CellPress
OPEN ACCESS

Neuron

Review

Neuronal autophagy in the control of synapse function

Anna Karpova,[1,10,15] P. Robin Hiesinger,[2,15] Marijn Kuijpers,[3,4,15] Anne Albrecht,[9,10,14,15] Janine Kirstein,[5,6,15] Maria Andres-Alonso,[1,12] Alexander Biermeier,[8] Britta J. Eickholt,[7] Marina Mikhaylova,[8] Marta Maglione,[2] Carolina Montenegro-Venegas,[1,11] Stephan J. Sigrist,[2,7] Eckart D. Gundelfinger,[1,10,11] Volker Haucke,[2,4,7,*] and Michael R. Kreutz[1,10,12,13,*]

[1]Leibniz Institute for Neurobiology (LIN), 39118 Magdeburg, Germany
[2]Faculty of Biology, Chemistry, Pharmacy, Freie Universität Berlin, 14195 Berlin, Germany
[3]Donders Institute for Brain, Cognition and Behaviour, Radboud University, Nijmegen, the Netherlands
[4]Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), Robert-Rössle-Straße 10, 13125 Berlin, Germany
[5]Leibniz Institute on Aging-Fritz-Lipmann-Institute, 07754 Jena, Germany
[6]Friedrich-Schiller-Universität, Institute for Biochemistry & Biophysics, 07745 Jena, Germany
[7]Institute of Molecular Biology and Biochemistry, Charité Universitätsmedizin Berlin, 10117 Berlin, Germany
[8]Institute of Biology, Humboldt Universität zu Berlin, 10115 Berlin, Germany
[9]Institute of Anatomy, Medical Faculty, Otto-von-Guericke University Magdeburg, 39120 Magdeburg, Germany
[10]Center for Behavioral Brain Sciences, Otto-von-Guericke-University, 39120 Magdeburg, Germany
[11]Institute for Pharmacology and Toxicology, Medical Faculty, Otto-von-Guericke University Magdeburg, 39120 Magdeburg, Germany
[12]Leibniz Group "Dendritic Organelles and Synaptic Function", Center for Molecular Neurobiology, ZMNH, University Medical Center Hamburg-Eppendorf, 20251 Hamburg, Germany
[13]German Center for Neurodegenerative Diseases (DZNE), Site Magdeburg, 39120 Magdeburg, Germany
[14]German Center for Mental Health (DZPG), partner site Halle-Jena-Magdeburg, Germany
[15]These authors contributed equally
*Correspondence: haucke@fmp-berlin.de (V.H.), michael.kreutz@lin-magdeburg.de (M.R.K.)
https://doi.org/10.1016/j.neuron.2025.01.019

SUMMARY

Neurons are long-lived postmitotic cells that capitalize on autophagy to remove toxic or defective proteins and organelles to maintain neurotransmission and the integrity of their functional proteome. Mutations in autophagy genes cause congenital diseases, sharing prominent brain dysfunctions including epilepsy, intellectual disability, and neurodegeneration. Ablation of core autophagy genes in neurons or glia disrupts normal behavior, leading to motor deficits, memory impairment, altered sociability, and epilepsy, which are associated with defects in synapse maturation, plasticity, and neurotransmitter release. In spite of the importance of autophagy for brain physiology, the substrates of neuronal autophagy and the mechanisms by which defects in autophagy affect synaptic function in health and disease remain controversial. Here, we summarize the current state of knowledge on neuronal autophagy, address the existing controversies and inconsistencies in the field, and provide a roadmap for future research on the role of autophagy in the control of synaptic function.

INTRODUCTION

Neurons are highly polarized cells, with often long axonal and dendritic arborizations. The number of synapses a neuron forms can be huge; the molecular makeup of synapses can be extraordinarily complex; and the distance of synapses from the cell body, where most protein synthesis occurs, can be very large, up to about a meter in humans. Because neurons are postmitotic long-lived cells, maintaining the integrity of their proteome is of particular importance. Mammalian forebrain synapses comprise more than a 1,000 different proteins, and this complex proteome creates a unique situation with respect to the molecular dynamics of protein exchange and turnover,[1,2] in particular within the presynaptic compartment. As presynaptic neurotransmission involves the fusion and endocytic recycling of synaptic vesicles (SVs),[3–6] local membrane flux is exceptionally high at axonal terminals, and accordingly, the presynapse represents a region of high energy demand[7] and active membrane dynamics. How protein turnover is controlled in axons and axon terminals and whether this occurs locally (i.e., at the synapse) or in the soma are questions of key importance in molecular neuroscience.[1]

One major degradation route, tailored to the degradation of large protein complexes, toxic aggregates, and entire organelles, is autophagy.[8,9] Landmark studies have shown that neuronal integrity particularly depends on basal autophagy, likely because misfolded proteins and damaged organelles cannot be diluted through cell division and will thus accumulate in neurites and within soma.[10–13]

Here, we review the current state of knowledge regarding the mechanisms and mysteries of neuronal autophagy and how context-specific autophagic flux and cargo selection are regulated. We further discuss how basal synaptic function, plasticity,

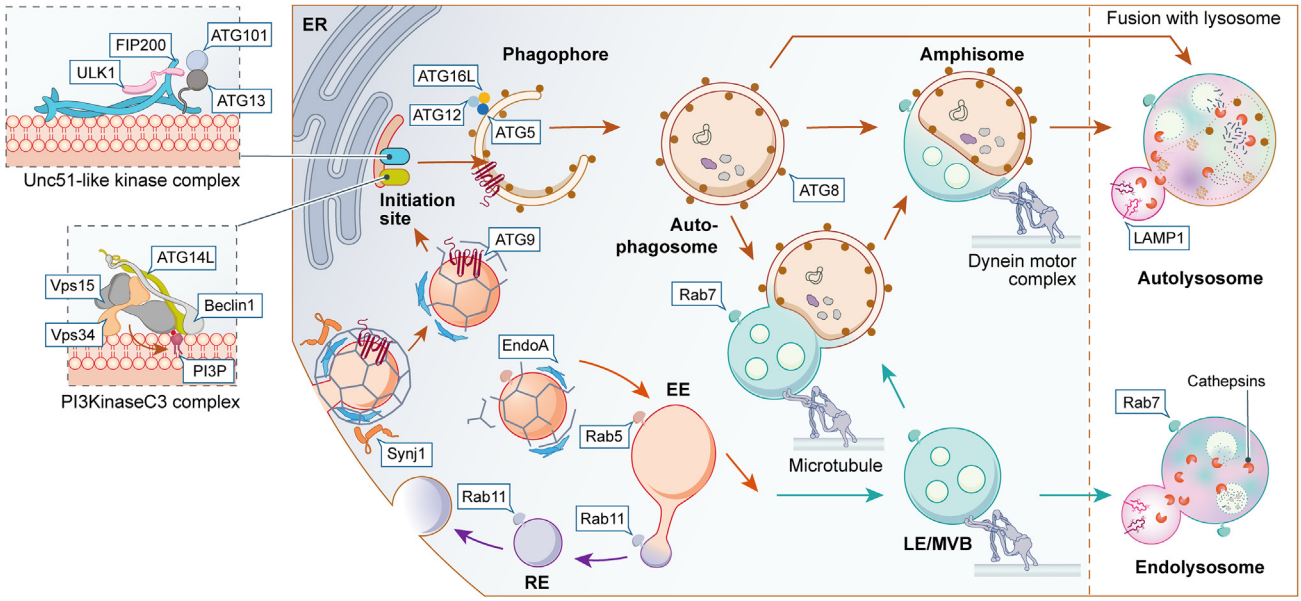

Figure 1. Mechanisms of neuronal autophagy and endolysosomal membrane dynamics

Autophagy is initiated by the assembly of a pre-autophagosomal structure referred to as the phagophore initiation site, primarily near the endoplasmic reticulum (ER). This process depends on the Unc51-like kinase complex and the PI3KC3 complex I, which synthesize the signaling lipid phosphatidylinositol 3-phosphate (PI(3)P). A further critical player in the initial stages of phagophore nucleation and expansion is the lipid scramblase ATG9, which is delivered via ATG9 containing vesicles. Endophilin A (EndoA) and synaptojanin-1 (Synj1) are involved in the recycling of ATG9 vesicles. A complex consisting of ATG5, ATG12, and ATG16L1 contributes to autophagosome formation by conjugating ATG8 family members (i.e., LC3 and GABARAP proteins) to phosphatidylethanolamine in the phagophore membrane. Sealed autophagosomes can form autolysosomes upon fusion with lysosomes or signaling organelles (amphisomes) upon fusion with Rab7-positive late endosomal/multivesicular bodies organelles (LE/MVBs). Autophagosome formation intersects with endocytosis and the sorting of proteins and lipids from early endosomes (EEs) to recycling endosomes (REs) or late endosomes (LEs) for degradation guided by small Rab GTPases. In neurons, LE/MVBs are transported retrogradely to the soma via dynein, where they fuse with degradative lysosomes to form endolysosomes. Amphisomes are formed through the fusion of LE/MVBs with autophagosomes, a crucial step that enables autophagosomes to acquire motors for retrograde transport. Upon reaching the soma, these organelles ultimately fuse with acidic lysosomes to form autolysosomes, facilitating cargo degradation.

and network function or dysfunction are affected by perturbations or enhancement of autophagy and how neuronal autophagy crosstalks with other pathways of regulated protein turnover to safeguard synaptic function. In the final section, we summarize recent work on non-canonical functions of autophagy in neurons and provide an outline for future studies. The relevance of neuronal autophagy for human brain diseases is only covered in specific cases, as excellent reviews have recently been published on this subject (e.g., Balusu et al.[14] and Nixon and Rubinsztein[15]).

MECHANISMS AND MYSTERIES OF NEURONAL AUTOPHAGY

Autophagy is a broad term that refers to evolutionary conserved degradation and recycling processes, which diverse cells, including neurons and glial cells, use to deliver cytoplasmic contents such as toxic or defective proteins and organelles to lysosomes for degradation.[16–22] Microautophagy is a form of autophagy during which cytoplasmic entities destined for degradation are directly taken up by the vacuole (in yeast and plants) via direct membrane invagination.[23] In cells from *D. melanogaster* and mammals, a similar mechanism referred to as endosomal microautophagy involves late endosomes and targets proteins (e.g., synaptic proteins) containing a KFERQ motif for late endosomal/lysosomal degradation.[24] It is related to chaperone-mediated autophagy (CMA), which involves the direct delivery of cytosolic proteins targeted for degradation to the lysosome.[25] In this review, we focus on macroautophagy (hereafter referred to simply as autophagy), a process that is initiated by the formation of a cup-shaped, double-membraned phagophore, whose edges extend and fuse to become an autophagosome (Figure 1). Cargos destined to be degraded by autophagy are captured by the forming autophagosome either through bulk engulfment of proteins or organelles or more selectively via adaptors (also referred to as receptors). Autophagosomes eventually fuse with lysosomes to enable cargo degradation by acid hydrolases contained in the lysosomal lumen to recycle amino acids and lipids such as cholesterol, which are important to sustain neuronal health (Figure 1). Strong evidence indicates that the endoplasmic reticulum (ER) serves as a primary source for phagophore formation, but various other membrane sources have also been suggested to contribute to autophagosome formation; these include endosomes, the ER/Golgi intermediate compartment, and the plasma membrane.[26–28] A key event in the early steps of autophagosome formation is the conjugation of members of the ubiquitin-like ATG8 family, which includes in vertebrates the LC3 and gamma-aminobutyric-acid-receptor-associated protein (GABARAP) subfamilies, to phosphatidylethanolamine in precursor membranes (Figure 1). In

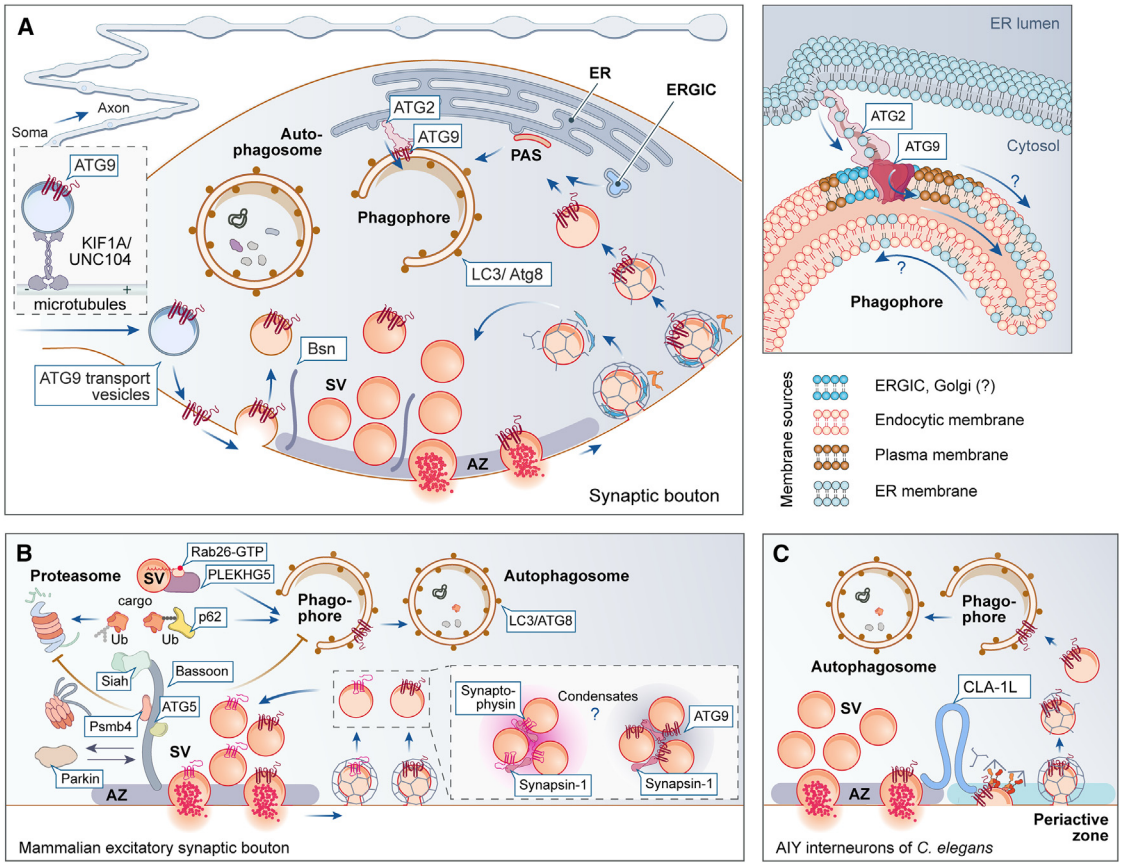

Figure 2. Biogenesis of autophagosomes in axons

(A) The lipid scramblase ATG9 is transported along axonal microtubules to presynaptic sites by the kinesin motor protein KIF1A/Unc104. ATG9-containing vesicles undergo activity-dependent cycling near the active zone (AZ) of transmitter release, mirroring the exocytosis and endocytosis steps of synaptic vesicles (SVs). Endocytosed ATG9 vesicles can acquire membranes from the ER/Golgi intermediate compartment (ERGIC) and possibly the *trans*-Golgi network (TGN) as well as the plasma membrane. Consequently, the membranes that seed the phagophore, initially formed at the phagophore initiation site at the ER, are derived from these diverse sources (indicated by different colors in magnified insert). Phagophore membrane expansion depends on a lipid transport conduit from the ER to autophagosomes formed by the rod-shaped bridge-like lipid transport protein ATG2 and the lipid scramblase ATG9 (magnified inset on the right). As the phagophore elongates, the opposing membranes fuse, forming a mature autophagosome.

(B) The giant AZ scaffolding protein Bassoon (BSN) regulates presynaptic proteostasis via its association with ATG5 and the proteasome subunit Psmb4. BSN also interacts with the ubiquitin (Ub) E3 ligase Siah1 and functionally interacts with Parkin-mediated degradation pathways. Entire organelles, such as SVs, may also be recruited to the phagophore via the small GTPase Rab26 and its guanine exchange factor PLEKHG5. SVs and ATG9 vesicles share similar appearances and exo-endocytosis pathways, yet they are distinct organelles. They may be separated at the perisynaptic endocytic zone by liquid phase separation, as the SV protein synaptophysin and ATG9 can form separate condensates with synapsin.[32]

(C) At the AZ of the *C. elegans* AIY neuron, the long isoform of the AZ protein clarinet (CLA-1L) acts as a positive regulator of presynaptic autophagy. CLA-1L links the AZ and the neighboring endocytic zone to promote the AP2- and AP180-mediated endocytosis of ATG9. Ablation of CLA-1L causes the accumulation of ATG9 at the periactive zone membrane.[33]

addition, evidence suggests that autophagosomal membrane growth involves lipid shuttling from the ER, which is mediated by a complex between the bridge-like lipid transfer protein ATG2 and the lipid scramblase ATG9,[29] the only transmembrane protein in the autophagy pathway (Figure 2A). While pathway conservation across species suggests that the core biology (i.e., about 40 so-called ATG proteins found in yeast) is similar in all cells,[17,30] it is likely that neuron-specific and possibly synapse-specific adaptations exist.[8,21,31]

Imaging studies in cultured hippocampal neurons and in *D. melanogaster* motoneurons have revealed that autophagosome biogenesis primarily occurs in the distal part of the axon[20,34,35] (as further discussed below) in a process that depends on the Unc51-like kinase complex (comprising ULK1, FIP200, ATG13, and ATG101) and on the synthesis of the signaling lipid phosphatidylinositol 3-phosphate (PI(3)P) by vacuolar sorting protein 34 (VPS34) complex I (composed of VPS34 kinase, VPS15, ATG14L, and Beclin-1; Figure 1).[36] Retrograde transport of distally formed autophagosomes via dynein-dynactin motors is accompanied by progressive acidification and maturation via fusion with Rab7-positive endosomes to form amphisomes. These eventually fuse with acidic lysosomes in the soma.[20] Whether degradative lysosomes containing active proteases travel into axons remains debated.[37] Axonal autophagosome formation appears to be a constitutive process,[38] although some studies[9,39–42] indicate that neuronal or synaptic

activity can alter autophagosome formation, suggesting that distinct types of autophagosomes may travel along axons. This pathway of axonal autophagosome biogenesis is consistent with the hypothesis that similar to other cell types, the ER, which is highly abundant in axons, also in neurons serves as a major membrane donor for autophagosome formation.[26,28] Consistently, neuron-specific loss of ATG5, a key factor for LC3/GABARAP lipidation, causes the accumulation of axonal ER in murine neurons.[43]

Our current knowledge of the physiological and pathophysiological roles of autophagy in neuronal and synaptic function derives from four major approaches: (1) genetic loss-of-function or, more rarely, gain-of-function studies in animal models and cultured neurons, (2) live imaging of autophagosome dynamics in neurons *in vivo*, (3) proteomic analyses of autophagic vesicles isolated from brain tissue or cultured neurons, and (4) of neuronal protein content following genetic perturbation of autophagy.

Genetic studies in mice have revealed that brain-specific conditional knockout (KO) of core autophagy genes, such as ATG5 and ATG7, using the neural progenitor cell-specific nestin-Cre driver, results in massive neurodegeneration and premature death of mice.[10,11] Similar neurodegenerative phenotypes were observed upon neural-specific deletion of the ULK1 complex subunit FIP200.[44] Virus-induced conditional deletion of ATG5 in neural progenitor cells in adult mice impaired survival of adult-generated neurons and delayed neuronal maturation.[45] Depletion of ATG5 in embryonic mouse brain compromised neural progenitor cell differentiation and neuromorphogenesis,[46] a phenotype recapitulated in hippocampal neurons from conditional ATG5 KO mice[47] and in one subtype of striatal spiny projection neurons.[48] In contrast, conditional loss of ATG5 in excitatory neurons in mice did not result in overt changes in neuronal morphology and only mildly impacted on neuronal viability,[43] while cell-type-selective ablation of ATG7 in Purkinje cells leads to axonal dystrophy and degeneration in adult mice.[12] Recent studies have uncovered cases of human patients with complex neurodevelopmental disorders linked to congenital reduction or complete absence of the ATG7 protein. These patients display cerebellar and corpus callosum abnormalities, varying facial dysmorphisms, and impaired autophagic activity.[49] Indeed, emerging evidence suggests genetic links between autophagy and neurodevelopmental disorders in humans.[50] Consistently, 4D imaging and genetic studies in the developing *D. melanogaster* brain have revealed that different neuron types activate or suppress synaptic autophagy to regulate synapse formation and brain connectivity.[51–53] Collectively, these findings suggest that the requirement for neuronal autophagy may be developmental stage specific and neuronal cell-type specific. Moreover, it appears that some forms of neuronal autophagy may proceed in the absence of core autophagy proteins such as ATG5, e.g., via alternative ATG5-independent mechanisms of autophagy[54] that may involve PI(3)P binding proteins such as WIPI3.[55]

While autophagosome formation has been shown to predominantly occur in distal axons in vertebrate and invertebrate neurons,[34,35,56,57] evidence exists for similar processes in other cellular compartments, although their regulation may be different. For example, p62/SQSTM1, a widely used marker for autophagy that acts as a receptor for aggregated proteins (i.e., aggrephagy), accumulates in neuronal somata but not in axons when autophagy is compromised.[43,44] Conversely, impairment of autophagy caused by a loss of neuronal ATG5 results in the massive accumulation of tubular ER in axons but does not impact ER in somata and dendrites.[43] Why somatic and dendritic ER appear to be unaffected by the neuron-wide blockade of ATG5-dependent autophagosome formation, although the ER is thought to be a continuous single-copy organelle, remains enigmatic. Lastly, accumulating evidence indicates that autophagy can also be initiated in the somatodendritic compartment where it functions in the turnover of postsynaptic proteins to regulate long-term depression (LTD)[58–60] and excitatory neurotransmission[61] (see below). Notably, the movement of autophagic organelles within dendrites can also be influenced by synaptic activity.[62]

Beyond the question of where autophagosomes are formed, further riddles arise from the proteomic analyses of the putative substrates for neuronal and synaptic autophagy. One approach is based on the rationale that genetic perturbation of neuronal autophagy should cause the accumulation of its major substrate proteins. Quantitative proteomic analysis of cultured neurons engineered to enable the acute conditional loss of ATG5 revealed the accumulation of tubular sheet ER proteins, including reticulons, REEPs, and the ryanodine receptor, in addition to well-known substrates such as p62/SQSTM1.[43] Surprisingly, this analysis failed to detect alterations in the levels or localization of presynaptic and postsynaptic proteins or in SV numbers. In contrast, studies in hippocampal neurons depleted of the giant active zone (AZ) scaffold proteins Bassoon and Piccolo displayed signs of severe neurodegeneration caused by the presynaptic turnover of SVs via pathways potentially involving ATG5-dependent autophagy.[57,63] These studies are consistent with the hypothesis that the presynaptic function and SV cycling are linked to the induction of the autophagy pathway via poorly understood mechanisms that may involve ubiquitination,[21,57,64] the endocytic proteins endophilin A[35] and AP2,[47] and the activity-dependent and endocytosis of the lipid scramblase ATG9[65] (further discussed below). Recent proteomic analyses of autophagosomes isolated from brain or cultured human and mouse neurons have confirmed the presence of ER membrane proteins in neuronal autophagosomes. In addition, these studies have identified many other potential cargos for neuronal autophagy, such as mitochondria and mitochondrial nucleoids and SV proteins as well as aggregation-prone proteins with roles in age-related neurodegeneration.[66–68] The relative enrichment of these various cargos differs between studies and appears to further depend on developmental status and age of the mice used as donors.[67] It is likely that autophagic cargo further varies in a neuron subtype-specific manner. In agreement, it has been shown that autophagy differentially controls neuronal excitability by regulating axonal ER turnover in cortical and hippocampal glutamatergic neurons,[43] while in distinct GABAergic striatal spiny projection neurons, excitability depends on the autophagic regulation of the inwardly rectifying potassium channel Kir2.[48]

These examples illustrate that in spite of our detailed knowledge about the molecular machinery for neuronal autophagy,

major riddles regarding the physiological substrates and mechanisms that control autophagy at synapses remain.

MECHANISMS AND REGULATION OF AUTOPHAGOSOME FORMATION AT SYNAPSES

Constitutive formation of autophagosomes has been observed in developing axons of mammalian neurons in primary culture[34,38] and in *C. elegans in vivo*[56] and has been proposed to be important for the maintenance of neuronal and synaptic homeostasis. In contrast, the question whether autophagy in the presynaptic compartment can be induced by starvation, the major inducer of autophagy in non-neuronal cells, has been discussed controversially.[20,38,69,70] Evidently, in addition to constitutive processes, multiple autophagy-inducing pathways can affect synaptic structure and function. Treatment with rapamycin, an inhibitor of the protein kinase complex mechanistic target of rapamycin complex 1 (mTORC1), induces formation of autophagic vacuoles in dopaminergic axons and impairs neurotransmitter release.[71] In hippocampal primary neurons, nutrient limitation, which activates mTOR-dependent autophagy, induces structural and functional presynaptic alterations.[72] Locally, autophagy can also be induced in presynaptic boutons by the production of reactive oxygen species (ROS).[73] Moreover, KCl-mediated depolarization has been suggested to increase the number of LC3-positive structures in axon terminals and in other neuronal compartments of cultured rodent hippocampal neurons.[40] Activity-induced increase of autophagosome formation has further been observed at presynapses of AIY neurons in *C. elegans in vivo*.[74] Somewhat discrepant with these studies is the finding that chemical induction of LTD (chem-LTD) by low-dose application of NMDA[75] or chronic silencing via TTX[76] can also globally induce autophagy in primary rodent neurons, presumably via mTORC1 inhibition.

Work on the neuromuscular junction (NMJ) of *D. melanogaster* larvae shows that amino acid starvation and neuronal activity can both stimulate autophagosome biogenesis within the presynapse[35,77,78] and that activity-induced calcium influx and presynaptic autophagy are coupled.[42] According to a recent preprint, neuronal activity-dependent and starvation-induced synaptic autophagy appears to be regulated via distinct molecular mechanisms.[79] At the *D. melanogaster* larval NMJ, the SV protein synaptogyrin is essential for activity-driven autophagy without affecting starvation-induced autophagy. In contrast, the membrane-deforming protein endophilin B participates in autophagy induced by starvation while being dispensable for activity-induced autophagosome formation.[79,80]

An interesting yet poorly understood feature of presynaptic autophagosome formation is its apparent link with the SV cycle. At fly NMJs, the induction of presynaptic autophagy depends on the membrane-deforming protein endophilin A (i.e., a close relative of endophilin B mentioned above) and the endophilin-associated signaling lipid phosphatase synaptojanin-1 (Synj1), proteins demonstrated to be of key importance for the endocytic recycling of SV membranes.[35,41,78,81] A physical and functional link between neuronal autophagy and the SV cycle is underscored by the fact that numerous components of the autophagic machinery are present in the presynaptic compartment and associated with SVs[82] (Figure 2; for review, see Gundelfinger et al.[21]). These include, for example, subunits of the PI3KC1 complex (e.g., Beclin-1 and Vps34), ATG5, ATG16L1, the cargo adaptor p62/SQSTM1,[57] and the lipid transfer protein ATG2.[83] ATG2 transfers lipids from donor membranes (e.g., ER) to ATG9 vesicles to enable autophagosome membrane growth[84] (Figure 2A). The lipid scramblase ATG9, isoform ATG9A in vertebrates, is contained in small vesicles in axons and at synapses that undergo exo-endocytic cycling in parallel to the exocytosis and endocytosis of SVs (Figure 2; for review, see Choi et al.[32] and Holzer et al.[85]). Studies in *C. elegans* and in rat brain have demonstrated that ATG9-containing vesicles are distinct from SVs, in spite of their similar appearance in electron microscopy and the partial co-purification of ATG9 with SVs.[82,86–88] Interestingly, the presynaptic abundance and sorting of ATG9 vesicles may vary with synaptic activity.[87] After AP3 adaptor complex-mediated formation at the *trans*-Golgi network, a major secretory station of neurons, ATG9 vesicles are transported in axons along microtubules via the kinesin KIF1A/Unc104 to presynapses[56,89] (Figure 2A). This pathway seems closely related to the transport of SV precursors.[90]

At synapses, ATG9 vesicles undergo activity-dependent cycles of exocytosis and endocytosis.[89] Presynaptic accumulation of ATG9 near clathrin-rich sites is observed in *C. elegans* mutants for dynein 1 or endophilin A and in mutant nematodes either lacking Synj1 or expressing a missense mutant that corresponds to a human early-onset Parkinson mutation in Synj1.[65] When SV exocytosis is blocked, e.g., in mutants for the key exocytic proteins UNC13/Munc13, UNC10/RIM, or UNC18/Munc18,[87] ATG9 vesicles fail to accumulate at the periactive zone. As ATG9 vesicle-mediated membrane delivery is crucial for autophagosome formation and growth[84,91] (see Choi et al.[32] for a detailed review), these data suggest that presynaptic autophagosome formation via ATG9 may be triggered by the activity-dependent exo-endocytic cycling of SV membranes. Consistently, it has been shown that disruption of ATG9 trafficking results in impairment of activity-dependent but not basal autophagosome biogenesis at synapses.[33,65,87] Upon ectopic expression in non-neuronal cells, ATG9A vesicles can form condensates with the SV-associated protein synapsin, which are distinct from condensates formed by synapsin with the SV integral membrane protein synaptophysin.[32,86] This mechanism may contribute to the segregation of SVs from ATG9A vesicles at synapses. Whether and how a similar mechanism acts at endocytic sites of the periactive zone to separate material destined for autophagic degradation from recycling material needs to be examined. Moreover, how and where synaptic activity-regulated and constitutive pathways of ATG9 cycling are separated remains an important question for future studies.

A further link between presynaptic autophagy and activity-dependent SV cycling is related to the integrity of the AZ, e.g., the nanoscale sites where SV fusion occurs. Large scaffolding proteins at the AZ for neurotransmitter release, such as *C. elegans* clarinet-1 (CLA-1L), a member of the RIM superfamily,[87] or the giant protein Bassoon in vertebrates, have major functions in coordinating SV recycling and presynaptic autophagy (Figures 2B and 2C). The long isoform of CLA-1L is essential for activity-induced autophagy in *C. elegans* nerve terminals

by controlling the sorting of ATG9 vesicles.[87] Disruption of CLA-1L function causes ATG9 accumulation at endocytic sites and impairs activity-induced autophagosome formation, akin to a loss of Synj1 or endophilin A. In contrast, presynaptic autophagy is repressed in rodent brain synapses by the giant AZ protein Bassoon.[57,64] Bassoon-deficiency leads to the accumulation of LC3/ATG8 at presynaptic sites and in axons, concomitant with impaired neurotransmitter release and increased ubiquitination of presynaptic proteins (Figure 2B). How exactly Bassoon interacts with the autophagic machinery is not well understood. Bassoon was shown to bind ATG5 and overexpression of the ATG5 binding region of Bassoon suppresses autophagy overshoot in primary hippocampal neurons depleted of both Bassoon and the closely related Piccolo protein (Figure 2B). In these neurons, ATG5 is recruited to the presynapse only after induction of autophagy.[57] It is currently unknown whether and, if so, how Bassoon affects trafficking of ATG9 vesicles. Assessment of the function of Bassoon in presynaptic autophagy is complicated by the fact that Bassoon and its paralog Piccolo are involved in multiple pathways of presynaptic proteostasis by interacting with the ubiquitin-proteasome system (UPS) and the endolysosomal system[63,92–94] (Figure 2B). A reflection of the interplay between presynaptic autophagy and the UPS may be the fact that depletion of the Parkinson's disease-related protein Parkin, an E3 ubiquitin ligase implicated in mitophagy,[95,96] can ameliorate excessive presynaptic autophagy in neurons depleted of Bassoon.[64]

Another pathway of autophagy-mediated removal of abandoned presynaptic material involves the inclusion of entire organelles, such as SVs, into autophagosomes. Mechanistically, this can be mediated by the small GTPase Rab26 and PLEKHG5, a guanine exchange factor (GEF). PLEKHG5 can directly bind PI(3)P, induce GTP binding to Rab26, and in turn trigger the formation of the ATG5-ATG12-ATG16L complex[97–99] (Figure 2B). Surprisingly, a null mutant for Rab26 showed no phenotype in *D. melanogaster* and has normal neurotransmission.[100]

Collectively, distinct modes of autophagy operate in axons and within the presynaptic and postsynaptic compartments. Apart from a constitutive pathway that may support the steady-state turnover of neuronal proteins (e.g., the axonal ER), stress- or starvation-related pathways may also operate at certain types of synapses to help keep them functional and resilient to insults and metabolic challenges. In addition, activity-induced pathways of presynaptic and postsynaptic autophagy exist, which can be triggered by SV cycling and/or mechanisms of synaptic plasticity, and are crucial for higher brain functions.

CONTEXTUAL SPECIFICITY OF AUTOPHAGIC CARGO SELECTION IN NEURONS

The characterization of neuronal autophagy at steady-state conditions or its induction upon alteration of synaptic activity raises the question of how cargo specificity is achieved. One can assume that least three distinct factors may contribute to cargo availability for engulfment independent of the mechanisms of autophagy induction: first, the relative abundance of cargo, which is closely related to protein half-life; second, the time and location of autophagosome biogenesis relative to the localization of the cargo; and third, the direct or indirect molecular interaction of cargo with early-acting components of the autophagic machinery (e.g., autophagy adaptors or receptors) during phagophore formation (Figure 3A).

A recent proteomic study identified nearly 3,000 proteins significantly enriched in mouse brain-derived LC3-positive autophagosomal vesicles (AVs).[67] This large number gives some indication of the breadth of material that can be engulfed and raises questions about how many of these proteins are individually selected for degradation. As shown in that study, >60% of the brain AV content was shared with the content of non-neuronal cell lines, whereas 38% could be considered as "brain-specific." Where does this specificity for hundreds of brain-specific proteins come from? The relative abundance of proteins in neurons, compared with other cell types, may be part of the answer. A quantitative proteome map of the human body disclosed that the brain has the largest number of enriched and specific proteins, compared with that of other organs.[101] All other factors being equal, autophagosomes are more likely to engulf proteins and subcellular structures that are more abundant. The many proteins that are specific or enriched in neurons thus likely skew the specificity profile. However, relative abundance most likely does not account for the 38% of brain-specific proteins in the neuronal degradome and particularly for the surprisingly high number of synaptic proteins of which many display a comparably long half-life.[102]

As neurons exhibit anatomically, functionally, and molecularly highly divergent sub-compartments, the proteome of the presynaptic nerve terminal differs substantially from that of the cell body or dendrites. Growing phagophores likely engulf proteins and subcellular structures in the vicinity of their origin of biogenesis. However, the origin of biogenesis does not need to be related to the cargo that will be degraded. For instance, in the case of bulk autophagy (such as mTORC1-dependent autophagy), the trigger of autophagosome formation and local engulfment is not the cargo. In developing fly photoreceptor axon terminals, autophagosomes are selectively generated at the tips of synaptogenic filopodia, suggesting that the place and time of autophagosome formation are highly regulated[52] (Figure 3B). The tip of a filopodium is arguably a small space that provides considerable specificity to what a developing phagophore may engulf. Not surprisingly, autophagosome formation in these filopodia typically leads to filopodial collapse, providing a regulatory mechanism for synapse formation.[52] Similarly, autophagy has been linked to synapse formation in mice[103] and nematodes.[56] Different developing neuron types may employ local autophagy at different times and places, leading to distinct cargo selectivity.[51] Hence, the timing of when and where autophagosomes form greatly restricts what cargo proteins are available to be engulfed. Consequently, one can assume that the subcompartment-specific proteome quantitatively affects cargo composition, particularly when paired with spatially and temporally controlled autophagosome induction (Figure 3C). For example, the activity-dependent induction of autophagosome formation in the vicinity of presynaptic

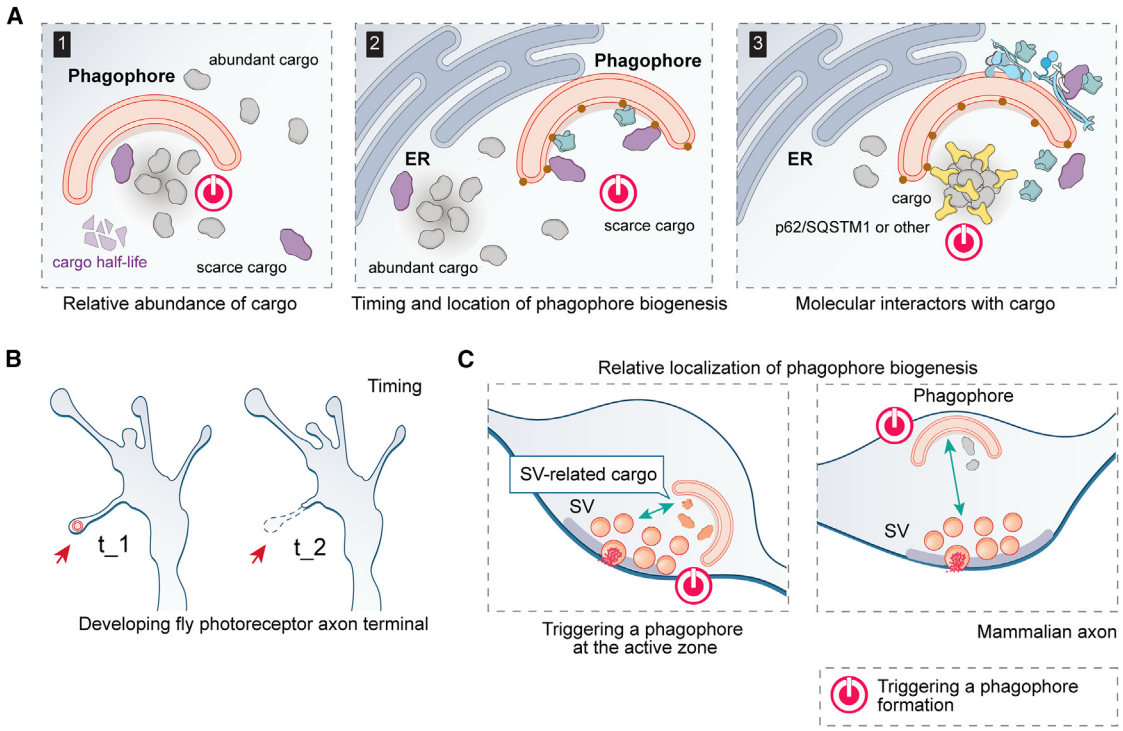

Figure 3. Contextual specificity of phagophore formation and cargo engulfment

(A) Factors that might contribute concurrently to the selection of engulfed cargo are the following: (1) the relative abundance of cargo proteins influences the availability of cargo for engulfment by the expanding phagophore. (2) The timing and localization of phagophore biogenesis significantly restrict the available cargo for engulfment. (3) Direct or indirect binding of cargo to early autophagy machinery during phagophore formation can enhance cargo accessibility and in turn promote phagophore formation in proximity to cargo. The decoration of cargo with an "eat me" signal, recognized by an autophagy receptor, can facilitate phagophore recruitment to cargo proteins, aggregates, and/or organelles. Receptors, such as p62/SQSTM1, direct phagophore recruitment and can directly or indirectly promote autophagosome formation.

(B) Autophagosome formation in the small area of the filopodial tips of developing axonal terminals in photoreceptors typically leads to filopodia collapse, suggesting a process of bulk engulfment of available cargo.

(C) Phagophore formation triggered near synaptic release sites is more likely to engulf SV-related cargo, compared with phagophores formed at more distal locations. This observation can serve as a counterexample to the notion that the cargo itself initiates the process.

release sites will increase the likelihood of SV-related proteins in the autophagosomal degradome (Figure 3C).

Individual proteins and whole organelles can increase the likelihood of being engulfed with dedicated "eat me" signals. The best known "eat me" signal is ubiquitination, which can also mark proteins for proteasomal degradation. Autophagosomes are recruited to ubiquitinated proteins, aggregates, or organelles through the autophagy receptor p62/SQSTM1. Since the initial characterization of p62, dozens of selective autophagy receptors have been described,[104] some of which are soluble, like p62, and others permanently bound to putative cargo. "Unifying criteria" for all autophagy receptors have been named as follows: (1) binding to cargo and (2) binding to ATG8 family members (LC3/GABARAPs) on the inner surface of the maturing phagophore membrane with an LC3-interacting region (LIR) domain.[105] Many LIR-LC3/GABARAP interactions facilitate the engulfment of large cargo by the phagophore. This is best characterized for aggrephagy, where large p62-positive cargo aggregates are selectively engulfed and degraded. However, this mechanism does not explain the induction of autophagy, as LC3/GABARAPs mark elongating phagophore membranes downstream of phagophore biogenesis. LIR domains and selective autophagy based on LC3/GABARAP interactions may therefore increase the probability of a cargo to be recognized by an existing phagophore that formed independent of cargo. Indeed, many proteins contain LIR domains but are not enriched in the autophagosomal degradome, and overexpression of LIR-domain proteins and their expression or interaction with ATG8 proteins do not necessarily increase autophagosome formation.[67] In the mechanistically distinct process of endosomal microautophagy, proteins containing KFERQ motifs, including synaptic proteins, are targeted for degradation via late endosomes in an activity-dependent manner.[24]

A core idea of selective autophagy posits that autophagy receptors do not only increase the likelihood of being engulfed by an autophagosome that formed elsewhere, but they can directly or indirectly promote autophagosome formation. Phagophore assembly sites most typically require a phosphatidylinositol synthase-rich ER domain, ATG9-positive vesicles, and two kinase complexes (the ULK1/2 and PI3KC3-C1 complexes; Figure 1).[106] Both ULK1/2 and the PI3KC3-C1 complex components ATG14, Beclin-1, and Vps34 contain LIR domains that bind LC3/GABARAPs.[107] Since LIR-dependent binding to LC3/GABARAPs occurs

downstream of phagophore biogenesis, a feedback loop has been proposed whereby autophagy receptors exert the additional function of recruiting core autophagy machinery to the site of action. In the best-studied example, p62 forms aggregates together with cargo proteins, and these co-assemblies recruit the ULK1 complex and ATG9 vesicles to induce a phagophore that can readily engulf the aggregate itself. LIR-LC3/GABARAP binding is required for this mechanism and may operate post-induction to promote engulfment. Yet, a reversal of the order of these interactions is also conceivable and would ultimately place the cargo-autophagy receptor complex at the initiation steps of phagophore formation. In this scenario, cargo-autophagy receptor complexes *first* recruit the core autophagy machinery and subsequently utilize the LIR-LC3/GABARAP interaction to push phagophore-mediated engulfment around the aggregate itself.[108] Overexpression of autophagy receptors typically is not sufficient to induce autophagy, although examples where p62 induces autophagy exist in nematodes[109] and flies.[110]

To what extent selective autophagy marks the induction of autophagosome formation, as opposed to increasing the probability of a given cargo to be engulfed based on autophagosomes that were induced by a different mechanism, remains unclear. In either case, the observation that the neuronal autophagosomal degradome contains thousands of proteins, including hundreds of brain-specific proteins, highlights that autophagy receptors can only be part of a composite of factors that lead to cargo specificity. There are dozens of autophagy receptors but not hundreds or thousands. Consistently, no brain-specific autophagy receptor has been found to date. Rather, autophagy receptors that function in selective autophagy throughout the organism are more likely to execute brain-specific roles due to context, e.g., the specific environment of the presynaptic axon terminal. That said, some level of cargo specificity may arise from differential localization, abundance, and autophagy receptor specificity of the distinct members of the LC3/GABARAP family of ATG8-related proteins.

In conclusion, cargo availability may determine cargo specificity, a concept we refer to as *contextual specificity*. Availability can be increased by (1) overall abundance in neurons and their subcompartments; (2) the precise location and timing of phagophore biogenesis relative to the cargo; (3) the decoration of the cargo with an ‘‘eat me’’ signal to be recognized by an autophagy receptor; and (4) finally, the specific isotype of autophagosome formed, e.g., LC3b-coated autophagosomes may differ from those coated by GABARAP proteins (Figures 3A–3C). Induction may be linked to autophagy receptors but may also be mechanistically separate. Experimentally controlled induction, e.g., through increased neuronal activity, could differentially affect any of these four factors and thereby alter cargo specificity of neuronal autophagy.

Thus, we propose that so-called ‘‘bulk’’ autophagy as well as autophagy induction in the specific contexts of normal development and function may not be unspecific after all. Based on these considerations, bulk autophagy and selective autophagy may not be mutually exclusive but rather form a mechanistically overlapping continuum based on contextual specificity. Future studies are needed to rigorously test this hypothesis.

PROTEOLYTIC CROSSTALK AT THE SYNAPSE

The investigation and interpretation of contextual specificity is further complicated by proteolytic crosstalk at the synapse. The synaptic proteome is regulated by both protein synthesis and proteolytic clearance. As outlined above, the main proteolytic pathways involved in this process include autophagy and the endolysosomal pathway as well as the UPS. Proteomic analyses have revealed that all of these proteolytic systems are present at the synapse.[111,112] The half-lives of synaptic proteins vary significantly, ranging from hours to more than 20 days.[102,113] As aging progresses, the half-lives of proteins increase by approximately 20%.[2] Notably, this increase in half-life affects selected proteins (1) that are neuroprotective and play a role in maintaining proteostasis with age and whose absence or reduced activity is linked to neurodegenerative diseases; (2) that are rich in basic amino acids and unstructured regions (these proteins are often degraded by the autophagy-lysosome pathway, which declines in capacity with aging); and (3) that require more energy for synthesis, such as larger proteins or those enriched in amino acids with complex biosynthesis pathways (e.g., Cys, Asp, or Arg).[2] Neuronal activity promotes the recruitment and activation of proteasomes at synapses through calcium-calmodulin-dependent kinase IIα (CaMKIIα), which then regulates synaptic strength and plasticity.[114] An increasing number of studies provide evidence for crosstalk between proteolytic pathways.[115] A player in the interplay of degradative pathways in the synapse seems to be the presynaptic scaffolding protein Bassoon, which interacts with both, components of the proteasomal and the autophagosomal systems (see above; Figure 2B). Regulatory nodes include molecular chaperones, the ubiquitin code involving ubiquitin linkage and branching, and signaling cascades.[116–118] A recent study demonstrated that the regulatory 19S and the catalytic 20S particles of the proteasome are differentially distributed in rat cortical neurons.[119] The 19S particle is highly abundant in dendrites and axons, whereas the 20S particle is more abundant in the soma. Notably, the 19S particles near synapses did not associate with 20S particles. The regulatory 19S particle possesses deubiquitination activity.[120] Surprisingly, free 19S can bind to and deubiquitinate Lys63-ubiquitinated protein substrates. This deubiquitinating activity can be attributed to the Uch37/Uchl5 deubiquitinating enzymes, which are associated with the 19S particle and much less with the complete 26S proteasome. Lys63-ubiquitination is linked to autophagy, trafficking, and nuclear factor κB (NF-κB) signaling.[121] Analysis of these Lys63-ubiquitinated proteins revealed an enrichment of synaptic proteins, such as synaptogyrin, syntaxin, and synaptotagmin, as well as neurotransmitter receptors and ion channels including AMPA receptors (AMPARs). Inhibition of the deubiquitinase activity of the 19S particle led to changes in excitatory synaptic transmission. The 19S deubiquitinating activity regulates the AMPAR life cycle at multiple levels: it stabilizes AMPAR at the synapse by suppressing its degradation and promotes both its internalization and exocytosis.[119] These findings suggest that an excess of 19S proteasome subunits could function as a deubiquitinase, removing ubiquitin from protein substrates destined for

autophagosomal degradation, thereby stabilizing substrates involved in protein trafficking and signaling events.

AUTOPHAGY CONTROLS SYNAPTIC PLASTICITY AND COGNITIVE FUNCTION

Genetic reduction of key autophagy factors leads to severe neurodevelopmental and cognitive deficits and can lead to mild or severe forms of neurodegeneration and to epileptiform activity.[122,123] Conversely, overexpression of essential autophagy genes has been shown to protect from age-dependent memory impairment.[124] It is unlikely that the exclusive failure of presynaptic mechanisms is causally linked to these interventions. In a series of studies, a central contribution of autophagy to specific forms of synaptic plasticity, in particular to LTD, has been established (Figure 4A). During LTD, the efficacy of signal transmission at synapses is reduced following low-frequency stimulation. Synaptic efficacy is tightly regulated by presynaptic and postsynaptic modification of glutamatergic signaling involving NMDA, AMPA, and metabotropic glutamate receptor subtypes. Reducing AMPAR numbers at the postsynaptic site by endocytosis is a central LTD mechanism that leads to reduced excitatory synaptic transmission. Factors that induce autophagy such as the ULK1 complex or ATG5 appear to facilitate LTD by enabling the degradation of AMPARs and PSD-95 and by preventing their re-mobilization to the cell surface[58,60,125] (Figure 4A). However, conflicting evidence has been reported suggesting that LTD might inhibit autophagy,[56] and it is at present unclear whether and how different experimental conditions might contribute to these discrepant results. In addition, autophagy may dampen AMPAR-dependent excitatory transmission via regulation of protein kinase A signaling[61] (Figure 4A).

On the other hand, it has also been shown that gross elevation of neuronal autophagy induced by brain-derived neurotrophic factor (BDNF) genetic targeting leads to defective CA3-CA1 postsynaptic long-term potentiation (LTP) in adult mice, a form of plasticity where synaptic efficacy is lastingly enhanced upon high-frequency stimulation. In BDNF-mediated synaptic plasticity, postsynaptic scaffolding proteins, such as SHANK3, PSD-95, and PICK1, have been identified as cargos for autophagic degradation, suggesting that neuronal autophagy might play a role in (post)synaptic plasticity by targeting postsynaptic proteins for lysosomal turnover.[126] Interestingly, loss of neuronal autophagy in the absence of ATG5 has been demonstrated to completely block presynaptic LTP at mossy fiber synapses (mf-LTP) in the hippocampal CA3 area via a presynaptic mechanism involving ryanodine receptor-mediated calcium release from axonal ER stores[43] (Figure 4B). *In vivo*, a gradient of autophagic vesicles in the hippocampal CA1 region, a prime location for the formation of long-term memory, may determine the susceptibility of proximal vs. distal dendritic synapses to induce LTD.[59]

In general, disturbing the cellular machinery for inducing LTD or LTP impairs the formation of memory. The exact contribution of adapting synaptic strength by LTD- and LTP-like processes in relevant circuits for learning and memory *in vivo* remains a target of intensive investigations.[127–129] Genetic and local pharmacological interventions within the hippocampus—a key brain structure for the formation of long-term memories—demonstrate a significant role for autophagy in hippocampus-dependent long-term memory formation. Hippocampal CA1-specific ATG5 KO mice show a deficit for long-term contextual fear memory, although their cognitive flexibility improved in a non-aversive learning task through an LTD-independent mechanism.[58] Knockdown of either Beclin-1 or ATG12 impaired not only contextual fear memory but also spatial object recognition as a non-aversive hippocampus-dependent task.[124] Conversely, induction of autophagy by administration of Beclin-1 peptides to the hippocampus of mice increased memory strength in both tasks—importantly only during training, but not retrieval—suggesting a contribution of autophagy to memory consolidation processes.[124]

The precise molecular mechanisms within local circuits that are essential for memory formation remain unclear. Future studies will need to investigate these mechanisms once cell-specific readouts and suitable interventional tools for autophagy become available.

NEURONAL AUTOPHAGY IN BRAIN AGING

With the course of neuronal and brain aging, a dwindling capacity of neuronal autophagy was detected in various model organisms (*C. elegans*, *D. melanogaster*, and rodents) and cultivated human neurons (for reviews, see Aman et al.,[16] Rubinsztein et al.,[130] Cuervo and Dice,[131] Chang et al.,[132] and Leidal et al.[133]). Moreover, it was demonstrated that the rate of autophagosome biogenesis significantly decreases in aged cultured neurons, along with notable morphological defects in autophagosomes.[134] These defects include stalled isolation membranes that fail to mature into functional autophagosomes. Notably, the same study showed that expressing WIPI2B in aged neurons effectively restored autophagosome biogenesis. These data are consistent with the observation that the levels of key autophagy proteins such as Beclin-1 decrease with age.[135] Mechanistically, decreasing the levels of autophagic proteins might ultimately cause inefficient autophagosome formation and maturation and/or lead to defective autophagosome-lysosome fusion in aged neurons.[67,136–140]

In addition to the progressive loss of the ability of the aging brain to induce autophagy and/or to age-related defects in autophagic flux, for instance, due to impaired axonal transport (e.g., as demonstrated for murine dorsal root ganglia [DRG] neurons[140]), old neurons may alter the repertoire of cargos targeted for autophagy-mediated turnover. Consistent with this idea, a recent proteomics study has provided compelling evidence for the increased sequestration of (pre)synaptic proteins into autophagosomes in adult and aged mice, compared with adolescents, suggesting changes of autophagic cargo selectivity in aging brains.[67]

An important question is whether and to which degree restoring autophagy may counteract age-related memory decline. Although the answer to this question cannot be given at present, recent observations suggest that restoring neuronal autophagy may be beneficial in old age. Rodent studies administering autophagosome-inducing membrane-permeant Beclin-1 peptides into the hippocampus demonstrates a rescue of cognitive function in

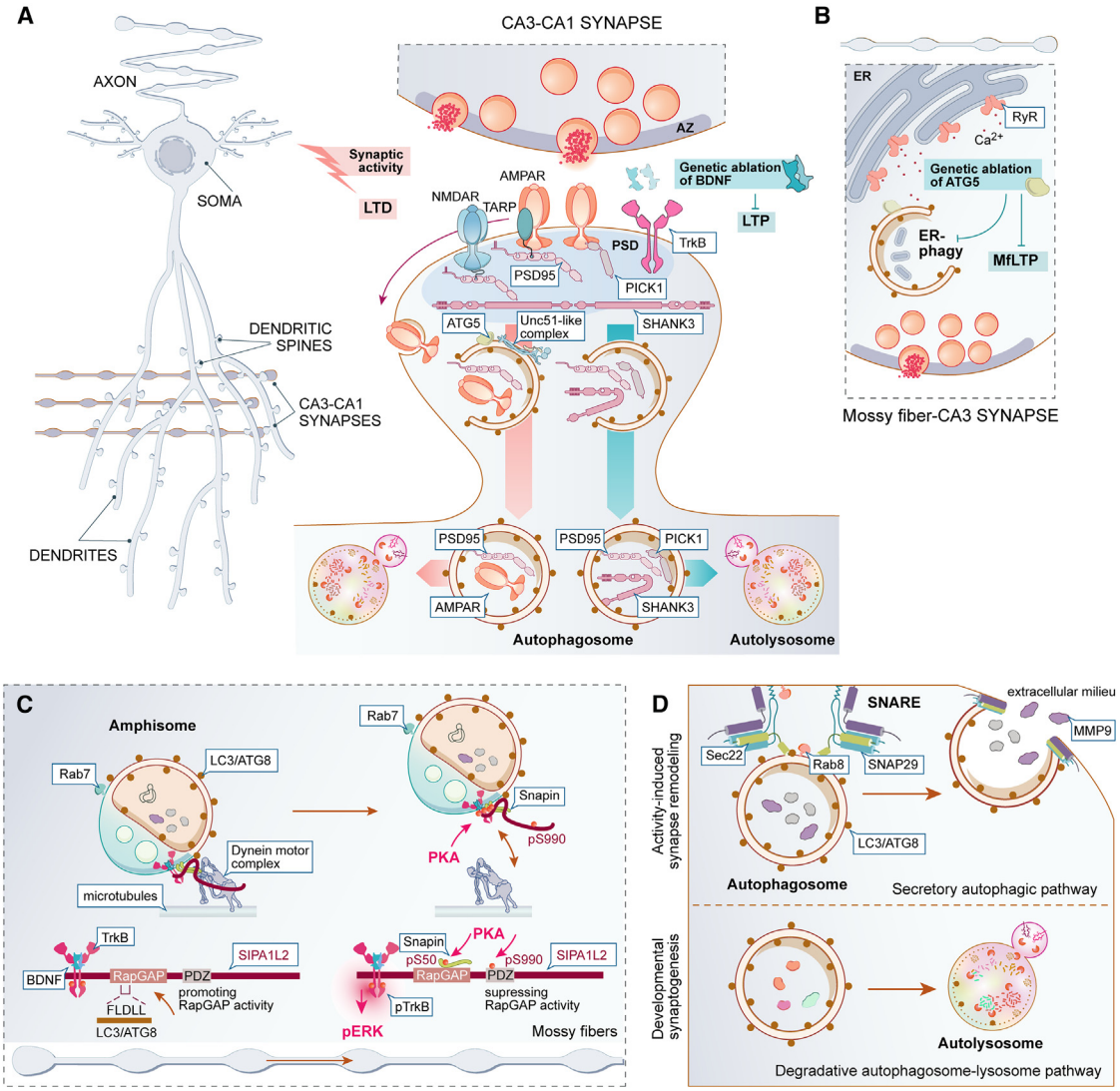

Figure 4. Regulation of autophagy in the context of synaptic plasticity

(A) At the postsynapse, autophagic vesicles can be generated following the induction of long-term depression (LTD). Factors that promote autophagy, such as the ULK1 complex or ATG5, facilitate LTD. This mechanism contributes to the removal of PSD-95 from synapses, subsequently increasing the surface mobility of AMPA receptors (AMPARs) and facilitating their removal and degradation. Elevation of neuronal autophagy induced by BDNF genetic targeting results in defective CA3-CA1 postsynaptic long-term potentiation (LTP). In the context of BDNF-mediated synaptic plasticity, postsynaptic scaffolding proteins such as SHANK3, PSD-95, and PICK1 have been identified as cargo for autophagic degradation. This suggests that neuronal autophagy may play a role in (post)synaptic plasticity by targeting postsynaptic proteins for lysosomal turnover.

(B) Neuronal ER-phagy plays a crucial role in regulating presynaptic excitatory neurotransmission by controlling ryanodine receptor (RyR)-mediated calcium release from axonal ER stores. The loss of autophagy due to the ablation of neuronal ATG5 has been shown to completely block presynaptic long-term potentiation mossy fiber synapses (mf-LTP) in the hippocampal CA3 region.

(C and D) Non-canonical roles of neuronal autophagy. (C) Amphisomes facilitate local TrkB signaling at synaptic boutons to promote neurotransmission and activity-dependent synaptic changes via the Rap GTPase-activating protein SIPA1L2. SIPA1L2 binds to LC3/ATG8 and links TrkB amphisomes to dynein motors. LC3/ATG8 regulates the RapGAP activity of SIPA1L2 and control the speed of retrograde trafficking and local TrkB signaling. Following induction of presynaptic plasticity, amphisomes dissociate from dynein motors at synapses due to PKA-dependent phosphorylation of the dynein adaptor protein snapin. PKA phosphorylation of SIPA1L2 reduces its GAP activity and facilitates local signaling at synaptic boutons, which results in enhanced neurotransmitter release. SIPA1L2 knockout mice exhibit impaired BDNF-dependent presynaptic plasticity.

(D) In fly larval neuromuscular junctions (NMJs), activity-induced synapse remodeling relies on a secretory autophagic pathway involving the SNARE proteins SNAP29 and Sec22, as well as the small GTPase Rab8. In contrast, developmental synaptogenesis depends on protein turnover via a degradative autophagosome-lysosome pathway.

aged (i.e., 16-month-old) mice.[124] Moreover, spermidine, a body-endogenous polyamine that can induce and restore efficient autophagy in aging tissues, has been tested in clinical trials as a potential therapeutic treatment for age-related memory decline.[141–143] In aged mice, 6 months of dietary spermidine supplementation partially rescued the age-dependent decrease in presynaptic

LTP at mossy fiber-CA3 synapses.[144] In a mouse model of mild cognitive impairment, the treatment with spermidine or application of an autophagy enhancing TAT-Beclin-1 peptide restored memory capacity.[124] TAT-Beclin-1 as well as spermidine also induce the posttranslational modification of the AMPAR subunit GluA1 by favoring the autophagy/lysosomal-mediated degradation of amyloid fibrils.[145] While the detailed mechanisms of spermidine action are a subject of investigation, *in vivo* analysis of aging *D. melanogaster* suggests that the age-protective role of spermidine with respect to cognitive functions is mediated via boosting autophagy and mitochondrial respiratory function in aging brains.[146–149] These protective effects seem to relate to a brain-wide form of early aging-associated presynaptic AZ plasticity, entailing increases of AZ scaffold proteins and SV release factors.[150] Interestingly, in the *D. melanogaster* brain, autophagy defects were found to impair presynaptic plasticity in a non-cell-autonomous manner via a mechanism involving NPY-type neuropeptide signaling,[151] adding a further dimension to the interaction between autophagy and synaptic plasticity in the aging brain.

NON-CANONICAL ROLES OF NEURONAL AUTOPHAGY

Recent research indicates that neuronal autophagy might serve cellular functions beyond its role as a degradative clearance pathway. Amphisomes are organelles of the autophagy pathway that result from the fusion of autophagosomes with late endosomes. Evidence provided by two studies suggests that amphisomes are required for the long-range retrograde BDNF/TrkB trafficking along axons and subsequent gene expression[47] and enable local BDNF/TrkB signaling at neighboring *en passant* presynapses to modulate neurotransmission[152] (Figure 4C). The latter function is orchestrated by the Rap GTPase-activating protein (RapGAP) SIPA1L2, which connects TrkB amphisomes to dynein motors. The autophagosomal protein LC3 regulates the RapGAP activity of SIPA1L2 and controls retrograde trafficking and local signaling of TrkB. Following induction of presynaptic plasticity, amphisomes dissociate from dynein at boutons to enable local signaling and promote neurotransmitter release (Figure 4C). SIPA1L2 KO mice suffer from impaired BDNF-dependent presynaptic plasticity.[152] Hence, neuronal autophagy via formation and long-range retrograde transport of signaling amphisomes convey BDNF/TrkB signals from distal axons and synapses to the nucleus to regulate presynaptic plasticity,[152] neuronal branching, and neuronal survival.[47] Signaling amphisomes may thus constitute stable entities capable of a dual, degradative, and signaling function where signaling receptors at the outer membrane would be physically segregated from degradative cargo. Future studies should clarify how and whether biogenesis of amphisomes at boutons is different from LC3-associated phagocytosis and endocytosis (LANDO).[153] The conjugation of ATG8 with single-membrane phagosomes and Rab5-positive early endosomes has so far only been described in glia, although the machinery is present in neurons.

Another emerging non-canonical function of neuronal autophagy is the secretion of specific cargos to the extracellular space (Figure 4D). For example, α-synuclein, a protein implicated in the pathogenesis of Parkinson's disease, can be released via secretory autophagy to prevent its intracellular accumulation and toxicity.[154,155] In addition to the release of toxic proteins, secretory autophagy may also serve as a rapid response mechanism that facilitates the release of signaling molecules and extracellular vesicles to mediate synaptic plasticity and neuronal survival.[156] Work in fly larval NMJs using RNAi-based genetic screening revealed that distinct autophagic mechanisms contribute to synapse development and to the modulation of synaptic plasticity.[157] This study showed that activity-induced synapse remodeling relies on a secretory autophagic pathway that involves the SNARE proteins SNAP29 and Sec22 as well as the small GTPase Rab8, while developmental synaptogenesis depends upon protein turnover via a degradative autophagosome-lysosome pathway. Interestingly, the latter is downregulated upon induction of neuronal activity.[157] Furthermore, secretory autophagy has been implicated in the release of inflammation-related cargo, such as interleukin (IL)-1β, HMGB1, and matrix metalloproteinase 9 (MMP9), and organelles such as mitochondria, into the extracellular space.[158–161] The release of MMP9 might impact neuronal function by enhancing the cleavage of pro-BDNF (proBDNF) to its mature form.[160] Moreover, many of these secreted molecules can influence neuronal plasticity and survival, highlighting a potential link between autophagy, immune response, and neuronal function. Interestingly, the SKA2-FKBP5 signaling pathway has recently been shown to play a key role in secretory autophagy in microglia. Knockdown of SKA2 in hippocampus resulted in increased secretion of IL-1β, neuroinflammation, and deleterious effects in neurons. The pathway is not specific to microglia, and it would be very interesting to investigate the role of this machinery in neurons.[162] Finally, secretory autophagy also has been linked to mitochondrial quality control,[161] and very recent work points to a role of secretory autophagy in lower motoneuron disease.[163]

CHALLENGES AND FUTURE DIRECTIONS

Collectively current evidence suggests that neuronal autophagy has adopted specific functions that serve the needs of a highly polarized cell with thousands of synaptic contacts. It is, however, still unclear how synaptic neurotransmission directly impacts autophagy in mechanistic terms. In this context, we propose that contextual specificity, i.e., local differences in cargo availability relative to when and where autophagosome formation is triggered, significantly contributes to cargo selection. Contextual specificity might also explain at least in part the apparent discrepancies in content profiling of autophagosomes in different proteomic studies[43,66–68] and the divergent phenotypes of transgenic KO mice targeting neuronal autophagy.[10–12,43–46,48]

Moreover, there is still a surprising paucity of data on the potential role of synaptic autophagy for protein replacement in particular at presynapses. Among the gaps in our knowledge, which likely will be closed in the future, are the following questions:

- Which degradative pathways are involved in turnover of certain synaptic proteins?
- How are synaptic proteins sorted for specific degradative pathways, and how is sorting itself accomplished?

- How do different pathways for synaptic protein turnover contribute to shaping and adapting the presynaptic proteome, and how are these pathways controlled?
- How does synaptic activity affect client protein degradation?
- Which presynaptic sensing mechanisms identify protein "damage"?
- And, finally, is degradation of local organelles at boutons, like mitochondria by mitophagy, governed by similar principles?

A central question is also whether autophagosomes can form in all parts of a neuron, e.g., in dendritic spines and within presynaptic boutons. Under resting steady-state conditions, autophagic structures are rarely found at presynapses in the mammalian brain. In contrast, closed autophagosomes have been observed in large boutons with multiple synaptic contact sites under conditions of starvation, upon induction of neuronal activity,[35,42] or when autophagy is unphysiologically enhanced, e.g., upon depletion of Bassoon and Piccolo.[57,63,64] Sonic hedgehog signaling (via ShhN treatment) has also been shown to induce the formation of phagophores and autophagic vacuoles in presynaptic terminals.[164]

In spite of the promising potential of autophagy enhancement as a potential therapeutic strategy to counteract aging and neurodegenerative diseases, several challenges remain. One challenge is the need for specificity at the levels of both tissue and cell type: systemic activation of autophagy could have unintended effects on other tissues and organs. Therefore, targeted delivery of autophagy enhancers to the brain is a critical area of research. Nanotechnology and advanced drug delivery systems are being explored to achieve such specificity.[165,166] Moreover, while elevated autophagy may be beneficial in one type of neuron or in microglia, it may turn out to exhibit adverse effects in some other types of neurons. Clearly, a better understanding of the cell-type-specific functions of autophagy in the brain is required to pursue this strategy further. Another challenge is to dissect which level of autophagy induction may be beneficial to patients. While insufficient autophagy contributes to neurodegeneration, excessive autophagy can also be detrimental, leading to cell death.[167] Restoring juvenile but not excessive levels of brain autophagy appears to be most desirable.

Therefore, therapeutic strategies must aim to restore autophagy to physiological levels, rather than indiscriminately boosting it.[168] Finally, in complex neuronal circuits relying on a well-balanced excitatory/inhibitory interaction of different cell types and their plastic synaptic interactions, circuit-specific interventional tools may be required to address physiological levels of autophagy without disturbing circuit interactions.

ACKNOWLEDGMENTS

We thank the Deutsche Forschungsgemeinschaft (DFG) for funding (DFG-FOR5228: AL 2561/1-1, EI 849/7-1, GU238/7-1, HA2686/22-1, HI 1886/8-1, KI 1988/7-1, KR 1879/14-1, KU 4199/1-1, MA 9763/1-1, and MI 1923/5-1).

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Giandomenico, S.L., Alvarez-Castelao, B., and Schuman, E.M. (2022). Proteostatic regulation in neuronal compartments. Trends Neurosci. *45*, 41–52. https://doi.org/10.1016/j.tins.2021.08.002.

2. Kluever, V., Russo, B., Mandad, S., Kumar, N.H., Alevra, M., Ori, A., Rizzoli, S.O., Urlaub, H., Schneider, A., and Fornasiero, E.F. (2022). Protein lifetimes in aged brains reveal a proteostatic adaptation linking physiological aging to neurodegeneration. Sci. Adv. *8*, eabn4437. https://doi.org/10.1126/sciadv.abn4437.

3. Chanaday, N.L., Cousin, M.A., Milosevic, I., Watanabe, S., and Morgan, J.R. (2019). The synaptic vesicle cycle revisited: new insights into the modes and mechanisms. J. Neurosci. *39*, 8209–8216. https://doi.org/10.1523/JNEUROSCI.1158-19.2019.

4. Rizzoli, S.O. (2014). Synaptic vesicle recycling: steps and principles. EMBO J. *33*, 788–822. https://doi.org/10.1002/embj.201386357.

5. Soykan, T., Maritzen, T., and Haucke, V. (2016). Modes and mechanisms of synaptic vesicle recycling. Curr. Opin. Neurobiol. *39*, 17–23. https://doi.org/10.1016/j.conb.2016.03.005.

6. Sudhof, T.C. (2004). The synaptic vesicle cycle. Annu. Rev. Neurosci. *27*, 509–547. https://doi.org/10.1146/annurev.neuro.26.041002.131412.

7. Li, S., and Sheng, Z.H. (2022). Energy matters: presynaptic metabolism and the maintenance of synaptic transmission. Nat. Rev. Neurosci. *23*, 4–22. https://doi.org/10.1038/s41583-021-00535-8.

8. Fleming, A., Bourdenx, M., Fujimaki, M., Karabiyik, C., Krause, G.J., Lopez, A., Martín-Segura, A., Puri, C., Scrivo, A., Skidmore, J., et al. (2022). The different autophagy degradation pathways and neurodegeneration. Neuron *110*, 935–966. https://doi.org/10.1016/j.neuron.2022.01.017.

9. Hill, S.E., and Colón-Ramos, D.A. (2020). The journey of the synaptic autophagosome: A cell biological perspective. Neuron *105*, 961–973. https://doi.org/10.1016/j.neuron.2020.01.018.

10. Hara, T., Nakamura, K., Matsui, M., Yamamoto, A., Nakahara, Y., Suzuki-Migishima, R., Yokoyama, M., Mishima, K., Saito, I., Okano, H., et al. (2006). Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. Nature *441*, 885–889. https://doi.org/10.1038/nature04724.

11. Komatsu, M., Waguri, S., Chiba, T., Murata, S., Iwata, J., Tanida, I., Ueno, T., Koike, M., Uchiyama, Y., Kominami, E., et al. (2006). Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature *441*, 880–884. https://doi.org/10.1038/nature04723.

12. Komatsu, M., Wang, Q.J., Holstein, G.R., Friedrich, V.L., Jr., Iwata, J., Kominami, E., Chait, B.T., Tanaka, K., and Yue, Z. (2007). Essential role for autophagy protein Atg7 in the maintenance of axonal homeostasis and the prevention of axonal degeneration. Proc. Natl. Acad. Sci. USA *104*, 14489–14494. https://doi.org/10.1073/pnas.0701311104.

13. Menzies, F.M., Fleming, A., Caricasole, A., Bento, C.F., Andrews, S.P., Ashkenazi, A., Füllgrabe, J., Jackson, A., Jimenez Sanchez, M., Karabiyik, C., et al. (2017). Autophagy and neurodegeneration: pathogenic mechanisms and therapeutic opportunities. Neuron *93*, 1015–1034. https://doi.org/10.1016/j.neuron.2017.01.022.

14. Balusu, S., Praschberger, R., Lauwers, E., De Strooper, B., and Verstreken, P. (2023). Neurodegeneration cell per cell. Neuron *111*, 767–786. https://doi.org/10.1016/j.neuron.2023.01.016.

15. Nixon, R.A., and Rubinsztein, D.C. (2024). Mechanisms of autophagy-lysosome dysfunction in neurodegenerative diseases. Nat. Rev. Mol. Cell Biol. *25*, 926–946. https://doi.org/10.1038/s41580-024-00757-5.

16. Aman, Y., Schmauck-Medina, T., Hansen, M., Morimoto, R.I., Simon, A.K., Bjedov, I., Palikaras, K., Simonsen, A., Johansen, T., Tavernarakis, N., et al. (2021). Autophagy in healthy aging and disease. Nat Aging *1*, 634–650. https://doi.org/10.1038/s43587-021-00098-4.

17. Mizushima, N., and Komatsu, M. (2011). Autophagy: renovation of cells and tissues. Cell *147*, 728–741. https://doi.org/10.1016/j.cell.2011.10.026.

18. Lee, K.M., Hwang, S.K., and Lee, J.A. (2013). Neuronal autophagy and neurodevelopmental disorders. Exp. Neurobiol. *22*, 133–142. https://doi.org/10.5607/en.2013.22.3.133.

19. Dikic, I., and Elazar, Z. (2018). Mechanism and medical implications of mammalian autophagy. Nat. Rev. Mol. Cell Biol. *19*, 349–364. https://doi.org/10.1038/s41580-018-0003-4.

20. Stavoe, A.K.H., and Holzbaur, E.L.F. (2019). Autophagy in neurons. Annu. Rev. Cell Dev. Biol. *35*, 477–500. https://doi.org/10.1146/annurev-cellbio-100818-125242.

21. Gundelfinger, E.D., Karpova, A., Pielot, R., Garner, C.C., and Kreutz, M.R. (2022). Organization of presynaptic autophagy-related processes. Front. Synaptic Neurosci. *14*, 829354. https://doi.org/10.3389/fnsyn.2022.829354.

22. Galluzzi, L., Baehrecke, E.H., Ballabio, A., Boya, P., Bravo-San Pedro, J.M., Cecconi, F., Choi, A.M., Chu, C.T., Codogno, P., Colombo, M.I., et al. (2017). Molecular definitions of autophagy and related processes. EMBO J. *36*, 1811–1836. https://doi.org/10.15252/embj.201796697.

23. Uttenweiler, A., and Mayer, A. (2008). Microautophagy in the yeast Saccharomyces cerevisiae. Methods Mol. Biol. *445*, 245–259. https://doi.org/10.1007/978-1-59745-157-4_16.

24. Uytterhoeven, V., Lauwers, E., Maes, I., Miskiewicz, K., Melo, M.N., Swerts, J., Kuenen, S., Wittocx, R., Corthout, N., Marrink, S.J., et al. (2015). Hsc70-4 deforms membranes to promote synaptic protein turnover by endosomal microautophagy. Neuron *88*, 735–748. https://doi.org/10.1016/j.neuron.2015.10.012.

25. Kaushik, S., and Cuervo, A.M. (2012). Chaperone-mediated autophagy: a unique way to enter the lysosome world. Trends Cell Biol. *22*, 407–417. https://doi.org/10.1016/j.tcb.2012.05.006.

26. Axe, E.L., Walker, S.A., Manifava, M., Chandra, P., Roderick, H.L., Habermann, A., Griffiths, G., and Ktistakis, N.T. (2008). Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. J. Cell Biol. *182*, 685–701. https://doi.org/10.1083/jcb.200803137.

27. Puri, C., Renna, M., Bento, C.F., Moreau, K., and Rubinsztein, D.C. (2013). Diverse autophagosome membrane sources coalesce in recycling endosomes. Cell *154*, 1285–1299. https://doi.org/10.1016/j.cell.2013.08.044.

28. Ge, L., Zhang, M., and Schekman, R. (2014). Phosphatidylinositol 3-kinase and COPII generate LC3 lipidation vesicles from the ER-Golgi intermediate compartment. eLife *3*, e04135. https://doi.org/10.7554/eLife.04135.

29. van Vliet, A.R., Chiduza, G.N., Maslen, S.L., Pye, V.E., Joshi, D., De Tito, S., Jefferies, H.B.J., Christodoulou, E., Roustan, C., Punch, E., et al. (2022). ATG9A and ATG2A form a heteromeric complex essential for autophagosome formation. Mol. Cell *82*, 4324–4339.e8. https://doi.org/10.1016/j.molcel.2022.10.017.

30. Ohsumi, Y. (2014). Historical landmarks of autophagy research. Cell Res. *24*, 9–23. https://doi.org/10.1038/cr.2013.169.

31. Grochowska, K.M., Andres-Alonso, M., Karpova, A., and Kreutz, M.R. (2022). The needs of a synapse-How local organelles serve synaptic proteostasis. EMBO J. *41*, e110057. https://doi.org/10.15252/embj.2021110057.

32. Choi, J., Jang, H., Xuan, Z., and Park, D. (2024). Emerging roles of ATG9/ATG9A in autophagy: implications for cell and neurobiology. Autophagy *20*, 2373–2387. https://doi.org/10.1080/15548627.2024.2384349.

33. Xuan, Z., and Colón-Ramos, D.A. (2023). The active zone protein CLA-1 (Clarinet) bridges two subsynaptic domains to regulate presynaptic sorting of ATG-9. Autophagy *19*, 2807–2808. https://doi.org/10.1080/15548627.2023.2229227.

34. Maday, S., and Holzbaur, E.L.F. (2014). Autophagosome biogenesis in primary neurons follows an ordered and spatially regulated pathway. Dev. Cell *30*, 71–85. https://doi.org/10.1016/j.devcel.2014.06.001.

35. Soukup, S.F., Kuenen, S., Vanhauwaert, R., Manetsberger, J., Hernández-Díaz, S., Swerts, J., Schoovaerts, N., Vilain, S., Gounko, N.V., Vints, K., et al. (2016). A LRRK2-dependent EndophilinA phosphoswitch is critical for macroautophagy at presynaptic terminals. Neuron *92*, 829–844. https://doi.org/10.1016/j.neuron.2016.09.037.

36. Matsunaga, K., Morita, E., Saitoh, T., Akira, S., Ktistakis, N.T., Izumi, T., Noda, T., and Yoshimori, T. (2010). Autophagy requires endoplasmic reticulum targeting of the PI3-kinase complex via Atg14L. J. Cell Biol. *190*, 511–521. https://doi.org/10.1083/jcb.200911141.

37. Lie, P.P.Y., Yang, D.S., Stavrides, P., Goulbourne, C.N., Zheng, P., Mohan, P.S., Cataldo, A.M., and Nixon, R.A. (2021). Post-Golgi carriers, not lysosomes, confer lysosomal properties to pre-degradative organelles in normal and dystrophic axons. Cell Rep. *35*, 109034. https://doi.org/10.1016/j.celrep.2021.109034.

38. Maday, S., and Holzbaur, E.L.F. (2016). Compartment-specific regulation of autophagy in primary neurons. J. Neurosci. *36*, 5933–5945. https://doi.org/10.1523/JNEUROSCI.4401-15.2016.

39. Katsumata, K., Nishiyama, J., Inoue, T., Mizushima, N., Takeda, J., and Yuzaki, M. (2010). Dynein- and activity-dependent retrograde transport of autophagosomes in neuronal axons. Autophagy *6*, 378–385. https://doi.org/10.4161/auto.6.3.11262.

40. Wang, T., Martin, S., Papadopulos, A., Harper, C.B., Mavlyutov, T.A., Niranjan, D., Glass, N.R., Cooper-White, J.J., Sibarita, J.B., Choquet, D., et al. (2015). Control of autophagosome axonal retrograde flux by presynaptic activity unveiled using botulinum neurotoxin type a. J. Neurosci. *35*, 6179–6194. https://doi.org/10.1523/JNEUROSCI.3757-14.2015.

41. Vijayan, V., and Verstreken, P. (2017). Autophagy in the presynaptic compartment in health and disease. J. Cell Biol. *216*, 1895–1906. https://doi.org/10.1083/jcb.201611113.

42. Bademosi, A.T., Decet, M., Kuenen, S., Calatayud, C., Swerts, J., Gallego, S.F., Schoovaerts, N., Karamanou, S., Louros, N., Martin, E., et al. (2023). EndophilinA-dependent coupling between activity-induced calcium influx and synaptic autophagy is disrupted by a Parkinson-risk mutation. Neuron *111*, 1402–1422.e13. https://doi.org/10.1016/j.neuron.2023.02.001.

43. Kuijpers, M., Kochlamazashvili, G., Stumpf, A., Puchkov, D., Swaminathan, A., Lucht, M.T., Krause, E., Maritzen, T., Schmitz, D., and Haucke, V. (2021). Neuronal autophagy regulates presynaptic neurotransmission by controlling the axonal endoplasmic reticulum. Neuron *109*, 299–313.e9. https://doi.org/10.1016/j.neuron.2020.10.005.

44. Liang, C.C., Wang, C., Peng, X., Gan, B., and Guan, J.L. (2010). Neural-specific deletion of FIP200 leads to cerebellar degeneration caused by increased neuronal death and axon degeneration. J. Biol. Chem. *285*, 3499–3509. https://doi.org/10.1074/jbc.M109.072389.

45. Xi, Y., Dhaliwal, J.S., Ceizar, M., Vaculik, M., Kumar, K.L., and Lagace, D.C. (2016). Knockout of Atg5 delays the maturation and reduces the survival of adult-generated neurons in the hippocampus. Cell Death Dis. *7*, e2127. https://doi.org/10.1038/cddis.2015.406.

46. Lv, X., Jiang, H., Li, B., Liang, Q., Wang, S., Zhao, Q., and Jiao, J. (2014). The crucial role of Atg5 in cortical neurogenesis during early brain development. Sci. Rep. *4*, 6010. https://doi.org/10.1038/srep06010.

47. Kononenko, N.L., Claßen, G.A., Kuijpers, M., Puchkov, D., Maritzen, T., Tempes, A., Malik, A.R., Skalecka, A., Bera, S., Jaworski, J., et al. (2017). Retrograde transport of TrkB-containing autophagosomes via the adaptor AP-2 mediates neuronal complexity and prevents neurodegeneration. Nat. Commun. *8*, 14819. https://doi.org/10.1038/ncomms14819.

48. Lieberman, O.J., Frier, M.D., McGuirt, A.F., Griffey, C.J., Rafikian, E., Yang, M., Yamamoto, A., Borgkvist, A., Santini, E., and Sulzer, D. (2020). Cell-type-specific regulation of neuronal intrinsic excitability by macroautophagy. eLife *9*, e50843. https://doi.org/10.7554/eLife.50843.

49. Collier, J.J., Guissart, C., Oláhová, M., Sasorith, S., Piron-Prunier, F., Suomi, F., Zhang, D., Martinez-Lopez, N., Leboucq, N., Bahr, A., et al. (2021). Developmental consequences of defective ATG7-mediated autophagy in humans. N. Engl. J. Med. *384*, 2406–2417. https://doi.org/10.1056/NEJMoa1915722.

50. Deng, Z., Zhou, X., Lu, J.H., and Yue, Z. (2021). Autophagy deficiency in neurodevelopmental disorders. Cell Biosci. *11*, 214. https://doi.org/10.1186/s13578-021-00726-x.

51. Hassan, B.A., and Hiesinger, P.R. (2023). Autophagy in synapse formation and brain wiring. Autophagy *19*, 2814–2816. https://doi.org/10.1080/15548627.2023.2179778.

52. Kiral, F.R., Linneweber, G.A., Mathejczyk, T., Georgiev, S.V., Wernet, M.F., Hassan, B.A., von Kleist, M., and Hiesinger, P.R. (2020). Autophagy-dependent filopodial kinetics restrict synaptic partner choice during Drosophila brain wiring. Nat. Commun. *11*, 1325. https://doi.org/10.1038/s41467-020-14781-4.

53. Dutta, S.B., Linneweber, G.A., Andriatsilavo, M., Hiesinger, P.R., and Hassan, B.A. (2023). EGFR-dependent suppression of synaptic autophagy is required for neuronal circuit development. Curr. Biol. *33*, 517–532.e5. https://doi.org/10.1016/j.cub.2022.12.039.

54. Nishida, Y., Arakawa, S., Fujitani, K., Yamaguchi, H., Mizuta, T., Kanaseki, T., Komatsu, M., Otsu, K., Tsujimoto, Y., and Shimizu, S. (2009). Discovery of Atg5/Atg7-independent alternative macroautophagy. Nature *461*, 654–658. https://doi.org/10.1038/nature08455.

55. Yamaguchi, H., Honda, S., Torii, S., Shimizu, K., Katoh, K., Miyake, K., Miyake, N., Fujikake, N., Sakurai, H.T., Arakawa, S., et al. (2020). Wipi3 is essential for alternative autophagy and its loss causes neurodegeneration. Nat. Commun. *11*, 5311. https://doi.org/10.1038/s41467-020-18892-w.

56. Stavoe, A.K.H., Hill, S.E., Hall, D.H., and Colón-Ramos, D.A. (2016). KIF1A/UNC-104 transports ATG-9 to regulate neurodevelopment and autophagy at synapses. Dev. Cell *38*, 171–185. https://doi.org/10.1016/j.devcel.2016.06.012.

57. Okerlund, N.D., Schneider, K., Leal-Ortiz, S., Montenegro-Venegas, C., Kim, S.A., Garner, L.C., Waites, C.L., Gundelfinger, E.D., Reimer, R.J., and Garner, C.C. (2017). Bassoon controls presynaptic autophagy through Atg5. Neuron *93*, 897–913.e7. https://doi.org/10.1016/j.neuron.2017.01.026.

58. Kallergi, E., Daskalaki, A.D., Kolaxi, A., Camus, C., Ioannou, E., Mercaldo, V., Haberkant, P., Stein, F., Sidiropoulou, K., Dalezios, Y., et al. (2022). Dendritic autophagy degrades postsynaptic proteins and is required for long-term synaptic depression in mice. Nat. Commun. *13*, 680. https://doi.org/10.1038/s41467-022-28301-z.

59. Keary, K.M., 3rd, Gu, Q.H., Chen, J., and Li, Z. (2023). Dendritic distribution of autophagosomes underlies pathway-selective induction of LTD. Cell Rep. *42*, 112898. https://doi.org/10.1016/j.celrep.2023.112898.

60. Shen, H., Zhu, H., Panja, D., Gu, Q., and Li, Z. (2020). Autophagy controls the induction and developmental decline of NMDAR-LTD through endocytic recycling. Nat. Commun. *11*, 2979. https://doi.org/10.1038/s41467-020-16794-5.

61. Overhoff, M., Tellkamp, F., Hess, S., Tolve, M., Tutas, J., Faerfers, M., Ickert, L., Mohammadi, M., De Bruyckere, E., Kallergi, E., et al. (2022). Autophagy regulates neuronal excitability by controlling cAMP/protein kinase A signaling at the synapse. EMBO J. *41*, e110963. https://doi.org/10.15252/embj.2022110963.

62. Kulkarni, V.V., Anand, A., Herr, J.B., Miranda, C., Vogel, M.C., and Maday, S. (2021). Synaptic activity controls autophagic vacuole motility and function in dendrites. J. Cell Biol. *220*, e202002084. https://doi.org/10.1083/jcb.202002084.

63. Waites, C.L., Leal-Ortiz, S.A., Okerlund, N., Dalke, H., Fejtova, A., Altrock, W.D., Gundelfinger, E.D., and Garner, C.C. (2013). Bassoon and Piccolo maintain synapse integrity by regulating protein ubiquitination and degradation. EMBO J. *32*, 954–969. https://doi.org/10.1038/emboj.2013.27.

64. Hoffmann-Conaway, S., Brockmann, M.M., Schneider, K., Annamneedi, A., Rahman, K.A., Bruns, C., Textoris-Taube, K., Trimbuch, T., Smalla, K.H., Rosenmund, C., et al. (2020). Parkin contributes to synaptic vesicle autophagy in Bassoon-deficient mice. eLife *9*, e56590. https://doi.org/10.7554/eLife.56590.

65. Yang, S., Park, D., Manning, L., Hill, S.E., Cao, M., Xuan, Z., Gonzalez, I., Dong, Y., Clark, B., Shao, L., et al. (2022). Presynaptic autophagy is coupled to the synaptic vesicle cycle via ATG-9. Neuron *110*, 824–840.e10. https://doi.org/10.1016/j.neuron.2021.12.031.

66. Goldsmith, J., Ordureau, A., Harper, J.W., and Holzbaur, E.L.F. (2022). Brain-derived autophagosome profiling reveals the engulfment of nucleoid-enriched mitochondrial fragments by basal autophagy in neurons. Neuron *110*, 967–976.e8. https://doi.org/10.1016/j.neuron.2021.12.029.

67. Kallergi, E., Siva Sankar, D., Matera, A., Kolaxi, A., Paolicelli, R.C., Dengjel, J., and Nikoletopoulou, V. (2023). Profiling of purified autophagic vesicle degradome in the maturing and aging brain. Neuron *111*, 2329–2347.e7. https://doi.org/10.1016/j.neuron.2023.05.011.

68. Zhou, X., Lee, Y.K., Li, X., Kim, H., Sanchez-Priego, C., Han, X., Tan, H., Zhou, S., Fu, Y., Purtell, K., et al. (2024). Integrated proteomics reveals autophagy landscape and an autophagy receptor controlling PKA-RI complex homeostasis in neurons. Nat. Commun. *15*, 3113. https://doi.org/10.1038/s41467-024-47440-z.

69. Mizushima, N., Yamamoto, A., Matsui, M., Yoshimori, T., and Ohsumi, Y. (2004). In vivo analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker. Mol. Biol. Cell *15*, 1101–1111. https://doi.org/10.1091/mbc.e03-09-0704.

70. Young, J.E., Martinez, R.A., and La Spada, A.R. (2009). Nutrient deprivation induces neuronal autophagy and implicates reduced insulin signaling in neuroprotective autophagy activation. J. Biol. Chem. *284*, 2363–2373. https://doi.org/10.1074/jbc.M806088200.

71. Hernandez, D., Torres, C.A., Setlik, W., Cebrián, C., Mosharov, E.V., Tang, G., Cheng, H.C., Kholodilov, N., Yarygina, O., Burke, R.E., et al. (2012). Regulation of presynaptic neurotransmission by macroautophagy. Neuron *74*, 277–284. https://doi.org/10.1016/j.neuron.2012.02.020.

72. Catanese, A., Garrido, D., Walther, P., Roselli, F., and Boeckers, T.M. (2018). Nutrient limitation affects presynaptic structures through dissociable Bassoon autophagic degradation and impaired vesicle release. J. Cereb. Blood Flow Metab. *38*, 1924–1939. https://doi.org/10.1177/0271678X18786356.

73. Hoffmann, S., Orlando, M., Andrzejak, E., Bruns, C., Trimbuch, T., Rosenmund, C., Garner, C.C., and Ackermann, F. (2019). Light-activated ROS Production Induces synaptic autophagy. J. Neurosci. *39*, 2163–2183. https://doi.org/10.1523/JNEUROSCI.1317-18.2019.

74. Hill, S.E., Kauffman, K.J., Krout, M., Richmond, J.E., Melia, T.J., and Colón-Ramos, D.A. (2019). Maturation and clearance of autophagosomes in neurons depends on a specific cysteine protease isoform, ATG-4.2. Dev. Cell *49*, 251–266.e8. https://doi.org/10.1016/j.devcel.2019.02.013.

75. Shehata, M., Matsumura, H., Okubo-Suzuki, R., Ohkawa, N., and Inokuchi, K. (2012). Neuronal stimulation induces autophagy in hippocampal neurons that is involved in AMPA receptor degradation after chemical long-term depression. J. Neurosci. *32*, 10413–10422. https://doi.org/10.1523/JNEUROSCI.4533-11.2012.

76. Wang, Y., Lin, J., Li, J., Yan, L., Li, W., He, X., and Ma, H. (2023). Chronic neuronal inactivity utilizes the mTOR-TFEB pathway to drive transcription-dependent autophagy for homeostatic up-scaling. J. Neurosci. *43*, 2631–2652. https://doi.org/10.1523/JNEUROSCI.0146-23.2023.

77. Decet, M., and Verstreken, P. (2021). Presynaptic autophagy and the connection with neurotransmission. Front. Cell Dev. Biol. *9*, 790721. https://doi.org/10.3389/fcell.2021.790721.

78. Vanhauwaert, R., Kuenen, S., Masius, R., Bademosi, A., Manetsberger, J., Schoovaerts, N., Bounti, L., Gontcharenko, S., Swerts, J., Vilain, S., et al. (2017). The SAC1 domain in synaptojanin is required for autophagosome maturation at presynaptic terminals. EMBO J. *36*, 1392–1411. https://doi.org/10.15252/embj.201695773.

79. Hernandez-Diaz, S., Martinez-Olondo, P., Sanchez-Mirasierra, I., Montecinos, C., Ghimire, S., and Soukup, S.-F. (2023). Synaptogyrin regulates neuronal activity dependent autophagy to degrade synaptic vesicle components and pathological Tau. Prepint at. bioRxiv. https://doi.org/10.1101/2023.07.04.547658.

80. Hernandez-Diaz, S., Ghimire, S., Sanchez-Mirasierra, I., Montecinos-Oliva, C., Swerts, J., Kuenen, S., Verstreken, P., and Soukup, S.F.

(2022). Endophilin-B regulates autophagy during synapse development and neurodegeneration. Neurobiol. Dis. *163*, 105595. https://doi.org/10.1016/j.nbd.2021.105595.

81. Murdoch, J.D., Rostosky, C.M., Gowrisankaran, S., Arora, A.S., Soukup, S.F., Vidal, R., Capece, V., Freytag, S., Fischer, A., Verstreken, P., et al. (2016). Endophilin-A deficiency induces the Foxo3a-Fbxo32 network in the brain and causes dysregulation of autophagy and the ubiquitin-proteasome system. Cell Rep. *17*, 1071–1086. https://doi.org/10.1016/j.celrep.2016.09.058.

82. Taoufiq, Z., Ninov, M., Villar-Briones, A., Wang, H.Y., Sasaki, T., Roy, M.C., Beauchain, F., Mori, Y., Yoshida, T., Takamori, S., et al. (2020). Hidden proteome of synaptic vesicles in the mammalian brain. Proc. Natl. Acad. Sci. USA *117*, 33586–33596. https://doi.org/10.1073/pnas.2011870117.

83. Takamori, S., Holt, M., Stenius, K., Lemke, E.A., Grønborg, M., Riedel, D., Urlaub, H., Schenck, S., Brügger, B., Ringler, P., et al. (2006). Molecular anatomy of a trafficking organelle. Cell *127*, 831–846. https://doi.org/10.1016/j.cell.2006.10.030.

84. Sawa-Makarska, J., Baumann, V., Coudevylle, N., von Bülow, S., Nogellova, V., Abert, C., Schuschnig, M., Graef, M., Hummer, G., and Martens, S. (2020). Reconstitution of autophagosome nucleation defines Atg9 vesicles as seeds for membrane formation. Science *369*, eaaz7714. https://doi.org/10.1126/science.aaz7714.

85. Holzer, E., Martens, S., and Tulli, S. (2024). The role of ATG9 vesicles in autophagosome biogenesis. J. Mol. Biol. *436*, 168489. https://doi.org/10.1016/j.jmb.2024.168489.

86. Park, D., Wu, Y., Wang, X., Gowrishankar, S., Baublis, A., and De Camilli, P. (2023). Synaptic vesicle proteins and ATG9A self-organize in distinct vesicle phases within synapsin condensates. Nat. Commun. *14*, 455. https://doi.org/10.1038/s41467-023-36081-3.

87. Xuan, Z., Yang, S., Clark, B., Hill, S.E., Manning, L., and Colón-Ramos, D.A. (2023). The active zone protein Clarinet regulates synaptic sorting of ATG-9 and presynaptic autophagy. PLoS Biol. *21*, e3002030. https://doi.org/10.1371/journal.pbio.3002030.

88. Binotti, B., Ninov, M., Cepeda, A.P., Ganzella, M., Matti, U., Riedel, D., Urlaub, H., Sambandan, S., and Jahn, R. (2024). ATG9 resides on a unique population of small vesicles in presynaptic nerve terminals. Autophagy *20*, 883–901. https://doi.org/10.1080/15548627.2023.2274204.

89. Yang, S., and Colón-Ramos, D.A. (2022). Transmembrane protein ATG-9 links presynaptic autophagy with the synaptic vesicle cycle. Autophagy *18*, 1746–1747. https://doi.org/10.1080/15548627.2022.2049151.

90. Rizalar, F.S., Lucht, M.T., Petzoldt, A., Kong, S., Sun, J., Vines, J.H., Telugu, N.S., Diecke, S., Kaas, T., Bullmann, T., et al. (2023). Phosphatidylinositol 3,5-bisphosphate facilitates axonal vesicle transport and presynapse assembly. Science *382*, 223–230. https://doi.org/10.1126/science.adg1075.

91. Olivas, T.J., Wu, Y., Yu, S., Luan, L., Choi, P., Guinn, E.D., Nag, S., De Camilli, P.V., Gupta, K., and Melia, T.J. (2023). ATG9 vesicles comprise the seed membrane of mammalian autophagosomes. J. Cell Biol. *222*, e202208088. https://doi.org/10.1083/jcb.202208088.

92. Ackermann, F., Schink, K.O., Bruns, C., Izsvák, Z., Hamra, F.K., Rosenmund, C., and Garner, C.C. (2019). Critical role for Piccolo in synaptic vesicle retrieval. eLife *8*, e46629. https://doi.org/10.7554/eLife.46629.

93. Garner, C.C., and Ackermann, F. (2023). Synaptic logistics: the presynaptic scaffold protein Piccolo a nodal point tuning synaptic vesicle recycling, maintenance and integrity. Mol. Cell. Neurosci. *124*, 103795. https://doi.org/10.1016/j.mcn.2022.103795.

94. Montenegro-Venegas, C., Fienko, S., Anni, D., Pina-Fernández, E., Frischknecht, R., and Fejtova, A. (2021). Bassoon inhibits proteasome activity via interaction with PSMB4. Cell. Mol. Life Sci. *78*, 1545–1563. https://doi.org/10.1007/s00018-020-03590-z.

95. Kawabe, H., and Stegmüller, J. (2021). The role of E3 ubiquitin ligases in synapse function in the healthy and diseased brain. Mol. Cell. Neurosci. *112*, 103602. https://doi.org/10.1016/j.mcn.2021.103602.

96. Soukup, S.F., Vanhauwaert, R., and Verstreken, P. (2018). Parkinson's disease: convergence on synaptic homeostasis. EMBO J. *37*, e98960. https://doi.org/10.15252/embj.201898960.

97. Binotti, B., Pavlos, N.J., Riedel, D., Wenzel, D., Vorbrüggen, G., Schalk, A.M., Kühnel, K., Boyken, J., Erck, C., Martens, H., et al. (2015). The GTPase Rab26 links synaptic vesicles to the autophagy pathway. eLife *4*, e05597. https://doi.org/10.7554/eLife.05597.

98. Lüningschrör, P., Binotti, B., Dombert, B., Heimann, P., Perez-Lara, A., Slotta, C., Thau-Habermann, N., R von Collenberg, C., Karl, F., Damme, M., et al. (2017). Plekhg5-regulated autophagy of synaptic vesicles reveals a pathogenic mechanism in motoneuron disease. Nat. Commun. *8*, 678. https://doi.org/10.1038/s41467-017-00689-z.

99. Lüningschrör, P., and Sendtner, M. (2018). Autophagy in the presynaptic compartment. Curr. Opin. Neurobiol. *51*, 80–85. https://doi.org/10.1016/j.conb.2018.02.023.

100. Kohrs, F.E., Daumann, I.M., Pavlovic, B., Jin, E.J., Kiral, F.R., Lin, S.C., Port, F., Wolfenberg, H., Mathejczyk, T.F., Linneweber, G.A., et al. (2021). Systematic functional analysis of Rab GTPases reveals limits of neuronal robustness to environmental challenges in flies. eLife *10*, e59594. https://doi.org/10.7554/eLife.59594.

101. Jiang, L., Wang, M., Lin, S., Jian, R., Li, X., Chan, J., Dong, G., Fang, H., Robinson, A.E., Consortium, G.T., et al. (2020). A quantitative proteome map of the human body. Cell *183*, 269–283.e219. https://doi.org/10.1016/j.cell.2020.08.036.

102. Cohen, L.D., and Ziv, N.E. (2019). Neuronal and synaptic protein lifetimes. Curr. Opin. Neurobiol. *57*, 9–16. https://doi.org/10.1016/j.conb.2018.12.007.

103. Tang, G., Gudsnuk, K., Kuo, S.H., Cotrina, M.L., Rosoklija, G., Sosunov, A., Sonders, M.S., Kanter, E., Castagna, C., Yamamoto, A., et al. (2014). Loss of mTOR-dependent macroautophagy causes autistic-like synaptic pruning deficits. Neuron *83*, 1131–1143. https://doi.org/10.1016/j.neuron.2014.07.040.

104. Vargas, J.N.S., Hamasaki, M., Kawabata, T., Youle, R.J., and Yoshimori, T. (2023). The mechanisms and roles of selective autophagy in mammals. Nat. Rev. Mol. Cell Biol. *24*, 167–185. https://doi.org/10.1038/s41580-022-00542-2.

105. Lamark, T., and Johansen, T. (2021). Mechanisms of selective autophagy. Annu. Rev. Cell Dev. Biol. *37*, 143–169. https://doi.org/10.1146/annurev-cellbio-120219-035530.

106. Nishimura, T., Tamura, N., Kono, N., Shimanaka, Y., Arai, H., Yamamoto, H., and Mizushima, N. (2017). Autophagosome formation is initiated at phosphatidylinositol synthase-enriched ER subdomains. EMBO J. *36*, 1719–1735. https://doi.org/10.15252/embj.201695189.

107. Birgisdottir, Å.B., Mouilleron, S., Bhujabal, Z., Wirth, M., Sjøttem, E., Evjen, G., Zhang, W., Lee, R., O'Reilly, N., Tooze, S.A., et al. (2019). Members of the autophagy class III phosphatidylinositol 3-kinase complex I interact with GABARAP and GABARAPL1 via LIR motifs. Autophagy *15*, 1333–1355. https://doi.org/10.1080/15548627.2019.1581009.

108. Cóppola-Segovia, V., and Reggiori, F. (2024). Molecular insights into aggrephagy: their cellular functions in the context of neurodegenerative diseases. J. Mol. Biol. *436*, 168493. https://doi.org/10.1016/j.jmb.2024.168493.

109. Kumsta, C., Chang, J.T., Lee, R., Tan, E.P., Yang, Y., Loureiro, R., Choy, E.H., Lim, S.H.Y., Saez, I., Springhorn, A., et al. (2019). The autophagy receptor p62/SQST-1 promotes proteostasis and longevity in C. elegans by inducing autophagy. Nat. Commun. *10*, 5648. https://doi.org/10.1038/s41467-019-13540-4.

110. Aparicio, R., Rana, A., and Walker, D.W. (2019). Upregulation of the autophagy adaptor p62/SQSTM1 prolongs health and lifespan in middle-aged Drosophila. Cell Rep. *28*, 1029–1040.e5. https://doi.org/10.1016/j.celrep.2019.06.070.

111. Weingarten, J., Lassek, M., Mueller, B.F., Rohmer, M., Lunger, I., Baeumlisberger, D., Dudek, S., Gogesch, P., Karas, M., and Volknandt, W. (2014). The proteome of the presynaptic active zone from mouse brain. Mol. Cell. Neurosci. *59*, 106–118. https://doi.org/10.1016/j.mcn.2014.02.003.

112. van Oostrum, M., Blok, T.M., Giandomenico, S.L., Tom Dieck, S., Tushev, G., Fürst, N., Langer, J.D., and Schuman, E.M. (2023). The proteomic landscape of synaptic diversity across brain regions and cell types. Cell *186*, 5411–5427.e23. https://doi.org/10.1016/j.cell.2023.09.028.

113. Fornasiero, E.F., Mandad, S., Wildhagen, H., Alevra, M., Rammner, B., Keihani, S., Opazo, F., Urban, I., Ischebeck, T., Sakib, M.S., et al. (2018). Precisely measured protein lifetimes in the mouse brain reveal differences across tissues and subcellular fractions. Nat. Commun. *9*, 4230. https://doi.org/10.1038/s41467-018-06519-0.

114. Djakovic, S.N., Schwarz, L.A., Barylko, B., DeMartino, G.N., and Patrick, G.N. (2009). Regulation of the proteasome by neuronal activity and calcium/calmodulin-dependent protein kinase II. J. Biol. Chem. *284*, 26655–26665. https://doi.org/10.1074/jbc.M109.021956.

115. Korolchuk, V.I., Menzies, F.M., and Rubinsztein, D.C. (2010). Mechanisms of cross-talk between the ubiquitin-proteasome and autophagy-lysosome systems. FEBS Lett. *584*, 1393–1398. https://doi.org/10.1016/j.febslet.2009.12.047.

116. Rajalingam, K., and Dikic, I. (2016). SnapShot: expanding the ubiquitin code. Cell *164*, 1074–1074.e1. https://doi.org/10.1016/j.cell.2016.02.019.

117. Kwon, Y.T., and Ciechanover, A. (2017). The ubiquitin code in the ubiquitin-proteasome system and autophagy. Trends Biochem. Sci. *42*, 873–886. https://doi.org/10.1016/j.tibs.2017.09.002.

118. Cohen, L.D., and Ziv, N.E. (2017). Recent insights on principles of synaptic protein degradation. F1000Res *6*, 675. https://doi.org/10.12688/f1000research.10599.1.

119. Sun, C., Desch, K., Nassim-Assir, B., Giandomenico, S.L., Nemcova, P., Langer, J.D., and Schuman, E.M. (2023). An abundance of free regulatory (19S) proteasome particles regulates neuronal synapses. Science *380*, eadf2018. https://doi.org/10.1126/science.adf2018.

120. de Poot, S.A.H., Tian, G., and Finley, D. (2017). Meddling with fate: the proteasomal deubiquitinating enzymes. J. Mol. Biol. *429*, 3525–3545. https://doi.org/10.1016/j.jmb.2017.09.015.

121. Erpapazoglou, Z., Walker, O., and Haguenauer-Tsapis, R. (2014). Versatile roles of k63-linked ubiquitin chains in trafficking. Cells *3*, 1027–1088. https://doi.org/10.3390/cells3041027.

122. Ebrahimi-Fakhari, D., Saffari, A., Wahlster, L., Lu, J., Byrne, S., Hoffmann, G.F., Jungbluth, H., and Sahin, M. (2016). Congenital disorders of autophagy: an emerging novel class of inborn errors of neuro-metabolism. Brain *139*, 317–337. https://doi.org/10.1093/brain/awv371.

123. Tomoda, T., Yang, K., and Sawa, A. (2020). Neuronal autophagy in synaptic functions and psychiatric disorders. Biol. Psychiatry *87*, 787–796. https://doi.org/10.1016/j.biopsych.2019.07.018.

124. Glatigny, M., Moriceau, S., Rivagorda, M., Ramos-Brossier, M., Nascimbeni, A.C., Lante, F., Shanley, M.R., Boudarene, N., Rousseaud, A., Friedman, A.K., et al. (2019). Autophagy Is Required for Memory Formation and Reverses Age-Related Memory Decline. Curr. Biol. *29*, 435–448.e438. https://doi.org/10.1016/j.cub.2018.12.021.

125. Pan, Y., He, X., Li, C., Li, Y., Li, W., Zhang, H., Wang, Y., Zhou, G., Yang, J., Li, J., et al. (2021). Neuronal activity recruits the CRTC1/CREB axis to drive transcription-dependent autophagy for maintaining late-phase LTD. Cell Rep. *36*, 109398. https://doi.org/10.1016/j.celrep.2021.109398.

126. Nikoletopoulou, V., Sidiropoulou, K., Kallergi, E., Dalezios, Y., and Tavernarakis, N. (2017). Modulation of autophagy by BDNF underlies synaptic plasticity. Cell Metab. *26*, 230–242.e5. https://doi.org/10.1016/j.cmet.2017.06.005.

127. Connor, S.A., and Wang, Y.T. (2016). A place at the table: LTD as a mediator of memory genesis. Neuroscientist *22*, 359–371. https://doi.org/10.1177/1073858415588498.

128. Dringenberg, H.C. (2020). The history of long-term potentiation as a memory mechanism: controversies, confirmation, and some lessons to remember. Hippocampus *30*, 987–1012. https://doi.org/10.1002/hipo.23213.

129. Stacho, M., and Manahan-Vaughan, D. (2022). The intriguing contribution of hippocampal long-term depression to spatial learning and long-term memory. Front. Behav. Neurosci. *16*, 806356. https://doi.org/10.3389/fnbeh.2022.806356.

130. Rubinsztein, D.C., Mariño, G., and Kroemer, G. (2011). Autophagy and aging. Cell *146*, 682–695. https://doi.org/10.1016/j.cell.2011.07.030.

131. Cuervo, A.M., and Dice, J.F. (1998). How do intracellular proteolytic systems change with age? Front. Biosci. *3*, d25–d43. https://doi.org/10.2741/a264.

132. Chang, J.T., Kumsta, C., Hellman, A.B., Adams, L.M., and Hansen, M. (2017). Spatiotemporal regulation of autophagy during Caenorhabditis elegans aging. eLife *6*, e18459. https://doi.org/10.7554/eLife.18459.

133. Leidal, A.M., Levine, B., and Debnath, J. (2018). Autophagy and the cell biology of age-related disease. Nat. Cell Biol. *20*, 1338–1348. https://doi.org/10.1038/s41556-018-0235-8.

134. Stavoe, A.K., Gopal, P.P., Gubas, A., Tooze, S.A., and Holzbaur, E.L. (2019). Expression of WIPI2B counteracts age-related decline in autophagosome biogenesis in neurons. eLife *8*, e44219. https://doi.org/10.7554/eLife.44219.

135. Salminen, A., and Kaarniranta, K. (2009). Regulation of the aging process by autophagy. Trends Mol. Med. *15*, 217–224. https://doi.org/10.1016/j.molmed.2009.03.004.

136. Lipinski, M.M., Zheng, B., Lu, T., Yan, Z., Py, B.F., Ng, A., Xavier, R.J., Li, C., Yankner, B.A., Scherzer, C.R., et al. (2010). Genome-wide analysis reveals mechanisms modulating autophagy in normal brain aging and in Alzheimer's disease. Proc. Natl. Acad. Sci. USA *107*, 14164–14169. https://doi.org/10.1073/pnas.1009485107.

137. Kaushik, S., Arias, E., Kwon, H., Lopez, N.M., Athonvarangkul, D., Sahu, S., Schwartz, G.J., Pessin, J.E., and Singh, R. (2012). Loss of autophagy in hypothalamic POMC neurons impairs lipolysis. EMBO Rep. *13*, 258–265. https://doi.org/10.1038/embor.2011.260.

138. Ott, C., König, J., Höhn, A., Jung, T., and Grune, T. (2016). Macroautophagy is impaired in old murine brain tissue as well as in senescent human fibroblasts. Redox Biol. *10*, 266–273. https://doi.org/10.1016/j.redox.2016.10.015.

139. Stavoe, A.K.H., and Holzbaur, E.L.F. (2020). Neuronal autophagy declines substantially with age and is rescued by overexpression of WIPI2. Autophagy *16*, 371–372. https://doi.org/10.1080/15548627.2019.1695401.

140. Tsong, H., Holzbaur, E.L., and Stavoe, A.K. (2023). Aging differentially affects axonal autophagosome formation and maturation. Autophagy *19*, 3079–3095. https://doi.org/10.1080/15548627.2023.2236485.

141. Wirth, M., Benson, G., Schwarz, C., Köbe, T., Grittner, U., Schmitz, D., Sigrist, S.J., Bohlken, J., Stekovic, S., Madeo, F., et al. (2018). The effect of spermidine on memory performance in older adults at risk for dementia: A randomized controlled trial. Cortex *109*, 181–188. https://doi.org/10.1016/j.cortex.2018.09.014.

142. Schwarz, C., Horn, N., Benson, G., Wrachtrup Calzado, I., Wurdack, K., Pechlaner, R., Grittner, U., Wirth, M., and Flöel, A. (2020). Spermidine intake is associated with cortical thickness and hippocampal volume in older adults. Neuroimage *221*, 117132. https://doi.org/10.1016/j.neuroimage.2020.117132.

143. Schwarz, C., Benson, G.S., Horn, N., Wurdack, K., Grittner, U., Schilling, R., Märschenz, S., Köbe, T., Hofer, S.J., Magnes, C., et al. (2022). Effects of spermidine supplementation on cognition and biomarkers in older adults with subjective cognitive decline: A randomized clinical trial. JAMA Netw. Open *5*, e2213875. https://doi.org/10.1001/jamanetworkopen.2022.13875.

144. Maglione, M., Kochlamazashvili, G., Eisenberg, T., Rácz, B., Michael, E., Toppe, D., Stumpf, A., Wirth, A., Zeug, A., Müller, F.E., et al. (2019). Spermidine protects from age-related synaptic alterations at hippocampal mossy fiber-CA3 synapses. Sci. Rep. *9*, 19616. https://doi.org/10.1038/s41598-019-56133-3.

145. De Risi, M., Torromino, G., Tufano, M., Moriceau, S., Pignataro, A., Rivagorda, M., Carrano, N., Middei, S., Settembre, C., Ammassari-Teule, M.,

et al. (2020). Mechanisms by which autophagy regulates memory capacity in ageing. Aging Cell *19*, e13189. https://doi.org/10.1111/acel.13189.

146. Gupta, V.K., Scheunemann, L., Eisenberg, T., Mertel, S., Bhukel, A., Koemans, T.S., Kramer, J.M., Liu, K.S.Y., Schroeder, S., Stunnenberg, H.G., et al. (2013). Restoring polyamines protects from age-induced memory impairment in an autophagy-dependent manner. Nat. Neurosci. *16*, 1453–1460. https://doi.org/10.1038/nn.3512.

147. Sigrist, S.J., Carmona-Gutierrez, D., Gupta, V.K., Bhukel, A., Mertel, S., Eisenberg, T., and Madeo, F. (2014). Spermidine-triggered autophagy ameliorates memory during aging. Autophagy *10*, 178–179. https://doi.org/10.4161/auto.26918.

148. Schroeder, S., Hofer, S.J., Zimmermann, A., Pechlaner, R., Dammbrueck, C., Pendl, T., Marcello, G.M., Pogatschnigg, V., Bergmann, M., Müller, M., et al. (2021). Dietary spermidine improves cognitive function. Cell Rep. *35*, 108985. https://doi.org/10.1016/j.celrep.2021.108985.

149. Liang, Y., Piao, C., Beuschel, C.B., Toppe, D., Kollipara, L., Bogdanow, B., Maglione, M., Lützkendorf, J., See, J.C.K., Huang, S., et al. (2021). eIF5A hypusination, boosted by dietary spermidine, protects from premature brain aging and mitochondrial dysfunction. Cell Rep. *35*, 108941. https://doi.org/10.1016/j.celrep.2021.108941.

150. Gupta, V.K., Pech, U., Bhukel, A., Fulterer, A., Ender, A., Mauermann, S.F., Andlauer, T.F.M., Antwi-Adjei, E., Beuschel, C., Thriene, K., et al. (2016). Spermidine suppresses age-associated memory impairment by preventing adverse increase of presynaptic active zone size and release. PLoS Biol. *14*, e1002563. https://doi.org/10.1371/journal.pbio.1002563.

151. Bhukel, A., Beuschel, C.B., Maglione, M., Lehmann, M., Juhász, G., Madeo, F., and Sigrist, S.J. (2019). Autophagy within the mushroom body protects from synapse aging in a non-cell autonomous manner. Nat. Commun. *10*, 1318. https://doi.org/10.1038/s41467-019-09262-2.

152. Andres-Alonso, M., Ammar, M.R., Butnaru, I., Gomes, G.M., Acuña Sanhueza, G., Raman, R., Yuanxiang, P., Borgmeyer, M., Lopez-Rojas, J., Raza, S.A., et al. (2019). SIPA1L2 controls trafficking and local signaling of TrkB-containing amphisomes at presynaptic terminals. Nat. Commun. *10*, 5448. https://doi.org/10.1038/s41467-019-13224-z.

153. Magné, J., and Green, D.R. (2022). LC3-associated endocytosis and the functions of Rubicon and ATG16L1. Sci. Adv. *8*, eabo5600. https://doi.org/10.1126/sciadv.abo5600.

154. Ejlerskov, P., Rasmussen, I., Nielsen, T.T., Bergström, A.L., Tohyama, Y., Jensen, P.H., and Vilhardt, F. (2013). Tubulin polymerization-promoting protein (TPPP/p25alpha) promotes unconventional secretion of alpha-synuclein through exophagy by impairing autophagosome-lysosome fusion. J. Biol. Chem. *288*, 17313–17335. https://doi.org/10.1074/jbc.M112.401174.

155. Nakamura, Y., Sawai, T., Kakiuchi, K., and Arawaka, S. (2024). Neuronal activity promotes secretory autophagy for the extracellular release of alpha-synuclein. J. Biol. Chem. *300*, 107419. https://doi.org/10.1016/j.jbc.2024.107419.

156. Nieves Torres, D., and Lee, S.H. (2023). Inter-neuronal signaling mediated by small extracellular vesicles: wireless communication? Front. Mol. Neurosci. *16*, 1187300. https://doi.org/10.3389/fnmol.2023.1187300.

157. Chang, Y.C., Gao, Y., Lee, J.Y., Peng, Y.J., Langen, J., and Chang, K.T. (2024). Identification of secretory autophagy as a mechanism modulating activity-induced synaptic remodeling. Proc. Natl. Acad. Sci. USA *121*, e2315958121. https://doi.org/10.1073/pnas.2315958121.

158. Dupont, N., Jiang, S., Pilli, M., Ornatowski, W., Bhattacharya, D., and Deretic, V. (2011). Autophagy-based unconventional secretory pathway for extracellular delivery of IL-1beta. EMBO J. *30*, 4701–4711. https://doi.org/10.1038/emboj.2011.398.

159. Kim, Y.H., Kwak, M.S., Lee, B., Shin, J.M., Aum, S., Park, I.H., Lee, M.G., and Shin, J.S. (2021). Secretory autophagy machinery and vesicular trafficking are involved in HMGB1 secretion. Autophagy *17*, 2345–2362. https://doi.org/10.1080/15548627.2020.1826690.

160. Martinelli, S., Anderzhanova, E.A., Bajaj, T., Wiechmann, S., Dethloff, F., Weckmann, K., Heinz, D.E., Ebert, T., Hartmann, J., Geiger, T.M., et al. (2021). Stress-primed secretory autophagy promotes extracellular BDNF maturation by enhancing MMP9 secretion. Nat. Commun. *12*, 4643. https://doi.org/10.1038/s41467-021-24810-5.

161. Tan, H.W.S., Lu, G., Dong, H., Cho, Y.L., Natalia, A., Wang, L., Chan, C., Kappei, D., Taneja, R., Ling, S.C., et al. (2022). A degradative to secretory autophagy switch mediates mitochondria clearance in the absence of the mATG8-conjugation machinery. Nat. Commun. *13*, 3720. https://doi.org/10.1038/s41467-022-31213-7.

162. Hartmann, J., Bajaj, T., Otten, J., Klengel, C., Ebert, T., Gellner, A.K., Junglas, E., Hafner, K., Anderzhanova, E.A., Tang, F., et al. (2024). SKA2 regulated hyperactive secretory autophagy drives neuroinflammation-induced neurodegeneration. Nat. Commun. *15*, 2635. https://doi.org/10.1038/s41467-024-46953-x.

163. Hutchings, A.J., Hambrecht, B., Veh, A., Giridhar, N.J., Zare, A., Angerer, C., Ohnesorge, T., Schenke, M., Selvaraj, B.T., Chandran, S., et al. (2024). Plekhg5 controls the unconventional secretion of Sod1 by presynaptic secretory autophagy. Nat. Commun. *15*, 8622. https://doi.org/10.1038/s41467-024-52875-5.

164. Petralia, R.S., Schwartz, C.M., Wang, Y.X., Kawamoto, E.M., Mattson, M.P., and Yao, P.J. (2013). Sonic hedgehog promotes autophagy in hippocampal neurons. Biol. Open *2*, 499–504. https://doi.org/10.1242/bio.20134275.

165. Sarkar, S., Perlstein, E.O., Imarisio, S., Pineau, S., Cordenier, A., Maglathlin, R.L., Webster, J.A., Lewis, T.A., O'Kane, C.J., Schreiber, S.L., et al. (2007). Small molecules enhance autophagy and reduce toxicity in Huntington's disease models. Nat. Chem. Biol. *3*, 331–338. https://doi.org/10.1038/nchembio883.

166. Alvarez-Erviti, L., Seow, Y., Yin, H., Betts, C., Lakhal, S., and Wood, M.J.A. (2011). Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nat. Biotechnol. *29*, 341–345. https://doi.org/10.1038/nbt.1807.

167. Corti, O., Blomgren, K., Poletti, A., and Beart, P.M. (2020). Autophagy in neurodegeneration: new insights underpinning therapy for neurological diseases. J. Neurochem. *154*, 354–371. https://doi.org/10.1111/jnc.15002.

168. Lynch-Day, M.A., Mao, K., Wang, K., Zhao, M., and Klionsky, D.J. (2012). The role of autophagy in Parkinson's disease. Cold Spring Harb. Perspect. Med. *2*, a009357. https://doi.org/10.1101/cshperspect.a009357.