

ORIGINAL ARTICLE

Extensive Involvement of Autophagy in Alzheimer Disease: An Immuno-Electron Microscopy Study

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Abstract

The accumulation of lysosomes and their hydrolases within neurons is a well-established neuropathologic feature of Alzheimer disease (AD). Here we show that lysosomal pathology in AD brain involves extensive alterations of macroautophagy, an inducible pathway for the turnover of intracellular constituents, including organelles. Using immunogold labeling with compartmental markers and electron microscopy on neocortical biopsies from AD brain, we unequivocally identified autophagosomes and other prelysosomal autophagic vacuoles (AVs), which were morphologically and biochemically similar to AVs highly purified from mouse liver. AVs were uncommon in brains devoid of AD pathology but were abundant in AD brains particularly, within neuritic processes, including synaptic terminals. In dystrophic neurites, autophagosomes, multivesicular bodies, multilamellar bodies, and cathepsin-containing autophagolysosomes were the predominant organelles and accumulated in large numbers. These compartments were distinguishable from lysosomes and lysosomal dense bodies, previously shown also to be abundant in dystrophic neurites. Autophagy was evident in the perikarya of affected neurons, particularly in those with neurofibrillary pathology where it was associated with a relative depletion of mitochondria and other organelles. These observations provide the first evidence that macroautophagy is extensively involved in the neurodegenerative/regenerative process in AD. The striking accumulations of immature AV forms in dystrophic neurites suggest that the transport of AVs and their maturation to lysosomes may be impaired, thereby impeding the suspected neuroprotective functions of autophagy.

Key Words: Lysosomes, Neurodegeneration, Amyloid, Apoptosis, Necrosis

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INTRODUCTION

Alzheimer disease (AD) is characterized by the presence of intraneuronal neurofibrillary tangles, β -amyloid-containing neuritic plaques, and the loss of specific populations of neurons. Neuron death is preceded by retrograde degeneration of synaptic terminals, axons, and dendrites, which may evolve over many years (1–4). This degeneration is accompanied by attempts by neurons to repair and regenerate neuritic processes (5–7), which yields a highly characteristic pattern of enlarged dystrophic neurites. The molecular basis for this degenerative process and ultimate neuronal death is still poorly understood.

Growing attention has focused on proteases in both the early survival responses of neurons in AD and as agents of neurodegeneration, resulting either from inappropriate activation of proteases or from defective proteolysis, which allows buildup of toxic molecules. Widespread activation of calpains in neurons results in cleavage of key structural proteins and promotes cytoskeletal hyperphosphorylation by activating protein kinases, leading to neurofibrillary degeneration (8–10). Certain caspases are activated at low levels in vulnerable neuronal populations in the absence of a complete morphologic or biochemical pattern of neuronal apoptosis (11, 12). A decline in proteasome-mediated turnover of proteins during aging and AD (13, 14) is one factor that increases reliance on the lysosomal system, the principal alternative to the proteasome for intracellular turnover. Indeed, the synthesis of lysosomal system components, including cathepsins, is markedly upregulated early and progressively in AD (15–18). The function of the endocytic pathway, one major route to the lysosome, is altered before any other known pathologies in AD brain (19, 20), and this dysfunction may promote β -amyloid peptide generation (20–23) and reduce neuronal survival (24). Autophagy, the other major pathway to lysosomes, has received limited attention in relation to AD (25), although its importance as a mechanism for removing defective organelles and potentially toxic proteins (26, 27) and as a determinant of cell survival (28) in various disease settings is becoming appreciated (15, 29).

Autophagy is a tightly regulated process (30, 31), which is induced by nutritional or trophic deprivation under conditions of cell stress to provide substrates for energy or new synthesis by turning over nonessential cytoplasmic constituents, including organelles (32, 33). Autophagy is initiated when a region of cytoplasm and organelles within the cells is sequestered within a double membrane-limited vacuole, the autophagosome (34, 35). Autophagosomes mature to single

membrane phagolysosomes (30, 36–38) and become autolysosomes when they become acidified and acquire proteolytic enzymes by fusing with late endosomes or lysosomes (36, 38). Materials internalized by endocytosis also enter the autophagic pathway when endosomes fuse with autophagosomes (39, 40). The term autophagic vacuole (AV) is used here to refer to any of these prelysosomal compartments.

Autophagy is active during development to support major changes in cell size and morphology (25, 41). Autophagy may also act as a surveillance system in stressed or injured cells to remove damaged mitochondria and other organelles that have the potential to trigger apoptosis (42–44). On the other hand, autodigestion by acutely upregulating autophagy is a form of programmed cell death that is distinct from apoptosis but shares some of its features (45, 46). Autophagic cell death has become more frequently recognized as a caspase-independent form of apoptosis and several cathepsins have been shown to initiate or mediate aspects of apoptotic and necrotic cell death in various pathologic settings (15, 29). Autophagic vacuoles have recently been observed in experimental neurodegenerative states (47–50) and in dying striatal neurons in Parkinson disease (51), although information about the extent to which autophagy is involved in neurodegeneration and its pathogenic significance is limited (15, 25).

To investigate the possible involvement of autophagy in AD, we analyzed well-preserved biopsy specimens of neocortex from AD and non-AD control brains, which allowed us, for the first time, to identify and extensively characterize autophagosomes and related autophagic vacuoles and to differentiate them from other lysosome-related compartments known to accumulate in neurons in AD brain (52–54). Our results indicate that the lysosomal system mobilization previously shown to occur in AD involves, in part, the induction of macroautophagy in affected neurons, which is particularly evident within neuritic processes, including synaptic regions. Moreover, AV proliferation is unexpectedly robust in dystrophic neurites and includes grossly abnormal accumulations of immature forms of AVs such as autophagosomes, suggesting that, while autophagy may be induced in AD, AV transport and maturation are also impaired in affected neurons. These observations have implications for neurodegenerative mechanisms and β -amyloidogenesis in AD.

MATERIALS AND METHODS

Acquisition of Human Biopsy Brain and Postembedding for Electron Microscopy

Cortical biopsy specimens were studied from 7 patients with AD (age range, 71–86 years) as previously described (55). Cortical biopsies free of plaques and tangles from 3 subjects (age range, 67–72 years) were also examined (55). Clinicopathologic characteristics of these cases are described in the Table. Tissue was fixed in 3% glutaraldehyde/0.1 mol/L phosphate buffer, pH 7.4, and postfixed in 1% osmium tetroxide in Sorensen's phosphate buffer. After dehydration in ethyl alcohol, the tissue was embedded in Epon (EMS, Fort Washington, PA). Tissue blocks were cut serially into ultrathin

TABLE Clinicopathologic Characteristics of Cases Analyzed

Age (years)	Gender	Clinical Diagnosis	Pathologic Diagnosis	Cortical Region
74	Male	Dementia/dysphagia	Mild AD	Temporal
74	Male	Hydrocephalus/dyspraxic gait	Moderate AD	Frontal
78	Female	Hydrocephalus dementia	Moderate AD	Frontal
72	Female	R/O encephalitis	Mild to moderate AD	Temporal
71	Female	R/O encephalitis	Moderate AD	Temporal
86	Male	R/O encephalitis	Moderate AD	Temporal
75	Male	Dementia	Severe AD	Temporal
67	Female	Chronic meningitis	Control	Temporal
70	Female	R/O encephalitis	Control	Temporal
72	Male	R/O encephalitis	Control	Temporal

Encephalitis was not confirmed in cases selected. Neuropathologic diagnosis is based on numbers of senile plaques and neurofibrillary tangles per high power according to CERAD criteria (85). R/O, rule out (for the basis for obtaining the biopsy).

(0.06 μ m) sections. Ultrathin sections were stained with uranyl acetate and lead citrate.

Semiquantitative Analysis of AVs in AD Brain

For quantitative studies, the numbers of AVs in neuronal perikarya were counted from electron micrographs at 8,000 \times magnification. Neurons were selected for quantitative analyses from cortical lamina III. Every fourth neuron with its nucleus present in the cross section was photographed within each sector of the entire electron microscopy grid. To capture additional neurons from the same brain, every fourth ultrathin section was used for quantification to avoid selecting the same neuron twice. Glial cells were excluded on the basis of their nuclear morphology and chromatin patterns.

Immuno-Electron Microscopy

Ultrathin sections from Epon blocks were placed on nickel grids, air-dried, and etched briefly with 1% sodium meta-periodate in phosphate-buffered saline (PBS) followed by washing in filtered double-distilled deionized water and incubated in 1% bovine serum albumin in PBS for 2 hours. Sections were incubated overnight in primary antibody (calnexin 1:250 or protein disulfide isomerase 1:200), both from Stressgen (Victoria, BC, Canada); cathepsin D (1:20) from DAKO (Carpinteria, CA) in a humidified chamber at 4°C, washed several times in PBS, and incubated with 5 to 20 nm gold-conjugated secondary antibody (anti-mouse IgG or anti-rabbit IgG; Amersham Biosciences, Piscataway, NJ [1:50]) in PBS for 2 hours at room temperature. In negative control experiments, primary antibody was substituted with normal rabbit serum or normal mouse serum depending upon the primary antibody used (polyclonal or monoclonal). Grids

were washed again and briefly stained with uranyl acetate and lead citrate before being examined with a Philips CM 10 electron microscope.

Isolation of Autophagic Vacuoles from Mouse Liver and Postembedding for Electron Microscopy

To isolate liver AVs, three C57BL/6 mice were starved for 18 hours prior to death (56). Livers were harvested, minced, and homogenized using a polytron Teflon homogenizer and separated by differential centrifugation to produce enriched fractions containing cytoplasm, AVs, lysosomes, mitochondria, and a pellet containing the nuclear fraction and (up to 30%) unbroken cells (PNP). A cytosol fraction was obtained by separating the supernatant from the AV/lysosome/mitochondrial pellet by centrifugation at 100,000g for 1 hour at 4°C. Subsequently, lysosomes were separated from two AV fractions using a metrizamide discontinuous gradient (AV10, 10% metrizamide; and AV20, 20% metrizamide). Autophagic vacuoles were fixed in cold 4% paraformaldehyde and 0.25% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer (pH 7.2) overnight at 4°C. Following fixation, isolates were washed (3×) in cacodylate buffer, postfixed in 1% osmium tetroxide, and progressively dehydrated in ethanol. The sample was then embedded and polymerized in Epon 812 following infiltration.

Sections were cut and mounted onto copper grids that were examined using Philips CM 10 electron microscope.

RESULTS

Ultrastructure of Autophagy Pathology in AD Cortex

In AD brain, cathepsin D (cat D) and many other lysosomal proteases in active form have been shown to be concentrated in dystrophic neurites, which are frequently, but not exclusively, associated with senile plaques (57, 58). Our ultrastructural analyses of the neuropil in neocortical biopsies from individuals with AD revealed many neurites in which the normal cytoplasmic content of the enlarged process was almost completely replaced by vesicular organelles with varying structural features (Fig. 1a). The range of morphologies (Fig. 1a, d, e) was strikingly similar to that observed in highly purified populations of liver AVs isolated by metrizamide density gradient centrifugation from mice starved for 18 hours to induce AV accumulation (56, 59) (Fig. 1b, c). Previous studies (60) have shown that these purified AV fractions were highly enriched in the autophagosome markers, light chain 3 (LC3) (61, 62), and rab24 (63), and were distinct from lysosomes. The morphologies and composition of vesicles that accumulated in affected dystrophic neurites (Figs. 1, 2) corresponded to those of the vesicular compartments of the autophagic pathway. Many vesicles met standard

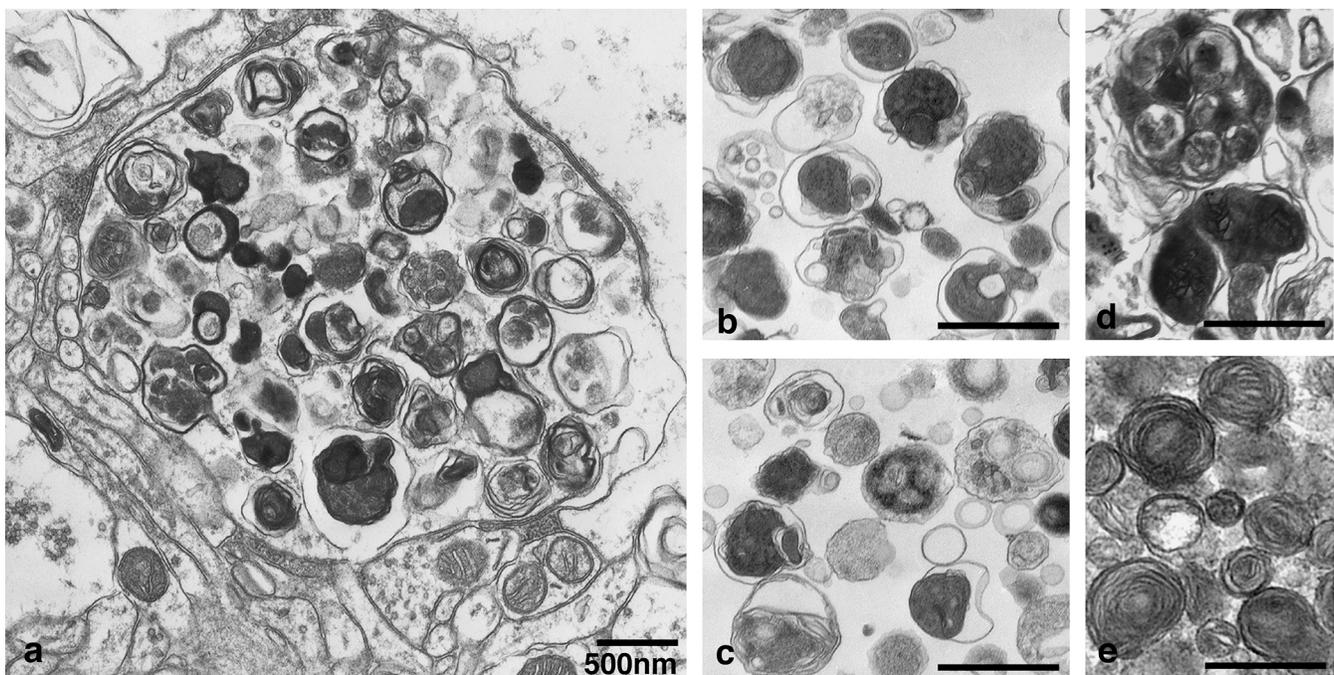


FIGURE 1. Ultrastructural appearance of autophagic vacuoles in AD brain and highly purified subcellular fractions from mouse liver (a). Dystrophic neurites contain abundant vesicles with a range of distinct morphologies similar to those of AVs highly purified from mouse liver by a well-established subcellular fractionation techniques (b, c). AV morphologies in brain include large double-membrane-limited vesicles containing multiple smaller double-membrane vesicles exhibiting heterogeneous intraluminal contents (d). Multilamellar bodies, another variant of AV (65), are also common in dystrophic neurites (e).

morphologic criteria for the immature and mature autophagosomes (37, 64), including a size $> 0.5 \mu\text{m}$ in diameter, a double-limiting membrane (immature), and the presence within a single vacuole of multiple membranous structures from mitochondria, Golgi, or endoplasmic reticulum, in addition to amorphous electron dense material. Multiple small double membrane-bound AVs were often contained within a larger AV (Fig. 1d), suggesting that autophagy in these neurites involves a continual process of AV consolidation. The vesicular contents of affected neurites, as well as the preparations of highly enriched AVs from liver, also often contained single membrane-limited multivesicular bodies containing light or dense amorphous material. Multilamellar bodies, which are autophagic in origin (65), also commonly appeared in some neurites (Figs. 1e and 2, inset). In regions of neuropil distant from β -amyloid deposits, frequent dystrophic neurites were present next to relatively normal-appearing neurites. While many of the dystrophic neurites contained abundant AVs of diverse morphology, AV populations of a particular morphologic type (e.g. multilamellar bodies [Fig. 2, inset arrows] or double-membrane-limited dense vesicles [Fig. 2, arrowheads]) often characterized specific abnormal neurites (Fig. 2).

Immuno-Electron Microscopy of Autophagic Vacuole Subtypes

Using immunogold electron microscopy, we further distinguished AVs from other organelles, such as endosomes and Golgi vesicles/tubules. The latter organelles are not expected to contain proteins that are resident to the endoplasmic

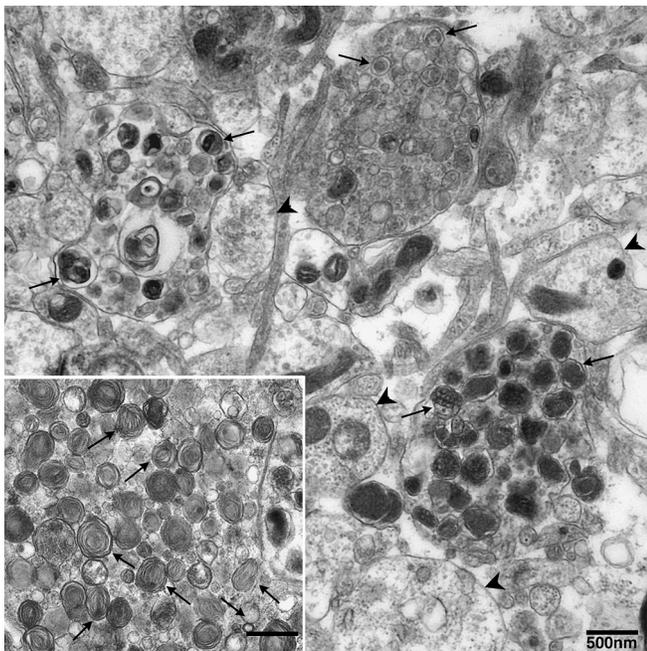


FIGURE 2. Different dystrophic neurites contain distinct AV populations. Abnormal neurites (long arrows) interspersed among normal-appearing neurites (arrowheads) often differ in the predominating AV subtype present and contain AVs of a specific morphologic type.

reticulum (ER). By contrast, autophagosomes have double membranes believed to originate from ER (36, 59, 66). Moreover, ER, like other vesicular organelles, is turned over by autophagy and is therefore expected to be part of the intraluminal contents of many AVs (59, 66, 67). Consistent with these expectations, the single or double membrane surfaces as well as the membranous contents of vesicles that we identified as AVs by morphologic criteria were also immunolabeled by antibodies to the resident ER proteins, protein disulfide isomerase (data not shown), and calnexin (Fig. 3a–c). Calnexin conjugated-immunogold, which selectively decorates ER (Fig. 3a, c), was present on the limiting membranes of autophagosomes (Fig. 3a, boxed area, b) and occasionally could be seen on ER membranes that are in the process of autophagic sequestration (Fig. 3a, arrows). The absence of gold in the surrounding neuropil confirmed the specificity of the immunolabeling. In contrast to the membrane labeling by anti-calnexin antibodies, immunogold labeling with cat D antibodies was seen mainly in the lumens of AVs (Fig. 3d–g). Immunogold labeling with antibodies to cat D distinguished immature AVs (presumably autophagosomes) lacking hydrolase from the more mature AVs that contained cathepsin immunoreactivity, reflecting late stages of AV maturation subsequent to endosomal/lysosomal fusion with AVs (Fig. 3d, e). The hydrolase-positive AVs at late stages of maturation were single membrane vesicles with amorphous dense intraluminal content (Fig. 3d, f), although some were double membrane-limited (Fig. 3e, arrow), possibly reflecting the active sequestration of multiple mature AVs within a single autophagosome-like vesicle. In contrast, multilamellar bodies (Fig. 3d, e arrowheads) were not immunolabeled with cat D. Many neurites contained high proportions of hydrolase-negative (immature) AVs. AVs were distinguishable from lysosomal dense bodies, which were generally smaller ($< 0.2 \mu\text{m}$), single membrane-limited structures of homogeneous density, which were strongly decorated by cathepsin antibodies (Fig. 3f). Residual bodies, such as lipofuscin, were not observed in neurites and were easily identified in perikarya by their bipartite organization of globoid electron-opaque lipopigment and homogeneous protein content that was strongly immunolabeled with cat D antibodies (Fig. 3g). In the absence of primary antibody, labeling of cat D-positive structures with immunogold secondary antibody was negligible (not shown). Cat D immunolabeling specificity was further indicated by the minimal labeling of other cytoplasmic organelles or ground substance and by the contrast between the intraluminal labeling by cat D antibodies (Fig. 3d–g) and the selective decoration of the double-limiting membrane of vesicles by anti-calnexin bodies (Fig. 3a, b).

Prevalence of Autophagic Pathology in AD Brain

In control cortical biopsies lacking detectable AD-related pathology, AVs were rarely observed in neurons and their processes (Fig. 4a). In AD brain, by contrast, AVs were frequently seen even in dendrites that were not markedly dystrophic (Fig. 4b), in terminal areas containing synaptic vesicles (Fig. 4c), and in distal dendritic processes as opposed

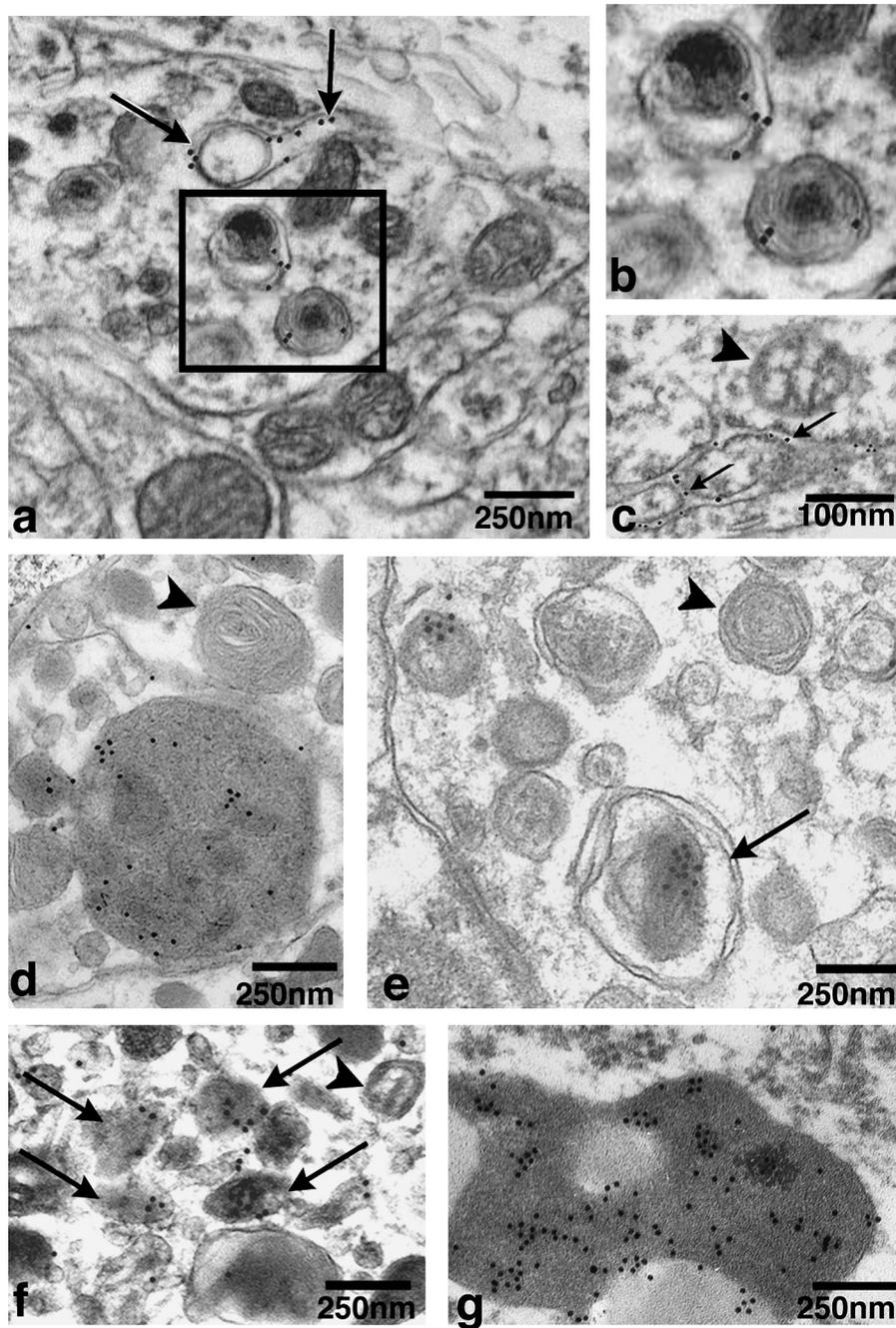


FIGURE 3. Immunogold electron microscopy of brain AVs. Highly selective immunogold labeling by anti-calnexin antibodies of the double-limiting membranes of AVs (**a**, boxed area; **b**) in abnormal neurites. A rare ER membrane profile in the process of autophagosome formation can also be seen (arrows). (**c**) Expected selective labeling by anti-calnexin immunogold of ER membranes (arrows) in the cytoplasm of a neuronal perikaryon. Mitochondria (arrowhead) and other cytoplasmic structures are unlabeled. The cat D antibodies mainly label electron dense intraluminal contents of structures with single-limiting membranes (**d**, arrow); micrographs in (**d**–**f**) are lightened to visualize gold more easily. Cat D rarely labeled multilamellar bodies (**d**, **e**, arrowheads) or double membrane-limited AVs lacking a dense core or containing clear membranous elements (**e**, arrow). The contents of small single-membrane dense bodies, corresponding to late AVs or lysosomes (**f**, arrow), are labeled (arrows) but not most double membrane-limited vesicles (arrowhead). The proteinaceous components of lipofuscin (**g**) are strongly immunolabeled by anti-cat D antibodies.

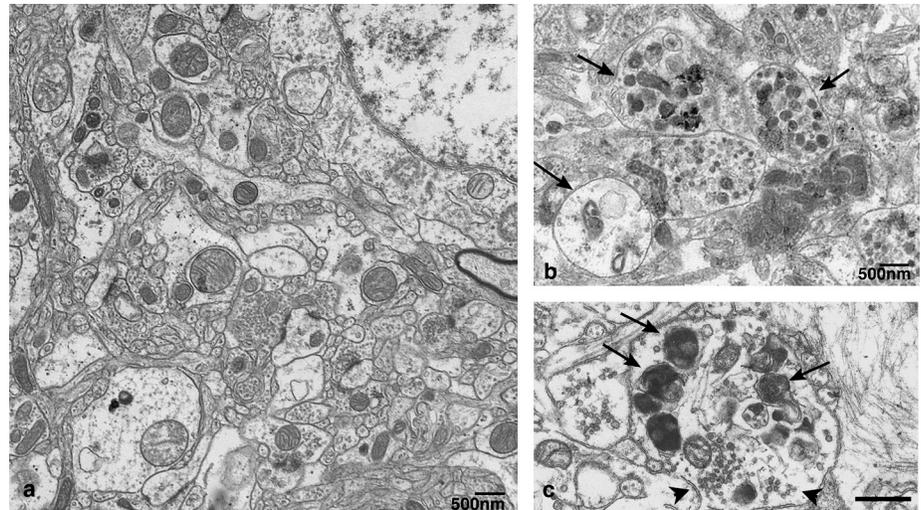
to normal-appearing neurons (Fig. 5). AV accumulation was most striking in dystrophic processes forming the neuritic network in proximity to senile plaques (Fig. 6), which have previously been shown to be strongly cat D immunoreactive (Fig. 6, inset) (58). Ultrastructural analysis reveals the extensive dystrophy of virtually all neurites in the vicinity of a β -amyloid deposit (Fig. 6) and within these neurites: the marked accumulation of vacuoles, most of which are AVs (Fig. 6, black arrows), and smaller numbers of swollen or condensed mitochondria (Fig. 6, arrowheads). The numbers of AVs in neuritic processes of AD brains far exceeded the incidence of

AVs in cell bodies, although AV numbers in neuronal perikarya were also markedly increased in AD.

Morphometric Analysis of AV Frequency in AD and Control Brains

In quantitative analyses of 137 randomly selected neurons in neocortex from three different control brains lacking AD pathology, AVs corresponding morphologically to autophagosomes, multilamellar bodies, and multivesicular bodies were observed at an average frequency of 0.117 ± 0.030 (mean \pm SE) per neuronal perikaryal cross section, and

FIGURE 4. AVs are normally rare in brain but common in neuronal processes and synaptic terminals in AD. In non-AD control brains, AVs are infrequently seen in neurites (**a**) and rarely seen in perikarya. In AD brain, AVs are common at relatively early stages of neuritic dystrophy in dendrites (**b**, arrows) and in synaptic terminals (**c**, arrows) identified based on the presence of collections of synaptic vesicles (**c**, arrowheads).



rarely appeared with a frequency of more than 1 per perikaryon. By contrast, in a sample of 130 randomly selected neocortical neurons from a total of 7 AD brains, AVs were identified at a more than 20-fold higher frequency (2.93 ± 0.19 per neuronal perikaryal cross section) (Fig. 7). The incidence of AVs and of dense lysosomes was particularly increased in the perikarya of neurons (Fig. 8a) that also contained paired helical filaments (Fig. 8a, inset). The example in Figure 8 shows a neuron with a relatively displaced normal-appearing nucleus, and no apparent perikaryal atrophy containing scattered bundles of paired helical filaments and numerous dense or multilamellar AVs in addition to lipofuscin granules and small dense lysosomes (Fig. 8b). Neuronal perikarya with elevated numbers of AVs commonly were relatively depleted of other organelles and particularly mitochondria (Fig. 8c, d; compare to the neuron in Fig. 5). Despite this increased incidence of AVs in perikarya, the number was relatively small compared with that in most affected neurites and was always outnumbered by dense lysosomes.

DISCUSSION

Our studies provide the first evidence supporting the extensive involvement of macroautophagy in AD pathogenesis. Previous studies have emphasized an early mobilization of the lysosomal system, including upregulated synthesis of lysosomal system components, secondary lysosome proliferation (68), and an accumulation of dense bodies in dystrophic neurites (52, 53) of vulnerable neuronal populations. Our data indicate that the induction of macroautophagy may contribute substantially to these downstream responses of the lysosomal system. We identified different vesicular compartments of the autophagic pathway (autophagosomes, autophagolysosomes, multilamellar bodies, and dense residual bodies) by various morphologic and biochemical criteria. These include vesicular subpopulations distinct from those described in earlier ultrastructural studies of Suzuki and Terry (52) and Terry et al (69). Autophagosomes were identified by their classic morphologic features of size greater than $0.5 \mu\text{m}$, double-limiting membrane, and heterogeneous intraluminal contents, which included intact organelles and organelle-

derived membranes (37, 64). Autophagosomes and other distinct vesicles of autophagic origin, such as multilamellar bodies (65), were shown to be prelysosomal based on an absence of immunogold labeling with cat D antibodies that strongly decorate lysosomal compartments. Importantly, AVs, which included a range of multivesicular morphologies, were excluded as being endosomes or multivesicular bodies of endosomal origin on the basis that the limiting membranes and intraluminal contents of many of these structures were immunogold labeled by antibodies to the resident ER proteins, calnexin, and protein disulfide isomerase (unpublished data). ER membranes are thought to be a source of membranes involved in autophagic sequestration (36, 59, 66), whereas compartments of the endocytic pathway should not be labeled by ER markers prior to fusion with autophagosomes.

In addition to the group of AVs representing early stages of macroautophagy, we identified more mature AVs, reflecting a stage after late endosome/lysosome fusion. Late AVs were more homogeneous in their intraluminal content than autophagosomes. They often, but not exclusively, exhibited single-limiting membranes, and contained cat D immunoreactivity by immuno-electron microscopy. These profiles were distinct from lysosomal dense bodies, which were small ($< 0.2 \mu\text{m}$), homogeneously dense, single membrane-limited structures, and strongly cat D immunoreactive. Collectively, cat D-positive compartments correspond to the dense bodies and acid phosphatase-containing vesicular structures that have previously been shown to be abundant in dystrophic neurites (52–54). The morphologic and biochemical features of early and late AVs in brain tissue closely corresponded to those of highly purified AVs from liver (60), which we showed to be enriched in LC3-II, a microtubule-associated protein thought to be a specific marker of autophagosomes (34, 62), and in rab24, a small GTPase reported to associate selectively with AVs (63). Moreover, in confirmation of our morphologic data, LC3-II levels are significantly elevated in AD brain (Yu et al, unpublished data).

AVs were rarely seen in neurons from the brains of individuals with no neuropathologic evidence of AD and were also uncommon in axons and dendrites. By contrast, AVs were

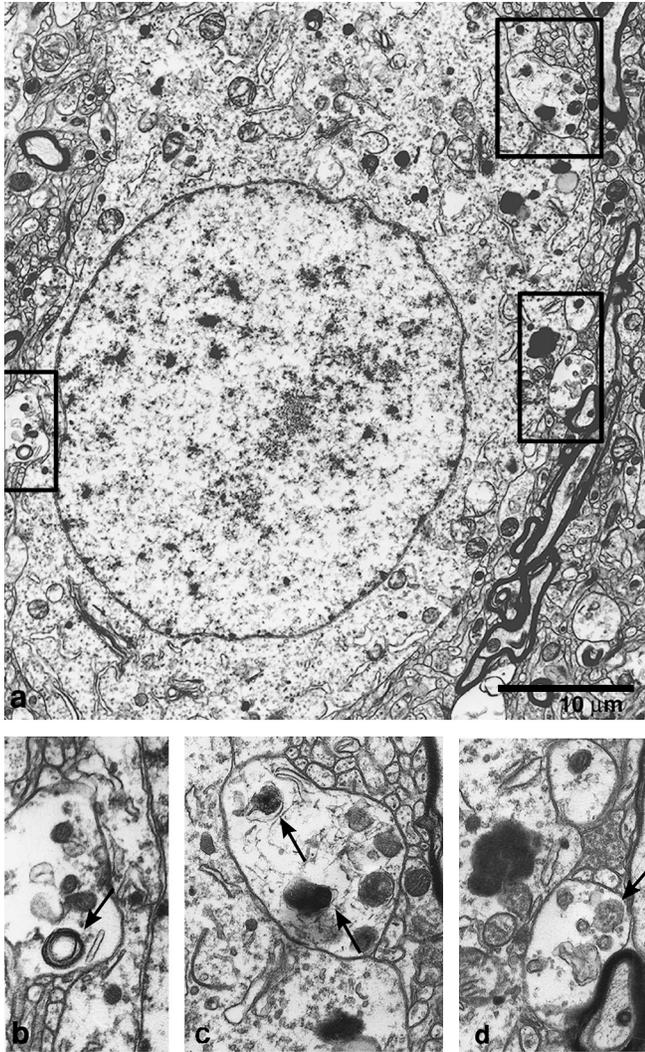


FIGURE 5. High incidence of AVs in nondystrophic terminal dendritic branches. Fine neuritic processes, which are apposed to normal-appearing neuronal perikarya (a), are intact and relatively normal in appearance but often contain AVs (b–d, arrows). Higher magnification images of the boxed areas in (a) are shown in (b–d).

frequently seen in otherwise normal-appearing neurites in the AD brain and were strikingly abundant in neurites that appeared swollen and dystrophic. These patterns were similar in all biopsy cases in this study. AVs were also more frequently observed in the perikarya of neurons in AD brains, particularly when these neurons contained paired-helical filaments. These observations accord with the extensive lysosomal hydrolase immunolabeling in AD brain (Fig. 8), which shows elevated neuropil staining and intense immunoreactive signal in neuritic plaques throughout affected regions (57, 70). Although AV accumulations are not specific to the degenerative phenomena of AD (47–49, 51), autophagic-lysosomal pathology in the brain is considerably more widespread and robust in AD than in other adult-onset neurodegenerative diseases (71). Specifically, the extensive neuritic dystrophy (53, 72) and the

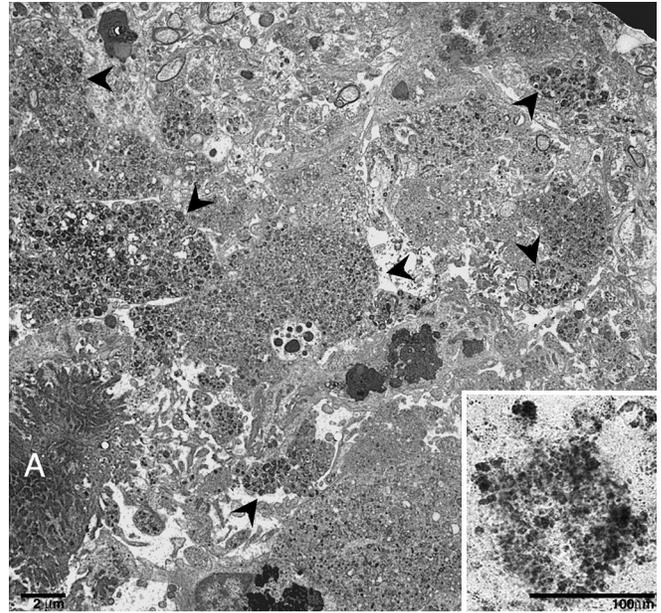


FIGURE 6. Marked AV accumulation in dystrophic neurites adjacent to amyloid deposits (A). Increased frequency and severity of neuritic dystrophy (arrowheads) within the vicinity of senile plaques, where at low magnification (inset) by light microscopic examination cat D immunoreactivity is particularly strong.

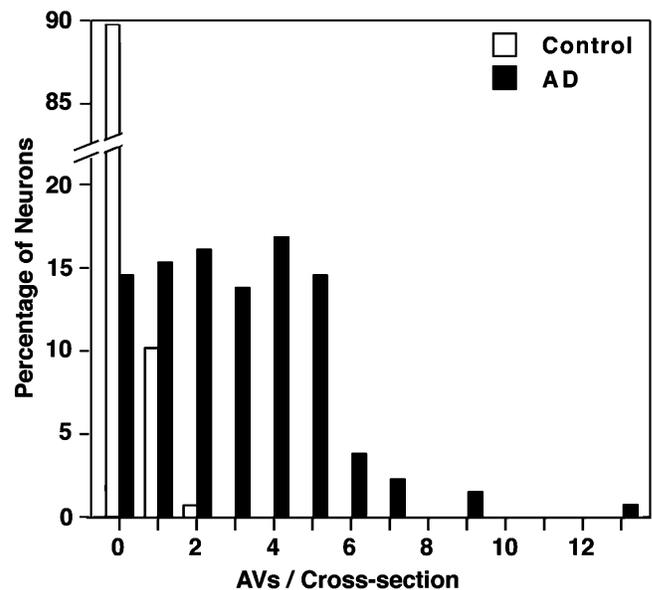


FIGURE 7. Autophagic vacuole frequency in neuronal perikarya in AD and control neocortex. AVs counted by visual inspection of electron micrographs in 130 AD neurons from 7 different AD brains and 137 control neurons from 3 control brains free of AD pathology. The percentages of neurons exhibiting a particular number of AVs in each group of cells are plotted. Note that no AVs were detected in 90% of control neurons.

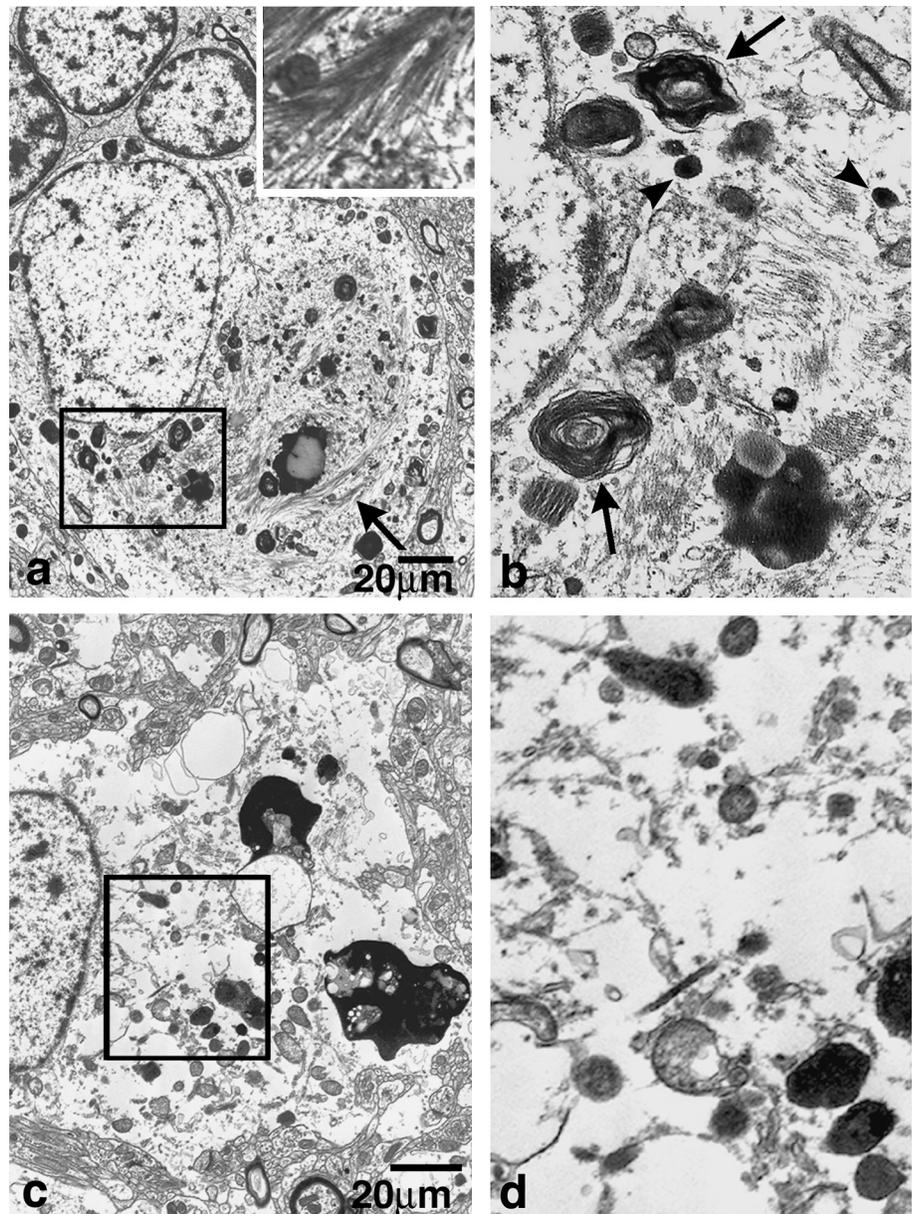


FIGURE 8. Autophagic vacuole frequency and depletion of other organelles in the perikarya of degenerating neurons. **(a)** A tangle-bearing neuron exhibiting scattered bundles of paired helical filaments (arrow and inset) and a peripherally displaced but otherwise normal nucleus. **(b)** The boxed area of **a** is shown at higher magnification. The perikaryon contains numerous AVs that include double-membrane dense structures and multilamellar bodies (arrows) as well as many small dense bodies or lysosomes (arrowheads). Autophagosome morphologies are rare in neuronal perikarya despite their abundance in neurites. **(c)** Mitochondria, Golgi, and ER are relatively depleted in neurons from AD brains (boxed area). These areas, seen at higher magnification in **(d)**, also display abnormal numbers of AVs and dense bodies compared with those seen in an intact neuron in Figure 5.

characteristic gross distension of these neurites in AD are not typical in other neurodegenerative diseases lacking β -amyloid (73). Moreover, the near replacement of cytoplasmic contents by AVs in these numerous dystrophic neurites, together with the high incidence of AVs in less obviously affected neurites, constitutes a uniquely large “burden” of autophagy-related compartments in the AD brain.

While macroautophagy seems to be induced in AD, the striking accumulation of AVs in dystrophic neurites suggests that some of the later steps of autophagy might be impaired in these neurons. Macroautophagy is normally active within the growing (regenerating) neurites of cultured neurons (41, 74, 75). This process involves the retrograde transport of immature AVs toward the cell body where they encounter and fuse with acidified hydrolase-containing compartments (41, 75).

AV maturation seems to be efficient under these conditions, resulting in rapid turnover of AV contents in lysosomes. The enormous accumulation of AVs, including autophagosomes and other immature AVs within dystrophic/degenerating neurites and the more modest accumulation in most neuronal perikarya, suggest that the transport of AVs is impeded or that autophagy locally within the dystrophic segments is particularly robust. These are not mutually exclusive possibilities. In addition, the coexistence of substantial numbers of both immature and acid-hydrolase-containing compartments suggests that immature AVs have access to hydrolase-containing compartments but may less efficiently carry out the final stages of AV maturation to lysosomes.

The large numbers of autophagic vacuoles in neurons of the AD brain have several possible implications for

pathogenesis in AD. AVs have been recently shown to contain the substrates and APP secretase activities required to generate A β and are particularly highly enriched in γ -secretase enzymatic activity and γ -secretase complex components (60). A β 40, A β 42, and β CTF have also been detected in purified AVs from livers of YAC transgenic mice overexpressing APP (60). Moreover, modulations of macroautophagy rates in cultured cells influence the rates of A β production, stimulating more than a doubling of A β production (Yu et al, unpublished data). These observations suggest that accumulated AVs in dystrophic neurites may contribute significantly to the local production of A β within plaques and that the generalized increase in autophagy in neuropil could be a significant source of A β overproduction in AD brain. Moreover, an impairment of macroautophagy that impedes the turnover of damaged mitochondria capable of triggering caspase activation or of oxidized membranes and proteins, which are a source of free radicals, is likely to promote neuronal degeneration (42). While local autophagy may be useful in the repair of damaged neurites and terminals, the stasis and accumulation of hydrolase-containing AVs in dystrophic neurites also represent a large reservoir of proteases that can potentially activate caspases directly and promote neurodegeneration (15, 28). The intriguing influences of macroautophagy on cellular aging mechanisms, cell survival, and protein handling that emerges from recent studies (26, 33, 76, 77) suggest that an understanding of its roles in neurons will be highly informative in defining pathogenic mechanisms in AD.

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