide toxicity and the pathogenesis of Alzheimer disease. Biochem 2009, 28(8):4755-4759.
22. Tsigelny IF, Crews L, Desplats P, Shaked GM, Sharikov Y, Mizuno H, Spencer B, Rockenstein E, Trejo M, Platsoukas D, et al: Mechanisms of Hybrid Oligomer Formation in the Pathogenesis of Combined Alzheimer’s and Parkinson’s Diseases. PLoS ONE 2008, 3(9):e3135.
23. Mandal P, Pettigrew J, Masliah E, Hamilton R, Mandal R: Interaction between Aβ Peptide and a Synuclein: Molecular Mechanisms in Overlapping Pathology of Alzheimer’s and Parkinson’s in Dementia with Lewy Body Disease. Neurochem Res 2006, 31(9):1153-1162.
24. Borchelt DR, Thirunagaran G, Eckman CB, Lee MK, Davenport F, Ratovitsky T, Prada CM, Kim G, Selkoe DJ, Yager D, et al: Familial Alzheimer’s disease-linked presenilin 1 variants elevate Aβ1-42/40 ratio in vitro and in vivo. J Neurosci 2006, 26(12):1005-1013.
25. Koo EH, Squazzo SL: Evidence that production and release of amyloid β-protein involves the endothocytic pathway. J Biol Chem 1994, 269(26):17386-17389.
26. Podlstry MB, Ottaszewski BL, Squazzo SL, Koo EH, Rydell RE, Teplow DB, Selkoe DJ: Aggregation of secreted amyloid β-protein into sodium dodecyl sulfate-stable oligomers in cell culture. J Biol Chem 1996, 271(16):9564-9570.
27. Jeffrey M, Halliday WG, Bell J, Johnston AR, MacLeod NK, Ingham C, Sayers AR, Brown DA, Fraser JR: Synapse loss associated with abnormal PrP precedes neuronal degeneration in the scrapie-infected murine hippocampus. Neuropath Appl Neurobiol 2000, 26(1):41-54.
28. Feiner I: Synaptic pathology and cell death in the cerebellum in Creutzfeldt-Jakob disease. Cereb Res 2002, 13(3):213-222.
29. Israili E, Sharon R: Beta-Synuclein occurs in vivo in lipid-associated oligomers and forms hetero-oligomers with alpha-synuclein. J Neurochem 2009, 108(2):465-474.
30. Hansen L, Salmon D, Galasko D, Masliah E, Katzman R, DeTeresa R, Thal L, Pay MM, Hofsatter R, Kluber M, et al: The Lewy body variant of Alzheimer’s disease: a clinical and pathologic entity. Neurology 1990, 40(1):1-8.
31. Lippa CF, Fujisawa H, Mann DM, Gasson B, Baba M, Schmidt ML, Nee LE, O’Connell B, Pollen DA, St George-Hyslop P, et al: Lewy bodies contain altered alpha-synuclein in brains of many familial Alzheimer’s disease
patients with mutations in presenilin and amyloid precursor protein genes. Am J Pathol 1998, 153(5):1365-1370.

37. Trojanowski JQ, Goelet M, Iwatsubo T, Lee VM: Fatal attractions: abnormal protein aggregation and neuron death in Parkinson's disease and Lewy body dementia. Cell Death Differ 1998, 5(10):832-837.

38. Hamilton RL: Lewy bodies in Alzheimer's disease: a neuropathological review of 145 cases using alpha-synuclein immunohistochemistry. Brain Pathol 2000, 10(3):378-384.

39. Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, Brett FM, Farrell MA, Rovan MJ, Lemere CA, et al: Amyloid-β protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. Nat Med 2008, 14(8):837-842.

40. Takahashi RH, Almeida CG, Kearney PF, Yu F, Lin MT, Milner TA, Gours G: Oligomerization of Alzheimer's β-Amyloid within Processes and Synapses of Cultured Neurons and Brain. J Neurosci 2004, 24(14):3592-3599.

41. Walsh DM, Klubin I, Fadeeva JV, Cullen WK, Amyyl R, Wolfe MS, Rovan MJ, Selkoe DJ: Naturally secreted oligomers of amyloid β protein potently inhibit hippocampal long-term potentiation in vivo. Nature 2002, 416(6880):535-539.

42. Iwai A, Masliah E, Sundsro MP, DeTeresa R, Mallory M, Salmon DP, Saitoh T: The synaptic protein NACP is abnormally expressed during the progression of Alzheimer's disease. Brain Res 1996, 7201-2730-274.

43. Creve L, Tigeltyn I, Hashimoto M, Masliah E: Role of Synucleins in Alzheimer's Disease. Neurotox Res 2009, 16(3):306-317.

44. Masliah E, Rockenstein E, Vincenzi S, Sagara Y, Mallory M, Hashimoto M, Mucke L: β-amyloid peptides enhance alpha-synuclein accumulation and neuronal deficits in a transgenic mouse model linking Alzheimer's disease and Parkinson's disease. Proc Natl Acad Sci USA 2001, 98(21):12245-12250.

45. Dahlgren KN, Manelli AM, Stine WB Jr, Baker LJ, Kraft GA, LaDu MJ: Oligomeric and Fibrillar Species of Amyloid-β Peptides Differentially Affect Neuronal Viability. JBColChem 2002, 277(35):32046-32053.

46. Pike CJ, Burdick D, Walenczuk AJ, Glabe CG, Cotman CW: Neurodegeneration induced by β-amyloid peptides in vitro: the role of peptide assembly state. J Neurosci 1993, 13(4):1676-1687.

47. Ebert RH, Barlow AK, Chmonty BA, Edwards C, Freed R, Liosatos M, Morgan TE, Rozovsky I, Trommer B, Viola KL, et al: Diffusible, nonfibrillar ligands derived from Aβ1-42 are potent central nervous system neurotoxins. Proc Natl Acad Sci USA 1998, 95(11):648-6453.

48. Lacor PN, Buriel MC, Furtow PW, Sanz Clemente A, Velasco PT, Wood M, Viola KL, Klein WU: Aβ Oligomer-Induced Alterations in Synapse Composition, Shape, and Density Provide a Molecular Basis for Loss of Connectivity in Alzheimer's Disease. J Neurosci 2007, 27(4):796-807.

49. Shaykhar GM, Bloodgood BL, Townsend M, Walsh DW, Selkoe DJ, Sabatini BL: Natural Oligomers of the Alzheimer Amyloid-β Protein Induce Reversible Synapse Loss by Modulating an NMDA-Type Glutamate Receptor-Dependent Signaling Pathway. J Neurosci 2007, 27(11):2866-2875.

50. Haass C, Selkoe DJ: Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid β-peptide. Nat Rev Mol Cell Biol 2007, 8(2):101-112.

51. Passer B, Pellegrini L, Russo C, Siegel RM, Lenardo MJ, Schettini G, Bachmann M, Talbott M, D'Adamo L: Generation of an Apoptotic Intracellular Peptide by γ-Secretase Cleavage of Alzheimer's Amyloid β-Protein Precursor. JAlzheimersDis 2000, 2:289-301.

52. Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N, Bird TD, Hardy J, Hutton M, Kirk W et al: Secreted amyloid β-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nat Med 1996, 2(8):864-870.

53. Salerno M, Morena M, Massagno T, Colombio L, Mazzenzoli G, Capobianco R, Diomede L, Thaler F, Mollica L, Musco G, et al: Structural properties of Gerstmann-Straussler-Scheinker disease amyloid protein. JBColChem 2003, 278(48):48146-48153.

54. Breuer GI: Isolation and culture of adult rat hippocampal neurons. J Neurosci Meth 1997, 17(2):143-155.

55. Klingauf J, Kavalik ET, Tsien RW: Kinetics and regulation of fast endocytosis at hippocampal synapses. Nature 1998, 394(6693):581-585.

56. Thais ME, Carqueja CL, Santos TG, Silva RV, Strech E, Machado RS, Walinheim DO, Bianchin NM, Sakamoto AC, Brentani RR, et al: Synaptosomal glutamate release and uptake in mice lacking the cellular prion protein. Brain Res 2006, 1075(1):13-19.

Cite this article as: Bate et al: α-synuclein induced synapse damage is enhanced by amyloid-β1-42: Molecular Neurodegeneration 2010 5.55.

Submit your next manuscript to BioMed Central
and take full advantage of:

• Convenient online submission
• Thorough peer review
• No space constraints or color figure charges
• Immediate publication on acceptance
• Inclusion in PubMed, CAS, Scopus and Google Scholar
• Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit