Supporting Online Material

Materials and Methods

Mice and genetic crosses

All genetic crosses were designed to produce experimental mice heterozygous for transgene insertions and/or gene deletions unless otherwise stated.

Mice expressing β-amyloid precursor protein (βAPP) bearing Swedish K670N/M671L(I) familial Alzheimer’s disease (FAD) mutations under the control of the murine prion protein promoter (Prp)(2, 3), Tg-swAPP^{Prp}(4), were generated in the C57BL/6J/C3H/HeJ and C57BL/6J genetic backgrounds by crossing Tg-swAPP^{Prp} to wildtype (WT) mice. Tg-swAPP^{Prp} mice were also crossed to mice expressing presenilin 1 (PS1) bearing the A246E(5) FAD mutation under murine Prp control, Tg-A246EPS1^{Prp}(4), to produce Tg-swAPP^{Prp} and Tg-swAPP^{Prp}; Tg-A246EPS1^{Prp} littermates in the C57BL/6J/C3H/HeJ and C57BL/6J genetic backgrounds. Mice expressing βAPP bearing both Swedish and London V717I(6) FAD mutations under the control of the murine Thy1.2 promoter (Thy1)(7), Tg-sw/lonAPP^{Thy1}(8), were generated in the C57BL/6J/DBA/Swiss Webster genetic background by crossing line 41 Tg-sw/lonAPP^{Thy1} to WT mice.

Mice homozygous for the deletion of APP, APP^null/APP^null(9), were generated in the C57BL/6J genetic background by crossing APP^{wt}/APP^null mice to each other. Mice heterozygous for the deletion of kinesin light chain 1 subunit of kinesin-I (KLC1), KLC1^{wt}/KLC1^null(10), were generated in the C57BL/6J genetic background by crossing KLC1^{wt}/KLC1^null mice to each other.
Reduced gene dosage of KLC1 in the Tg-swAPP<sup>Prp</sup> mice in the C57BL/6J/C3H/HeJ and C57BL/6J genetic backgrounds was achieved by first crossing Tg-swAPP<sup>Prp</sup> mice to either KLC1<sup>wt</sup>/KLC<sup>null</sup> or KLC1<sup>null</sup>/KLC<sup>null</sup> mice. The resulting Tg-swAPP<sup>Prp</sup>; KLC1<sup>wt</sup>/KLC<sup>null</sup> mice were then crossed with the WT; KLC1<sup>wt</sup>/KLC<sup>null</sup> mice to generate Tg-swAPP<sup>Prp</sup>; KLC1<sup>wt</sup>/KLC1<sup>wt</sup> and Tg-swAPP<sup>Prp</sup>; KLC1<sup>wt</sup>/KLC1<sup>null</sup> littermates (Fig.S2). Tg-swAPP<sup>Prp</sup> mice were also bred with mice deficient in low molecular weight neurofilament protein (NF-L), NF-L<sup>null</sup>/NF-L<sup>null</sup> (11), to obtain Tg-swAPP<sup>Prp</sup>; NF-L<sup>wt</sup>/NF-L<sup>null</sup> mice. These were crossed with NF-L<sup>wt</sup>/NF-L<sup>null</sup> mice to generate Tg-swAPP<sup>Prp</sup>; NF-L<sup>wt</sup>/NF-L<sup>wt</sup>, Tg-swAPP<sup>Prp</sup>; NF-L<sup>wt</sup>/NF-L<sup>null</sup> and Tg-swAPP<sup>Prp</sup>; NF-L<sup>null</sup>/NF-L<sup-null</sup> littermates in the C57BL/6J/C3H/HeJ genetic background.

To avoid the confounding effects of genetic background (12), Tg-swAPP<sup>Prp</sup> and Tg-swAPP<sup>Prp</sup> mice with and without A246EPS1<sup>Prp</sup> (Tg-swAPP<sup>Prp</sup>; Tg-A246EPS1<sup>Prp</sup>, Tg-swAPP<sup>Prp</sup>) or with and without one copy of KLC1 (KLC1<sup>wt</sup>/KLC<sup>wt</sup>, KLC1<sup>wt</sup>/KLC<sup>null</sup>) were examined both in the C57BL/6J/C3H/HeJ and C57BL/6J genetic backgrounds.

All mice were assigned to individual experiments randomly, age matched and whenever possible littermates were compared.

**Flies and genetic crosses**

All crosses were carried out at 29°C using standard genetic techniques (13).

Flies expressing βAPP linked to yellow fluorescent protein (YFP) (14) with reduced gene dosage of the kinesin light chain subunit of kinesin-I (KLC), Tg-APPYFP; KLC<sup>wt</sup>/KLC<sup>null</sup>, were generated by first crossing y<sup>1</sup>w<sup>1</sup>P{w<sup>mc</sup>=UAS-APP695::YFP}/y<sup>1</sup>w<sup>1</sup>P{w<sup>mc</sup>=UAS-APP695::YFP}; wt/wt; wt/wt; wt/wt(15) virgins to w<sup>+</sup>/Y; wt/wt; P{GawB}-2<sup>SG26.1</sup>/ P{GawB}-2<sup>SG26.1</sup>; wt/w(16) males where P{w<sup>mc</sup>=UAS-
APP695::YFP} and P{GawB} correspond to Hsap\APP\695.Scer\UAS.T:Avic\GFPYFP and P{Scer\GAL4\wB\w+mW,ks\Eco\ampR Ecol\ori= GawB}, respectively. The resulting y\textsuperscript{w}\textsuperscript{y}P{w\textsuperscript{+mC}=UAS-APP695::YFP}/Y; wt/wt; P{GawB}\textsuperscript{62B1-2\textsuperscript{SG26.1}}/wt; wt/wt flies were crossed with W\textsuperscript{y}/W\textsuperscript{y}; wt/wt; Df(3L)Klc\textsuperscript{Sex94}/TM6Btb; wt/wt(17) to generate y\textsuperscript{w}\textsuperscript{y}P{w\textsuperscript{+mC}=UAS-APP695::YFP}/Y\textsuperscript{*}; wt/wt; P{GawB}\textsuperscript{62B1-2\textsuperscript{SG26.1}/Df(3L)Klc\textsuperscript{Sex94}; wt/wt (Tg-APPPYFP; KLC\textsuperscript{wt}/KLC\textsuperscript{null}) flies. Due to the Tubby phenotype(13) y\textsuperscript{w}\textsuperscript{y}P{w\textsuperscript{+mC}=UAS-APP695::YFP}/Y\textsuperscript{*}; wt/wt; P{GawB}\textsuperscript{62B1-2\textsuperscript{SG26.1}}/wt; wt/wt females, rather than y\textsuperscript{w}\textsuperscript{y}P{w\textsuperscript{+mC}=UAS-APP695::YFP}/Y\textsuperscript{*}; wt/wt; P{GawB}\textsuperscript{62B1-2\textsuperscript{SG26.1}/TM6Btb; wt/wt littermates, were used as control (Tg-APPPYFP; KLC\textsuperscript{wt}/KLC\textsuperscript{wt}).

To generate flies expressing βAPP linked to YFP with reduced dosage of the kinesin heavy chain subunit of kinesin-I (KHC), Tg-APPPYFP; KHC\textsuperscript{wt}/KHC\textsuperscript{null}, y\textsuperscript{w}\textsuperscript{y}P{w\textsuperscript{+mC}=UAS-APP695::YFP}/Y; wt/wt; P{GawB}\textsuperscript{62B1-2\textsuperscript{SG26.1}}/wt; wt/wt males, obtained by a genetic cross identical to the one described for the generation of Tg-APPPYFP; KLC\textsuperscript{wt}/KLC\textsuperscript{null} flies, were crossed with y\textsuperscript{*}w\textsuperscript{*}/y\textsuperscript{*}w\textsuperscript{*}; CyO\textsuperscript{y}/Khc\textsuperscript{8}; wt/wt; wt/wt(18) females. This cross resulted in y\textsuperscript{w}\textsuperscript{y}P{w\textsuperscript{+mC}=UAS-APP695::YFP}y\textsuperscript{*}w\textsuperscript{*}; Khc\textsuperscript{8}/wt; P{GawB}\textsuperscript{62B1-2\textsuperscript{SG26.1}/wt; wt/wt (Tg-APPPYFP; KHC\textsuperscript{wt}/KHC\textsuperscript{null}) and y\textsuperscript{w}\textsuperscript{y}P{w\textsuperscript{+mC}=UAS-APP695::YFP}/y\textsuperscript{*}w\textsuperscript{*}; CyO\textsuperscript{y}/wt; P{GawB}\textsuperscript{621-2\textsuperscript{SG26.1}/wt; wt/wt (Tg-APPPYFP; KHC\textsuperscript{wt}/KHC\textsuperscript{wt}) littermates.

**Human subjects**

For pilot experiments we examined brains from an 82 year old female and a 69 year old male without prior history of neurological or psychiatric illness both scored as Alzheimer’s disease (AD) Braak stage(19) II by the Department of Pathology at the Albert Einstein College of Medicine. These experiments suggested that choline
acetyltransferase- (ChAT-IR) and phosphorylated tau-immunoreactive (phospho-tau-IR) swellings, akin to those found in mouse models of AD, may also be observed in early stage human AD.

To test this observation, we examined 3 female and 10 male brains from 65 to 100 year old subjects affiliated with the Alzheimer’s Disease Research Center (ADRC) of the University of California San Diego prior to death. 4 brains were scored as Braak stage 0, 6 as Braak stage I-III (3 Braak I, 2 Braak II and 1 Braak III) and 3 as Braak stage IV-VI (one for each Braak stage) by the Department of Pathology at the University of California San Diego. All 13 brains included in this study lacked Lewy bodies or neuropathological features characteristic of neurodegenerative diseases other than AD(20). Subjects with brains scored as Braak 0 had no prior history of neurological or psychiatric illness and an average mini-mental state examination (MMSE)(21) score of 29.5±0.5. Subjects with brains scored as Braak I-III and IV-VI had an average MMS of 22.83±4.17 and 10±5, respectively. All subjects with brains in the Braak IV-VI group had dementia and were diagnosed as probable AD (22, 23) by the ADRC.

**Primary hippocampal cell cultures**

Primary hippocampal cell cultures were generated from post-natal day 1 KLC1<sup>wt</sup>/KLC1<sup>wt</sup> and KLC1<sup>wt</sup>/KLC1<sup>null</sup> mice. In brief, hippocampi were excised in cold Hank’s balanced salt solution (HBBS, GIBCO), transferred into 10ml of cold HBSS, digested with a 0.22µ-filtered mixture of 45U papain (Worthington) in phosphate-buffered saline (Gibco), DL-cysteine HCL (Sigma), bovine serum albumin (BSA, Sigma) and D-glucose (Sigma) enriched with 0.05% of DNase (Boehringer Mannheim) for 15 minutes by shaking at 37ºC, washed twice with 10 ml of 500 µM L-glutamine (Sigma)
supplemented neurobasal medium (GIBCO), tritiated 20 times in 2 ml of growth media and plated at 60,000 cells per each well of a 24-well dish. Cell cultures were maintained in 500 μM L-glutamine and B27 (GIBCO) supplemented neurobasal media at 37°C in 5% CO₂ for an average of 14 days and then transfected with CMV promoter-driven APPYFP(14) using Lipofectamine 2000 according to manufacturer’s instructions (Invitrogen).

**Tissue preparation**

For light microscopy experiments, mice were transcardially perfused, brains post-fixed in 4% paraformaldehyde (PFA) buffered with Sörensen’s solution (pH 7.2) and cryoprotected in 20% sucrose. The only exception were experiments where one hemibrain was processed for βAPP and amyloid-β peptide (Aβ) measurements and one immersion-fixed in 4% PFA buffered with Sörensen’s solution (pH 7.2) for the estimation of the total number of senile plaques. Brain stems and cerebelli were removed from both mouse brains and hemibrains prior to sectioning. 40µ thick coronal brain sections were prepared using a Cryocut 1800 (Reichert-Jung) and all serially collected to allow for the generation of subseries of sections representative of the brain, hemibrain or brain structure of interest. To enable systematic random sampling(24), subseries of sections were generated by selecting its first section using a random number table(25) with the following sections equidistantly separated and parallel to the first one. To allow for unbiased scoring, subseries of sections were blind-coded prior to being randomly assigned to specific experiments, processed and uncoded only upon completion of the data collection.
Blocks of human basal forebrain were prepared from 2- to 20-hour post-mortem brains, formalin-fixed and cut using Vibratome Series 1000 Sectioning System (Technical Products International) into 50µ thick serial coronal sections representative of the nucleus basalis of Meynert (NMB). A subset of 3 to 4 sections were selected using a random number table to allow for independent-random sampling(26), blind-coded and treated in a similar fashion as the mouse samples.

For electron microscopy experiments, mice were transcardially perfused and brains post-fixed in 4% PFA, 0.5% glutaraldehyde (GA) buffered in Sörensen’s solution (pH 7.3). Vibratome cut 50µ thick coronal brain sections were collected in a systematic random fashion into subseries of sections representative of the entire NBM. Prior to immunohistochemical treatment, randomly selected subseries of sections were embedded into Epon 812 resin and cut into 0.7µ semithin and 70 to 80nm ultrathin sections.

βAPP and Aβ measurements

Hemibrains, or both sciatic nerves per mouse, were Dounce homogenized with 16 strokes in 4 times the volume of the sample’s weight using 5M guanidine hydrochloride, diluted 1 to 10 in 1X Dulbecco’s phosphate-buffered saline containing 5% BSA and protease inhibitors and centrifuged at 10,000g for 20 minutes. The resulting supernatants were loaded into well-established(27-36) commercially-available (Biosource International) human-specific anti-Aβ40- and anti-Aβ42 sandwich ELISA plates, which were processed according to the manufacturer’s instructions. Each sample was examined in duplicate or triplicate in 3 independent ELISA measurements using control Aβ40 and Aβ42 peptides as standards.
Brain extracts used for Aβ measurements were also electrophoresed on 10-20% gradient and 16% tricine gels (Invitrogen), blotted onto nitrocellulose and probed with antibodies against the C-terminus of full-length βAPP (AB5352, Chemicon) and the N-terminus of the human Aβ sequence of βAPP (6E10, Senetek PLC) in order to quantify levels of full-length βAPP. Quantification of βAPP was coupled to routine staining for kinesin light chains (63-90, courtesy of Dr. S. Brady), α-tubulin (DM1A, Sigma), calreticulin (Stressgen SPA-600) and Syntaxin 13 (Synaptic Systems 110-132) to confirm the genotypes obtained by PCR, to monitor for protein concentrations and to control for comparative loading of the samples. A control sample at 3 different protein concentrations was used along with experimental samples to verify linearity of the signal. Experimental samples with signal intensities within the linear range were quantified using Scion Image software (Scion Corporation).

**Histological and immunohistological techniques**

For histology, brain sections were routinely stained with either thionin (Sigma) or eosin and hematoxylin (both FD Neurotechnologies) to evaluate gross morphology and to facilitate the recognition of anatomical boundaries(37-40). Thioflavine S (Sigma) and modified Bielschowsky’s silver technique were used to visualize senile plaques. All stainings were carried out using standard histological procedures(41-45).

Immunohistochemistry for light microscopy was performed using routine protocols(42, 46). In brief, floating sections were quenched with 0.6% hydrogen peroxide, blocked and permeabilized in 3% normal donkey serum (NDS), 1% BSA, 0.25% Triton X-100 in Sörensen’s solution, incubated for 24 to 48 hours at 4ºC with primary antibodies raised against ChAT (AB144P, Chemicon) or phospho-tau (CP13,
courtesy of Dr. P. Davies and AT8, Innogenetics) and then with biotinylated secondary antibodies. Staining of antigens of interest was amplified with the avidin-biotin-peroxidase procedure employing Vectastain Elite ABC (Vector Labs) and visualized with diaminobenzidine tetrachloride (DAB). Phospho-tau staining in mice was achieved using the Vector M.O.M. system (Vector Labs) according to the manufacturer’s instructions.

For electron microscopy, the above immunohistochemical protocol was adjusted to allow optimal preembedding labeling(47). Sections were permeabilized in 0.05% Triton X-100 in Sörensen’s solution for 30 minutes at 4ºC and detergent was omitted from blocking and antibody solutions. 0.5% BSA and 1% NDS, 0.8% BSA, 1% gelatin in Sörensen’s solution were used as blocking solutions prior to incubation with primary and secondary antibodies, respectively. Development of the DAB reaction was brief and followed by a 10 minute incubation in 2% GA buffered with Sörensen’s solution prior to embedment into Epon 812 resin. Embedded sections were cut first into semithin sections, stained with 0.25% toluidine blue in 0.1% sodium borate to reveal the anatomy and then into 70 to 80 nm ultrathin sections, which were mounted onto copper grids and post-stained with uranyl acetate and lead citrate.

For immunofluorescence experiments, floating sections were first blocked and permeabilized using 5% NDS, 1% BSA and 0.25% Triton X-100 diluted in the Sörensen’s solution, incubated with the appropriate cocktail of primary antibodies raised against ChAT (as above), N-terminus of βAPP (APP44-63, RDI), C-terminus of βAPP (AB5352, Chemicon), N-terminus of the Aβ sequence of βAPP (6E10, Senetek PLC), C-terminus of Aβ40 (AB5364, Chemicon), C-terminus of Aβ42 (44-344, Biosource International)(32, 48), microtubule-associated protein-2 (MAP2, MAB3418, Chemicon)
and phosphorylated high molecular weight neurofilament protein (SMI31, Sternberger) for 24 to 48 hours at 4ºC and then with either fluorophore- or biotin-conjugated secondary antibodies (Jackson Labs) followed by fluorophore-conjugated streptavidin. Sections were counterstained with Neurotrace Fluorescent Nissl Stain (Molecular Probes) whenever appropriate. Pilot experiments testing the linearity of fluorescence intensities and signal to noise ratios of serially diluted primary antibodies were performed to identify an optimal concentration for each antibody used. Although antibodies against Aβ40 and Aβ42 have been previously characterized(32, 34, 49-51), we performed competition experiments with Aβ40 and Aβ42 to re-confirm their specificities (Fig. S10).

**Analysis of ChAT-IR fibers**

NBM was examined using an Axioplan microscope (Carl Zeiss) connected to an X-Y-Z stage (MSA 001-6, RSF Elektronik linked to Microcode II, Boeckler Instruments), a color video camera (Diagnostic Instruments) and controlled by the Bioquant image analysis software (Bioquant Image Analysis Corporation). In brief, a randomly placed line grid over the NBM was used to obtain random grid fields from which approximately 100 ChAT-IR fibers were examined per NBM. The section sampling fraction (ssf) was 1/5 and a comparable number of ChAT-IR fibers were examined from each section. Results from pilot experiments suggested that varicosities with diameters larger than 3µ within the ChAT-IR fibers of the NBM can be defined as swellings(52, 53). These results are based on the analysis of a large number of varicosities (>3000) visualized with ChAT antibody under several fixation and staining conditions in mice of different ages and genetic backgrounds. Although the diameters of varicosities varied with the fixation method, the concentration of ChAT antibody, the
time employed to develop the DAB reaction and with the genetic background in no circumstance did the average diameters of ChAT-IR varicosities (0.9-1.7µ) approach 3µ. The frequency of ChAT-IR fibers with swellings (N\textsuperscript{s}) was estimated as the sum of fibers with swellings (Σf\textsuperscript{s}) divided by the total number of fibers examined (n) and expressed as percentage using N\textsuperscript{s}= Σf\textsuperscript{s}/n. Methods to obtain total number of fibers within a defined volume would allow a more precise calculation of the number of fibers with swellings from the total number of fibers, but currently do not exist. Phospho-tau-IR swellings in the NBM were observed only in Tg-swAPP\textsuperscript{Ppr} mice; their consistently weak staining precluded rigorous quantification.

A similar approach was undertaken for the analysis of ChAT-IR and phospho-tau-IR fibers in the human NBM. N\textsuperscript{s}= Σf\textsuperscript{s}/n was particularly suitable for this analysis since it is not biased by the loss of cholinergic structures in the NBM in AD(54). Independent-random sampling, together with the lack of substantial phospho-tau-immunoreactivity (phospho-tau-IR) in normal compared to AD NBM, allowed us to estimate the extent of phospho-tau-IR swellings as the number of phospho-tau-IR swellings per mm\textsuperscript{2}. Moreover, phospho-tau-IR swellings often appeared as spheroids(52) precluding their estimation using N\textsuperscript{s}= Σf\textsuperscript{s}/n. The relative density of ChAT-IR fibers in mice and of phospho-tau-IR fibers in humans was approximated using the intersect analysis(55, 56).

The relationship between ChAT, phosphorylated high molecular weight neurofilament protein (phospho-NF-H) and kinesin light chain subunits of kinesin-I (KLC) within the varicosities and swellings was assessed using an Optiphot inverted microscope (Nikon) coupled to an MRC1024 confocal imaging system (BioRad). Fluorescence was quantified and the overlap between ChAT, NF-H and KLC analyzed
from projections of stacks of optical sections constructed from an average of 150-200 0.1µ z-sections (57). To map ChAT, NF-H and KLC intensities and to examine the overlap we also used an inverted wide-field microscope (TE200, Nikon) linked to a DeltaVision microscope system (Applied Precision). Images were processed and analyzed with an Octane workstation (Silicon Graphics) using the Softworx data inspector utility. Quantification of fluorescence was accomplished using only the linear range of the digital camera.

Estimates of the total number of cortical swellings/spheroids visualized with Bielschowsky’s silver were obtained by the fractionator method adjusted for rare events (see analysis of senile plaques).

For the ultrastructural analysis of the NBM, images were collected with an EM208S electron microscope (Philips). Comparable areas of the NBM within each ultrathin section were sampled in an equal number of ultrathin and thick sections in WT and Tg-swAPPPrp mice. Changes in fibers and ChAT-IR fibers were examined using Photoshop 5.5 (Adobe) and Bioquant image analysis software (Bioquant Image Analysis Corporation).

**In vivo imaging of βAPP**

In vivo transport of βAPP was imaged in segmental nerves of live dissected Drosophila larvae (15, 58, 59) and in primary hippocampal cultures kept at 37ºC in 5% CO2 using a heating stage (Harvard Apparatus) with an inverted epifluorescent microscope (TE-2000U, Nikon) connected to a Photometrics CoolSNAP_HQ-cooled CCD camera (Roper Scientific) and driven by MetaMorph 6.0 (Universal Imaging Corporation). Images were captured randomly at a speed of 10 frames per second at
100X magnification and 2x2 binning for each time-lapse stack. Stacks were converted to QuickTime movies, kymographs and montages and analyzed using MetaMorph (Universal Imaging Corporation).

In the segmental nerves of live Drosophila larvae, “flux” of APPYFP particles was approximated from a total of 150 images by superimposing a line perpendicularly to the major axis of the segmental nerve at a random location and counting the number of clearly defined APPYFP particles that crossed the line in the anterograde and retrograde direction within 15 seconds. APPYFPs that remained still for 15 seconds are shown in the kymographs, but were omitted from the flux analysis.

In primary hippocampal cultures, movement of APPYFP particles was approximated from the kymographs as a percentage of clearly defined stationary, anterogradely- or retrogradely-moving APPYFP particles out of all clearly defined APPYFP particles, which started and ended movement within the time and distance captured by the kymograph. The reported average percentages of stationary, anterogradely- or retrogradely-moving APPYFP particles (Fig.3E) derive from the analysis of a total of 193 and 292 APPYFP particles from 9 and 14 kymographs from 2 independently APPYFP-transfected primary hippocampal cultures from each KLC1<sup>wt</sup>/KLC1<sup>wt</sup> (n=3) and KLC1<sup>wt</sup>/KLC1<sup>null</sup> (n=4) mouse, respectively.

**Neuroimaging of βAPP**

βAPP, Aβ40 and Aβ42-immunoreactivities were analyzed with an Optiphot inverted microscope (Nikon) connected to an MRC102 confocal imaging system (BioRad). Fluorescence intensities were collected as 2µ thick Kalman-averaged (5x) confocal images, transformed into negative images by AdobePhotoshop 5.0 (Adobe) and
measured as integrated optical densities (IOD) by the Bioquant image analysis software (Bioquant Image Analysis Corporation). Linearity of the acquired fluorescence intensities was confirmed prior to the acquisition of each image by the MRC1024 confocal imaging system (BioRad). All IOD values were obtained from comparable areas of sensory cortex, entorhinal cortex, dentate gyrus and CA1. Each anatomical region was sampled 3 times from each section in 3 sections total per brain. In a pilot experiment we compared the above results with those obtained by measuring the fraction of the total area of the anatomical region under examination occupied by βAPP, Aβ40 and Aβ42-immunoreactivities(60). We obtained similar results with both methods.

**Analysis of senile plaques**

Estimates of the total number (N) of cortical swellings/spheroids visualized with Bielschowsky’s silver and senile plaques were calculated using the $N = \Sigma Q (1/ssf)(1/asf)(1/tsf)$ fractionator formula (61, 62). However, since pilot experiments indicated that both cortical swellings and senile plaques are rare in Tg-swAPP<sup>P3P</sup> brains, we adjusted the above formula for rare events. In brief, objects (Q) were at first searched for at low magnification (40X) and a line grid was used to ensure systematic sampling of the entire area under examination. Once an object was identified we counted the object when its top first came into focus within the z-axis at high magnification (100X). Objects in focus at the cut surface of the top of the section (exclusion surface) were ignored. Ssf was 1/12 with the average distance of the first and last systematic random sections on average 4.70±0.12 mm and 0.23±0.04 mm from the interaural line, respectively. There was no statistically significant difference in the distance of the first and last systematic random sections from the interaural line between mice of different genotypes examined.
Rather than sampling a fraction of the area (61), we sampled the entire area under examination so that the area sampling fraction (asf) equaled 1. The top and bottom of each section without the guard volume were used as the top and bottom of the disector(63) making the tissue sampling fraction equal 1. In summary, the fractionator used to estimate the N of cortical swellings and senile plaques corresponded to $\Sigma Q (1/ssf)$. A similar approach was undertaken to estimate N of swellings in different cortical regions and for senile plaques in different brain regions.

Average diameters and volumes of senile plaques were calculated with the Bioquant image analysis software (Bioquant Image Analysis Corporation). The volumes were obtained with the isotropic rotator(64). The average number of dystrophic neurites per senile plaque in Tg-swAPP$^{Prp}$/KLC1$^{wt}$/KLC1$^{wt}$ and Tg-swAPP$^{Prp}$/KLC1$^{wt}$/KLC1$^{null}$ mice was approximated as the average number of dystrophic neurites counted in 3 profiles of each senile plaque equidistantly separated from each other in the z-plane.

**Statistical analysis**

All samples were coded prior to the beginning of each experiment and scored blind to the identity of the sample. Only upon completion of the experiment and of the data collection were samples uncoded to allow statistical analysis.

Error bars in the graphs represent the standard error of the mean. All p-values in figure legends refer to asterisks in the corresponding figures.

The analysis of ChAT-IR fibers with varicosities of diameter larger than 3µ in all WT, Tg-SwAPP$^{Prp}$ and Tg-sw/lonAPP$^{Thy1}$ mice, together with the analysis of ChAT-IR fiber densities in all WT and Tg-swAPP$^{Prp}$ mice, was carried out using Mann-Whitney U test.
The analysis of ChAT-IR fibers with varicosities and phospho-tau-IR varicosities, together with the analysis of phospho-tau-IR fiber densities, in human samples was conducted using non-parametric statistical techniques. An exact Kruskal-Wallis (K-W) test was used to examine overall differences in the percentage of ChAT-IR fibers with varicosities of diameter larger than 3µ, the number of phospho-tau-IR swellings/mm², the density of phospho-tau-IR fibers/average area of the NBM/section and the MMSE among Braak groups 0, I-III and IV-VI. If the K-W test indicated an overall significant group effect, the differences between groups were analyzed further using Dunn’s multiple comparison procedure(65). The Hochberg’s correction was used to correct for multiple hypotheses and multiple comparisons. The p-values reported in the text and in the supplement represent p-values from the overall K-W test and the Hochberg’s adjustment of the Dunn’s p-value for multiple comparisons. Moreover, Spearman’s correlation analysis was conducted to assess the correlation between phospho-tau abnormalities and the MMSE scores. This analysis revealed a significant correlation between the number of phospho-tau-IR swellings, the density of phospho-tau-IR fibers and the MMSE scores. Rho values corresponded to 0.86, 0.82 and 0.61 for the correlation between the number of phospho-tau-IR swellings and the density of phospho-tau-IR fibers, the number of phospho-tau-IR swellings and the MMSE scores and the density of phospho-tau-IR fibers and the MMSE scores respectively.

Analysis of the number of swellings not associated with amyloid was carried out using the Mann-Whitney U test. Analyses of the in vivo axonal transport of APP in Drosophila larvae and in hippocampal cultures were conducted using the Mann-Whitney U test and the Student t-test respectively. Analysis of the average levels of full-length
βAPP was conducted using the Student’s t-test. Levels of Aβ, Aβ42/Aβ40 ratios and βAPP/Aβ immunoreactivities were analyzed using the Mann-Whitney U test. Analysis of the number of senile plaques, of the chronology of amyloid deposition and of their regional distribution was conducted using the Mann-Whitney U-test. Spearman’s correlation was used to test the association between the number of swellings not associated with amyloid and the number of senile plaques.
**Supplementary figures**

**Figure S1:** *Increase in the number of ChAT-IR fibers with swellings in Tg-sw/lonAPP<sup>Thy1</sup> mice.* Increased number of ChAT-IR fibers with varicosities of diameter larger than 3.0µ (swellings) in the NBM of 2.5-month old Tg-sw/lonAPP<sup>Thy1</sup> (n=4) compared to WT littermates (n=3, p<0.05). Comparable results were obtained from the analyses of 4-month old WT (n=3) and Tg-swAPP<sup>Ppy</sup> mice (n=4) in the C57Bl/J6 genetic background, thus excluding genetic background effects. In each experiment, at least 100 randomly selected fibers were examined per NBM.
**Figure S2:** *Amyloid-independent axonal swellings.* Representative image of the sensory cortex of Tg-swAPP<sup>Pp</sup> mice stained with Bielschowsky’s silver demonstrating that axonal swellings, besides decorating amyloid deposits (black arrows), also occur independently (red arrows) from the amyloid (bar 25µ).
**Figure S3:** *Increase in the density of phospho-tau-IR fibers in the NBM in AD brains.* We observed an overall significant difference (K-W test p<0.0005) in the density of phospho-tau-IR fibers between human brains in Braak groups 0, I-III and IV-VI. Dunn’s multiple comparison tests indicated an increased density of phospho-tau-IR fibers in the NBM in Braak groups I-III (n=6) and IV-VI (n=3) compared to Braak group 0 (n=3) human brains (p values of 0.09 and 0.003, respectively).
**Figure S4**: Punnett square showing the predicted genetic constitution of the mice from the genetic cross between Tg-swAPP<sup>Prp</sup>; KLC1<sup>wt</sup>/KLC1<sup>null</sup> and WT; KLC1<sup>wt</sup>/KLC1<sup>null</sup> mice.

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Predicted genetic constitution:

1/8 WT/WT;KLC1<sup>wt</sup>/KLC1<sup>wt</sup>

1/4 WT/WT;KLC1<sup>wt</sup>/KLC1<sup>null</sup>

1/8 WT/WT;KLC1<sup>null</sup>/KLC1<sup>null</sup>

1/8 Tg-swAPP<sup>Prp</sup>;KLC1<sup>wt</sup>/KLC1<sup>wt</sup>

1/4 Tg-swAPP<sup>Prp</sup;/KLC1<sup>wt</sup>/KLC1<sup>null</sup>

1/8 Tg-swAPP<sup>Prp</sup>/KLC1<sup>null</sup>/KLV1<sup>null</sup>
**Figure S5:** Comparison of the expected and observed frequencies of the genetic constitution of the mice from the genetic cross between Tg-swAPP$^{Prp}$; KLC1$^{wt}$/KLC1$^{null}$ and WT; KLC1$^{wt}$/KLC1$^{null}$ mice. Genotyping of 166 3 week-old mice (weaning) revealed that the observed and expected frequencies of each genetic constitution differed significantly (chi-square=24.53, p<.0005). The observed numbers of WT; KLC1$^{null}$/KLC$^{null}$ and Tg-swAPP$^{Prp}$/ KLC1$^{null}$/KLC$^{null}$ mice were significantly reduced when compared with the expected frequencies. When aged, mice completely lacking KLC1 exhibited severe brain defects, including axonal swellings and accelerated mortality. Since only a few poorly viable WT; KLC1$^{null}$/KLC$^{null}$ and Tg-swAPP$^{Prp}$/KLC1$^{null}$/KLC$^{null}$ mice were available, and given that their striking phenotype could confound subsequent analysis, we focused our experiments on mice harboring 50% reductions of KLC1 with or without Tg-swAPP$^{Prp}$ as these mice appeared viable and lacked major neurological defects.
Figure S6: Number of dystrophic neurites per senile plaque in Tg-swAPP<sup>Prp</sup>; KLC1<sup>wt</sup>/KLC1<sup>wt</sup> and Tg-swAPP<sup>Prp</sup>; KLC1<sup>wt</sup>/KLC1<sup>null</sup> mice. No difference in the average number of dystrophic neurites per senile plaque profile visualized with Bielschowsky’s silver between 19-21-month old Tg-swAPP<sup>Prp/WT</sup>; KLC1<sup>wt</sup>/KLC1<sup>wt</sup> (n=6) and Tg-swAPP<sup>Prp</sup>; KLC1<sup>wt</sup>/KLC1<sup>null</sup> mice (n=6).
**Figure S7**: Decreased APPYFP transport in the segmental nerves of Drosophila larvae with a 50% reduction in KLC or KHC. To directly test whether a relatively benign reduction in kinesin-I is sufficient to impair axonal transport of βAPP we attached yellow fluorescent protein to wildtype βAPP (APPYFP) and examined its transport in living nerves of Drosophila larvae carrying 50% reductions in kinesin-light chain (KLC) or kinesin-heavy chain (KHC). In this assay, the moderate expression of APPYFP alone did not significantly interfere with transport. However, when coupled to a 50% reduction in either KLC or KHC the expression of APPYFP gave rise to a dramatic impairment in the axonal transport of βAPP. The flux of APPYFP was significantly decreased in Tg-APPYFP; KLC<sup>wt</sup>/KLC<sup>null</sup> and Tg-APPYFP; KHC<sup>wt</sup>/KHC<sup>null</sup> compared with Tg-APPYFP; WT/WT larvae (Fig. S8). Previous work revealed that these 50% reductions in kinesin-I function have no or only a very minor phenotype on their own. 

**A.** Montages of the *in vivo* transport of APPYFP in the segmental nerves indicated that Drosophila larvae with a 50% reduction in KLC (Tg-APPYFP; KLC<sup>wt</sup>/KLC<sup>null</sup>, b, n=3), or a 50% reduction in KHC (Tg-APPYFP; KHC<sup>wt</sup>/KHC<sup>null</sup>, c, n=3, bar 10µ), exhibit a significant impairment in the βAPP transport compared with larvae having normal levels of KLC and KHC (Tg-APPYFP; WT/WT, a, n=4). 

**B.** Kymographs corresponding to montages representative of the *in vivo* transport of APPYFP in the WT (a, Movie S3), KLC-null mutant heterozygous (b, Movie S4) and KHC-null mutant heterozygous (c, Movie S5) larvae in A (vertical axis corresponds to time and the horizontal axis to distance).
**Figure S8:** Decreased flux of APPYFP in the segmental nerves of Drosophila larvae with a 50% reduction in KLC or KHC. The flux of APPYFP particles, defined as the number of APPYFP particles crossing a randomly positioned line perpendicular to the major axis of the segmental nerve axons in either direction (anterograde or retrograde) within 15 seconds, was significantly reduced in Tg-APPYFP; KLC^{wt}/KLC^{null} (n=3, p<0.05) and Tg-APPYFP; KHC^{wt}/KHC^{null} (n=3, p<0.0005) compared to Tg-APPYFP; WT/WT (n=4) larvae.
Figure S9: Comparison of Aβ42/Aβ40 ratios in Tg-swAPP<sup>P<sub>Prp</sub></sup> mice upon reduction of KLC1 or NF-L or upon addition of Tg-A246EPS1<sup>P<sub>Prp</sub></sup>. To exclude any confounding effect of genetic background on the role of kinesin-I in Aβ generation, we backcrossed mice into the C57Bl/J6 genetic background. In agreement with our previous measurements, 8-month old Tg-swAPP<sup>P<sub>Prp</sub></sup>; KLC1<sup>wt</sup>/KLC<sup>null</sup> mice in the C57Bl/J6 genetic background showed a significant increase in the Aβ42/Aβ40 ratios compared with the Tg-swAPP<sup>P<sub>Prp</sub></sup>; KLC1<sup>wt</sup>/KLC<sup>wt</sup> littermates. We also compared the increase in the Aβ42/Aβ40 ratio observed in Tg-swAPP<sup>P<sub>Prp</sub></sup> mice upon reduction of KLC1 to the well-established increase in Aβ42/Aβ40 ratios reported in the Tg-swAPP<sup>P<sub>Prp</sub></sup> mice upon addition of a PS1 transgene bearing FAD mutation. Comparable increases in the Aβ42/Aβ40 ratio were observed in 8-month old Tg-swAPP<sup>P<sub>Prp</sub></sup>; KLC1<sup>wt</sup>/KLC<sup>null</sup> and in 12-month old Tg-swAPP<sup>P<sub>Prp</sub></sup>; Tg-A246EPS1<sup>P<sub>Prp</sub></sup> mice. To test if reducing KLC1 enhanced amyloidogenic processing of βAPP by altering axonal structure or organization, we asked whether changes in Aβ akin to those observed upon reduction in kinesin-I would also be obtained when axonal structure was altered by other means. Since deletion of the low molecular weight neurofilament protein (NF-L) causes changes in microtubule organization and alters axonal structure, we examined levels of βAPP and the Aβ42/Aβ40 ratios in Tg-swAPP<sup>P<sub>Prp</sub></sup> mice deficient in NF-L. Levels of full-length βAPP and Aβ42/Aβ40 in 11-15-month old Tg-swAPP<sup>P<sub>Prp</sub></sup>; NF-L<sup>wt</sup>/NF-L<sup>null</sup> and Tg-swAPP<sup>P<sub>Prp</sub></sup>; NF-L<sup>null</sup>/NF-L<sup>null</sup> mice did not differ from the ones measured in Tg-swAPP<sup>P<sub>Prp</sub></sup>; NF-L<sup>wt</sup>/NF-L<sup>wt</sup> littermates nor from each other.

In summary, 8-month old Tg-swAPP<sup>P<sub>Prp</sub></sup>; KLC1<sup>wt</sup>/KLC<sup>null</sup> (C57Bl/J6, n=3) mice showed an 88% increase in the brain Aβ42/Aβ40 ratio compared with the Tg-swAPP<sup>P<sub>Prp</sub></sup>; KLC1<sup>wt</sup>/KLC<sup>wt</sup> (n=3) controls (p<0.05). This increase was equivalent to that seen in 12-
month old Tg-swAPP<sup>Prp</sup>; Tg-A246E-PS1<sup>Prp</sup> (n=3) mice compared with the Tg-swAPP<sup>Prp</sup> (n=3) littermates (p<0.05). Brain Aβ42/Aβ40 in 11-15-month old Tg-swAPP<sup>Prp</sup>; NF-L<sup>wt</sup>/NF-L<sup>null</sup> (n=3) and Tg-swAPP<sup>Prp</sup>; NF-L<sup>null</sup>/NF-L<sup>null</sup> (n=3) mice did not differ from that in Tg-swAPP<sup>Prp</sup>; NF-L<sup>wt</sup>/NF-L<sup>wt</sup> (n=3) mice.
**Figure S10**: Analysis of intracellular βAPP/Αβ. Staining for the C-terminus of βAPP (CAPP) was greater in Tg-swAPP<sup>Prp</sup> (b) than in WT (a, hippocampus) mice and overlapped with the staining for the N-terminus of βAPP (d, CAPP in red, NAPP in green and overlay in yellow). Staining for the C-terminus of βAPP (a and b, CAPP in red) is counterstained for microtubule-associated protein 2 (MAP2 in white). Antibodies for the Aβ sequence of βAPP (6E10) or the C-termini of Aβ40 and Aβ42 stained only sections from Tg-swAPP<sup>Prp</sup> (f, j and n), but not WT (e, i and m) or APP<sup>null</sup>/APP<sup>null</sup> (h, l and p), brains (hippocampus). Staining was substantially reduced when primary antibodies were omitted (c) or when they were pre-incubated with the appropriate synthetic Aβ (g, k and o, bar 50µ for all except d where 10µ).
Figure S11: Analysis of the average diameters and volumes of senile plaques in Tg-swAPP<sup>Prp</sup>; KLC1<sup>wt</sup>/KLC1<sup>wt</sup> and Tg-swAPP<sup>Prp</sup>; KLC1<sup>wt</sup>/KLC1<sup>null</sup> mice. 20 randomly chosen senile plaques per genotype were examined for morphological changes using Bielschowsky’s modified silver technique. 19-21 month-old Tg-swAPP<sup>Prp</sup>; KLC1<sup>wt</sup>/KLC1<sup>null</sup> mice (n=6) exhibited significantly larger diameters and volumes of senile plaques when compared to the age-matched or littermate Tg-swAPP<sup>Prp</sup>; KLC1<sup>wt</sup>/KLC1<sup>wt</sup> mice (n=6, p<0.05). The volumes were estimated using isotropic rotator.
**Figure S12**: Examples of diffuse extracellular $A\beta$-immunoreactivity in Tg-swAPP$^{Prp^p}$; $KLC1^{wt}/KLC1^{null}$ mice. Brain sections from Tg-swAPP$^{Prp^p}$; KLC1$^{wt}$/KLC1$^{null}$, but not Tg-swAPP$^{Prp^p}$; KLC1$^{wt}$/KLC1$^{wt}$, mice displayed extensive areas of diffuse $A\beta$40- and, albeit less frequently, $A\beta$42-immunoreactivity. Examples: 1) a lamina of diffuse “amyloid” in the superficial layers of the retrosplenium (a, b, c) and 2) densely packed in part compact amyloid and in part “diffuse amyloid” in the subiculum (d, e, f, bar 100µ).
**Figure S13**: Representative images of senile plaques in 11- and 19-month old sensory cortices from Tg-swAPP$^{Prp}$; KLC1$^{wt}$/KLC1$^{wt}$ and Tg-swAPP$^{Prp}$; KLC1$^{wt}$/KLC1$^{null}$ mice. No senile plaques were encountered in 11-month old Tg-swAPP$^{Prp}$; KLC1$^{wt}$/KLC1$^{wt}$ mice (n=2). Amyloid deposition (red arrow) in 11-month old Tg-swAPP$^{Prp}$; KLC1$^{wt}$/KLC1$^{null}$ mice (n=2) was comparable to that observed in 19-month old Tg-swAPP$^{Prp}$; KLC1$^{wt}$/KLC1$^{wt}$ mice (n=4, bar 50µ).
Figure S14: Correlation between the number of swellings not associated with amyloid and the number of senile plaques. Positive rank order correlation ($r^2=0.0891$, $p<0.0005$) between the number of swellings not associated with amyloid and the number of senile plaques in 17-24-month-old Tg-swAPP$^{Prp; KLC1^wt/KLC1^wt}$ (n=5) and Tg-swAPP$^{Prp; KLC1^wt/KLC1^{null}}$ mice (n=6).
1105681s1.mov: Axonal transport of APPYFP in the primary hippocampal cell culture from KLC1\textsuperscript{wt}/KLC1\textsuperscript{wt} mice.

1105681s2.mov: Axonal transport of APPYFP in the primary hippocampal cell culture from KLC1\textsuperscript{wt}/KLC1\textsuperscript{null} mice.

1105681s3.mov: Axonal transport of APPYFP in the segmental nerves of Drosophila larvae.

1105681s4.mov: Axonal transport of APPYFP in the segmental nerves of Drosophila larvae with a 50% reduction in KLC.

1105681s5.mov: Axonal transport of APPYFP in the segmental nerves of Drosophila larvae with a 50% reduction in KHC.
References

5. R. Sherrington et al., Nature 375, 754-60 (Jun 29, 1995).