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The Synaptic Autophagy Cycle

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Abstract

Macroautophagy (hereafter referred to as autophagy) is a evolutionarily conserved pathway in which proteins and organelles are delivered to the lysosome for degradation. In neurons, autophagy was originally described as associated with disease states and neuronal survival. Over the last decade, however, evidence has accumulated that autophagy controls synaptic function in both the axon and dendrite. Here, we review this literature, highlighting the role of autophagy in the pre- and postsynapse, synaptic plasticity, and behavior. We end by discussing open questions in the field of synaptic autophagy.

Keywords

Autophagy; synaptic transmission; synaptic plasticity; protein degradation; endocytosis

I. Introduction

Research over the past 40 years has defined roles for protein synthesis in neuronal plasticity and brain function [1]. Recently, however, the controlled degradation of proteins and organelles has also been appreciated to play critical roles in neurotransmission [2]. These steps occur via the degradation of specific cytosolic proteins through the ubiquitin / proteasome pathway and by multiple pathways that lead to degradation within the lysosome [3].

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Christian De Duve, who with Alex Novikoff, co-discovered the lysosome, introduced the term *autophagy* to describe how “portions of a cell somehow find their way inside the cell’s own lysosomes and are broken down” [4]. De Duve also coined the terms *lysosome*, *endocytosis* / *endosome*, *phagocytosis*/ *phagosome*, / *autophagosome* and *peroxisome* [5].

De Duve’s definition of autophagy encompasses the breakdown of any of a cell’s own components within the lysosome, but even in 1963 it was clear that proteins, lipids, and organelles could use multiple mechanisms of entry into the lysosomal lumen.

In the *endosomal-lysosomal* degradation pathway, membrane, lumenal or formerly extracellular components that were endocytosed are delivered to lysosomes by fusion with endosomes. In this highly regulated pathway, extracellular proteins and intrinsic proteins on the cell surface are internalized into small vesicles and sorted through a series of intermediate vesicular structures. Some components may be recycled to other membranes, while others that reach a late endosome can fuse with a lysosome that is capable of proteolysis and can degrade proteins in both the endosomal membrane and lumen [6,7].

A relatively newly discovered autophagic pathway is known as *chaperone-mediated autophagy* (CMA) [8], in which cytosolic proteins are bound by the chaperone Hsc70 and delivered to the lysosomal membrane, where they are unfolded and transported into the lysosomal lumen by a membrane protein, Lamp2A [9]. Proteins within the lumen are then exposed to lysosomal proteases and degraded. While a great deal of research has identified cytosolic proteins that play roles in synapses to be degraded by CMA including alpha-synuclein [10] and tau protein [11], there has been little research on CMA at synapses in contrast to in cell bodies.

In *endosomal microautophagy* (eMI), cytosolic proteins are similarly bound by Hsc70, but are internalized into small vesicles within endosomes which subsequently fuse with lysosomes for degradation [12]. These structures may also participate in producing exosomes secreted from the neurons, and so may participate in signaling and disease spread. As multivesicular bodies are very common in synapses and axons, these are likely to be important for synaptic turnover, and early evidence suggests that eMI may play a role in presynaptic function [13,14].

At this writing, most research has been conducted on roles for *macroautophagy* (which by convention will be called *autophagy* in this review), a process that can degrade cytosolic proteins, lipids and organelles. This form of autophagy has been particularly tractable because it involves the participation of large double membrane vesicles, termed *autophagosomes*, that fuse with lysosomes for degradation that can be examined by microscopy and because it can be manipulated by modulating the activity of genes initially characterized in yeast that are responsible for steps in the pathway.

Here, we will first review the molecular machinery involved in autophagy in mammalian cells. We will then briefly describe the history of the discovery of autophagy in neurons and explore how the machinery that mediates autophagy in dividing cells has been adapted to ensure appropriate autophagic protein degradation in post-mitotic neurons.

Recent work has highlighted a potential role for autophagy in the regulation of synaptic transmission, plasticity and behavior. These studies indicate that autophagy plays some roles that can be revealed simply by blocking specific steps in the pathway, while others that require both blockade and a triggering additional factor, particularly via cellular stress responses [15–18]. Both classes of synaptic autophagic function may regulate pre- and postsynaptic functions during normal neuronal development and in disease. We conclude with three outstanding questions that we feel are critical for the field to address to deepen our understanding of the role of autophagy in controlling neurotransmission.

II. Molecular Machinery of Autophagy

The molecular machinery that controls autophagosome formation, maturation and fusion with lysosomes were originally described in yeast by Yoshinori Ohsumi and colleagues [19]. Autophagy in mammalian cells is controlled by both proteins that are homologous to the yeast *a*u*t*opha*g*y (atg) proteins and additional mammalian-specific components [20].

In yeast and dividing mammalian cells, autophagy is strongly activated by nutrient or serum starvation and can be divided into distinct stages: (1) induction, (2) expansion, (3) maturation (Figure 1).

Autophagy is *induced* following nutrient or serum deprivation via the activity of the metabolic kinases, mTOR [21] and AMPK [22]. mTOR inhibits and AMPK activates the kinase ULK1, in complex with Atg13 and FIP200 [23–27]. ULK2, a ULK1 homolog in mammalian cells, may also control autophagy but it is presently unclear whether these kinases are redundant [24,28]. ULK complex activation leads to the phosphorylation of Beclin-1 and activation of the class iii phosphoinositol-3-kinase (PI3K), Vps34 [29,30]. The PI3K activity of Vps34 is critical both for endocytic activity [31,32] and autophagy [30] and is directed to a particular pathway by its interacting proteins. For example, when partnered with Atg14, Vps34 activity stimulates autophagy [33], and ULK1-dependent phosphorylation of Beclin-1 specifically activates Vps34 activity when Vps34 is complexed with Atg14 and Ambra1 [29].

Vps34 activity induces autophagy via local synthesis of phosphoinositol-3-phosphate (PI3P). The precise sites of PI3P synthesis and initial formation of the autophagic membrane may be at the endoplasmic reticulum [34–36], but contributions from plasma membrane [37], mitochondria [38], or recycling endosomes [39–42] are also involved. Subsequent recruitment of PI3P binding proteins, such as DFCP1 [34] and WIPI1-4 [39,43–45], initiate an enzymatic cascade that leads to the assembly of the autophagosomal membrane.

Expansion of the autophagosome membrane is driven by two ubiquitin-like conjugation systems. In the first, Atg12 is conjugated to Atg5 by the E1-like enzyme, Atg7, and the E2-like enzyme, Atg10 [46]. The Atg5-12 conjugate subsequently interacts with Atg16L1 [47]. In the second pathway, Atg8 is processed by the protease, Atg4 [48], and subsequently conjugated to phophatidylethanolamine (PE) by Atg7 and the E2-like enzyme, Atg3 [46]. Atg8 with PE conjugation requires the E3-like activity of Atg5-12 complexed with Atg16L1 [49,50].

Mammals have numerous Atg8 homologs, including the LC3 and GABARAP families [51]. The conjugated form of LC3 is referred to below as LC3-II and the unmodified protein is referred to LC3-I. Vesicles containing Atg9, a transmembrane protein, then fuse with the growing autophagosome and provide an additional membrane source [52].

How are proteins and organelles selected for autophagic degradation [53]? Upon nutrient deprivation, bulk cytosolic proteins can be sequestered into autophagosomes and degraded to increase the availability of amino acids [54–56]. Alternatively, proteins and organelles can be selectively degraded by binding to autophagy cargo adapters such as p62 [57,58], NBR1 [59], Tax1BP1 [60], optineurin [61] and NDP52 [62–65]. Proteins may contain an LC3-interacting motif that promotes binding to LC3 and sequestration within the autophagosome, such as Dishevelled-2 [66–68]. Proteins and organelles that are targeted for selective degradation can also be modified by post-translational modifications such as ubiquitination or acetylation, which mediate interactions with autophagy cargo proteins such as p62 [69].

Following cargo sequestration, autophagosomes close and are trafficked retrogradely to the perinuclear region, where most late endosomes and lysosomes are localized [70–72]. Retrograde trafficking of autophagosomes occurs on microtubules in a dynein-dependent manner [70]. Autophagosomes are linked to dynein via a Rab7, RILP, and OPR1L complex [73,74] and in the absence of these factors, mature autophagosomes accumulate. Interestingly, actin-dependent autophagosome transport has also been demonstrated, although this does not occur during starvation-induced autophagy [75]. Following retrograde transport, autophagosome fusion with lysosomes depends on the Qa SNARE, synataxin 17; the Qbc SNARE, SNAP29; and the R SNARE, VAMP8 [76]. This process also depends on the HOPS complex [77–79] and the autophagosome adapter PLEKHM1 [78]. The lysosomal GTPase Arl8 also controls autophagosomal and endosomal fusion with lysosomes [80–84] via recruitment of the HOPS complex [82] and lysosomal positioning [80]. The fusion of autophagosomes with lysosomes leads to degradation of autophagic cargo.

III. Autophagy in Neurons and Neurites

The initial studies of autophagy by De Duve, Novikoff and others were in dividing cells. Neurons have long been known to possess lipofuscin, neuromelanin, and in ceroid lipofuscinosis disorders, ceroid pigments, that are accumulated within autophagosomes [85]. Nevertheless, to our knowledge, the initial reports in neurons of autophagosomes (also known as autophagic vacuoles) were in two mid-1970s electron microscopy reports of pathological specimens from the brain of Huntington’s disease patients [86,87]. The latter study stressed a highly increased abundance of lipofuscin in the neurons, astrocytes and microglia of these patients, and conjectured that this was due to insufficient autophagy, an impression supported by many subsequent studies in human tissue and animal models. This same study further noted enlarged mitochondria devoid of cristae and suggested that this could be due to deficient autophagy associated with Huntington’s disease, as borne out by later research by Marianne DiFiglia and colleagues [88]. Cultured striatal neurons with mutant huntingtin gene were found to form prominent autophagosomes when stressed by dopamine-mediated oxidative stress [89], predicting one theme of this review: in synapses, some effects of autophagy become apparent under conditions of cellular stress. Mechanistic

studies by Ana Maria Cuervo and collaborators indicated that in the case of huntingtin mutations that this could result from a lack of appropriate autophagic cargo recognition [90].

In addition to the lipofuscin pigment, work by Luigi Zecca and collaborators has shown that the neuromelanin pigment of substantia nigra and locus coeruleus neurons is encased with autophagic organelles that apparently do not degrade these contents [85,91], leading to an initial focus in the field on neuronal autophagy as a product of disease and normal aging related stress [92]. Subsequent studies by Randy Nixon, Anne Cataldo and colleagues revealed the presence of neuronal autophagic vacuoles in tissue from Alzheimer's patients and other disorders [93–95] and further work by Anne Tolkovsky, David Rubinsztein, Zhenyu Yue, Richard Youle and many others have extended the observation of aberrant autophagy to a wide variety of associated neuronal disorders [96–98].

The presence of autophagic organelles in neurites and synapses awaited identification by Peter Hollenbeck [99], who observed retrograde axonal transport and lysosomal delivery of fluorescent cargo by autophagic vacuoles in cultured sympathetic neurons. This study suggested, in contrast to the other studies of the era, that autophagy might be associated with synaptic activity and may occur independently from disease or aging-oriented stress.

It took a long time for Hollenbeck's pioneering observation to be appreciated by the field at large. One reason is that in dividing cells, autophagy was typically initiated by nutrient deprivation [54–56]. While nutrient deprivation and starvation is a potent trigger for autophagic degradation in non-neuronal cells, it has little effect in most populations of central neurons [100]. An exception is in the hypothalamus [101,102], a brain region that is critical for the maintenance of energy homeostasis [103], where autophagy senses energy balance to control neuronal activity (see below). Another reason may be that the autophagosomes, particularly in neurites, may be relatively short-lived and transported rapidly.

An experimental tool that has vastly changed the study of autophagy in synaptic transmission is the use of fluorescently tagged LC3 to specifically label autophagosome membrane in living cells. A study from Erica Holzbaur and colleagues introduced an elegant model in which autophagosomes were primarily generated in the distal processes of neurons and then trafficked toward the cell body to fuse with lysosomes in proximal processes and the cell body [104–106]. Live imaging GFP-LC3 puncta in primary cultures of dorsal root ganglia (DRG) suggested that autophagosome formation occurs almost exclusively within the axon tip. This may not be surprising, as DRG neurons differ from most CNS axons in that they do not make *en passant* synapses but instead have a single release site from the distal axon [106]. Similar imaging studies in synaptically mature primary hippocampal neurons yielded a more complex picture, with autophagosome formation occurring predominantly in distal processes but also in the cell body and more proximally in dendrites and axons [106]. Shehata et al reported the activity-dependent formation of autophagosomes in dendrites of primary hippocampal neurons [15]. Similar findings suggesting distal formation of autophagosomes have been reported in *C. elegans* [107]. These data suggest that autophagosome formation occurs within pre- and post-synaptic compartments and

provide a means for retrograde transport of autophagic cargo from distal regions of the neuron to the cell body for degradation.

What specifies the location of autophagosome formation in neurons? Holzbaur and Maday proposed that PI3P synthesis occurs distally within axons, as DFCP1 puncta, which mark sites of PI3P synthesis and nascent autophagosome formation, were located distally in neuronal processes [106]. In *C. elegans*, Colon-Ramos and colleagues identified Atg9 trafficking events within the distal axon as being required for spatial organization of autophagosome formation [107]. Whether additional (neuron-specific) regulators are involved in orchestrating autophagosome formation and maturation within these highly complex cells remains to be seen.

The identification of autophagosomes and their regulated formation and trafficking, in neurons without ongoing pathology suggests that autophagy may play a role in the regulation of neurotransmission. To address this, transgenic mice lacking required autophagy genes could be used to identify changes in behavior and neurophysiology associated with loss of autophagy. Unfortunately, whole-body, constitutive knockout of many autophagy genes yield perinatal lethality [108]. Furthermore, the consequences of autophagy loss in the CNS could not be disambiguated from the effect of loss of autophagy in other organs.

Fundamental contributions to elucidating roles for autophagy in neurons were made by Hara, Komatsu and colleagues in the mid-2000's who addressed this issue by generating mice with conditional alleles of Atg5 or Atg7 that could be knocked out in genetically-defined cell types following expression of Cre recombinase [109,110]. CNS-specific knockout of Atg5 or Atg7 yielded similar age-dependent neurodegeneration, accumulation of cytoplasmic proteinaceous inclusions and motor dysfunction. These phenotypes were similar in both mouse lines, indicating that as in yeast, Atg5 and Atg7 function at similar steps in the mammalian CNS. These transgenic mouse lines have become critical tools that have enabled the identification of cell-type specific roles for autophagy in the CNS (see below).

IV. Autophagy and neurotransmission

Throughout the remainder of this review, we discuss the reciprocal roles of autophagic protein degradation in neurotransmission

1. Autophagy in synaptic development

As we and others have recently reviewed the role of autophagy in synaptic development, we limit our review of this topic to two particular studies specifically relevant to the role of autophagy in neurotransmission [111,112].

In *C. elegans*, clustering of postsynaptic $GABA_A$ receptors in muscle depends on presynaptic GABA release [113]. In the absence of presynaptic innervation by GABAergic terminals, $GABA_A$ receptors are diffusely localized on the plasma membrane of the muscle. Interestingly, however, the absence of both GABAergic and cholinergic inputs leads to endocytosis and degradation $GABA_A$ receptors via autophagy [16], while deletion of

autophagy in non-innervated muscles rescues deficits in neurotransmission. These data provide initial evidence that transmembrane postsynaptic receptors are degraded via autophagy, in an endocytosis-dependent manner. Notably, one family of mammalian Atg8 homologs, the GABARAPs, are critical for anterograde trafficking of $GABA_A$ receptors [114,115], suggesting that autophagy-associated proteins are critical regulators of biosynthesis and degradation of these receptors.

In addition to its role in degradation of neurotransmitter receptors during development, autophagy is also critical for the maturation of synaptic morphology. In *Drosophila*, developmental loss of autophagy decreases neuromuscular junction (NMJ) size, while enhancing neuronal autophagy by overexpression of Atg1, a homolog of ULK1, leads to an increased number of synaptic sites [116]. In contrast, however, autophagy is required for dendritic spine pruning during mouse cortical development [117] and plays a critical role in axon pathfinding in the developing CNS [118–120], indicating that autophagy has been adapted for a range of synaptic mechanisms.

Finally, the lysosomal GTPase Arl8 [121,122] contributes to the delivery of active zone and synaptic vesicle precursor proteins within non-degradative lysosome-like vesicle in *Drosophila*, *C. elegans* and in mammalian neurons [123–125]. Whether delivery of these components involves lysosomal degradation or intersects with autophagy remains to be seen.

Little is known how neuronal autophagy regulates synaptic structure. Autophagic degradation of neurotransmitter receptors or neurotransmitter release (see below) may contribute to activity-dependent synaptic development. Similarly, autophagic degradation of mitochondria or endoplasmic reticulum, organelles that regulate synapse development in part due to their position in neurites [126,127] may be critical for the establishment and maturation of synaptic contacts. Experiments in which the degradation of specific autophagic cargo are disrupted during CNS development will be required to distinguish between these mechanisms.

2. Autophagy and neurotransmitter release

Autophagosome formation at presynaptic terminals suggests that autophagy shapes neurotransmitter release. Our lab provided early evidence of a cell-autonomous role for autophagy in neurotransmitter release [128]. We took advantage of the fact that dopamine (DA) release can be directly measured using cyclic voltammetry, in contrast to the release of other neurotransmitters which depends on postsynaptic recordings of receptor activation, to unambiguously demonstrate that DA neurons lacking autophagy release more DA following electrical stimulation. This was a clear result of the role of autophagy *in axons*, as opposed to a role in cell bodies, as DA somata were not present in the brain slice preparation we used. Ultrastructural analysis of DA axons in the absence of autophagy revealed enlarged presynaptic terminals, and changes in the morphology and number of synaptic vesicles. These data implicated autophagy in the homeostasis of synaptic vesicles and neurotransmitter release machinery.

Recent work from Ackermann, Garner and colleagues further implicates autophagy in the clearance of damaged synaptic vesicles. Synaptic vesicles undergo rapid cycles of release

and reformation, and this has been hypothesized to lead to damage to synaptic vesicle associated proteins [129]. To address whether autophagy is involved in the clearance of synaptic vesicle proteins, Hoffman et al fused supernova, a protein which creates reactive oxygen species (ROS) in response to light [130], to synaptotagmin, synapsin or synaptophysin and demonstrated that ROS-induced damage leads to autophagosome formation within the activated presynaptic terminal and degradation of synaptic vesicle-associated proteins [131]. Activation of autophagy was required to counteract the damage caused by ROS on neurotransmitter release and synaptic vesicle endocytosis. These data suggest that autophagy contributes to synaptic homeostasis by degrading damaged synaptic vesicles on demand.

Does normal physiological activity also induce presynaptic autophagy? High-frequency stimulation of the *Drosophila* NMJ induces autophagosome formation within the presynaptic terminal, suggesting that autophagy may be required for the maintenance of presynaptic machinery during stimuli with elevated energy demands [132]. In combination, these data provide tantalizing evidence that autophagy is critical for the regulation of neurotransmitter release via a contribution to synaptic vesicle quality control.

Additional insights into the dynamics of presynaptic autophagy have arisen from experiments aimed at defining the role of presynaptic proteins in the control of autophagy. Synaptojanin is a lipid phosphatase that induces synaptic vesicle endocytosis via the dephosphorylation of $PI(4,5)P_2$. The SAC1 domain of synaptojanin has recently been implicated in the maturation of autophagosomes at the presynaptic terminal in *Drosophila* [133]. EndophilinA, another protein involved in synaptic vesicle endocytosis, has recently been found to promote presynaptic autophagy by recruiting autophagy-associated proteins to highly curved membranes [132]. Finally, the presynaptic proteins Bassoon and Piccolo suppress presynaptic autophagy by sequestering Atg5 [134]. In the absence of Bassoon and Piccolo, synaptic vesicle pools are depleted and synaptic sites are lost via activation of autophagy. These data suggest not only that autophagy plays important roles in presynaptic function, but also that presynaptic proteins contribute to autophagic function within the axon.

3. Autophagy and postsynaptic functio

Autophagy further controls synaptic transmission via postsynaptic mechanisms. Loss of autophagy in cortical pyramidal neurons or using nestin-cre to knockout autophagy throughout the nervous system leads to elevated levels of components of the postsynaptic density such as PSD-95, SHANK3, and PICK1 [102,117]. Furthermore, in a mouse model of Fragile X syndrome, elevations in PSD95 and the immediate early gene, Arc, can be rescued by activation of autophagy [135]. Whether these proteins are sequestered into nascent autophagosomes formed into the dendrite or their levels are affected by autophagy through an alternative mechanism remains unknown. The ability of autophagy to degrade postsynaptic scaffolding proteins provides a possible mechanism through which autophagy could control synapse morphology and the efficacy of synaptic transmission.

Autophagy also contributes to the degradation of neurotransmitter receptors. In addition to the degradation of $GABA_A$ receptors during *C. elegans* development [16], chemically-

induced long-term depression activates autophagic degradation of the AMPA receptor, GluR1 [15]. The mechanism by which autophagy contributes to GluR1 degradation and whether this is endocytosis-dependent, remains unknown.

4. Autophagy and synaptic plasticity

Changes in the strength of synaptic transmission, termed synaptic plasticity, are thought to represent a cellular correlate of learning and memory. Disrupted synaptic plasticity has been reported in mouse models lacking autophagy; however, much of the mechanism through which autophagy regulates synaptic plasticity remains unknown.

Long-term potentiation appears to be regulated by autophagy in some cases. Glatigny et al demonstrated that pharmacological inhibition of autophagy with Spautin-1 blocks theta burst stimulation-induced LTP in CA1 [136]. Nikoletopoulou and colleagues suggest that ongoing autophagy in the hippocampus is suppressed by brain-derived neurotrophic factor (BDNF) to permit LTP [102]. Autophagy may also play a role in long-term depression (LTD), as impaired autophagy has been implicated in exaggerated hippocampal mGluR-LTD in a mouse model of Fragile X syndrome [135].

Autophagy may contribute to synaptic plasticity in several ways. First, autophagy may actively degrade AMPA receptors to reduce synaptic strength during LTD [15]. Second, autophagy may degrade other synapse-associated proteins required for reorganization of the postsynaptic membrane during plasticity [102,117,135]. Autophagy may further regulate the levels of cytosolic calcium within the pre- or post-synaptic elements via degradation of mitochondria or endoplasmic reticulum [98,126]. It should also be noted that several kinases that regulate autophagic activity, including mTOR, Akt, and AMPK, are involved in synaptic plasticity [137,138], although whether these kinases act through autophagy to modulate synaptic plasticity remains a key question.

5. Autophagy contribution to behavior

The contribution of autophagy to brain function has also been explored through cell-type specific knockouts of autophagy genes.

a. Hypothalamus and energy homeostasis—The role of autophagy in hypothalamic control of food intake and energy homeostasis has been extensively studied. The arcuate nucleus of the hypothalamus (ARC) controls food intake in response to the status of an organism's energy store [103]. Two neuronal populations in the ARC, Agouti-related peptide (AgRP)-expressing (ARC^{AgRP}) and pre-opiomelanocortin (POMC)-expressing (ARC^{POMC}), oppositely control feeding [139–142]. ARC^{POMC} neurons release α-MSH, a cleavage product of POMC [143], which stimulates Melanocortin-4 Receptors (MC4R) on neurons in the paraventricular hypothalamus (PVH) [144–146], and other brain regions, to inhibit feeding. AgRP, which is also released in the PVH by ARC^{AgRP} neurons, is an antagonist of the MC4R and stimulates feeding [140,147]. Circulating hormones such as leptin, insulin and ghrelin, and extracellular levels of glucose and lipids alter ARC neuron firing to elicit feeding behavior or satiety [103]. These extrinsic cues activate intracellular biochemical cascades. In particular, mTOR and AMPK activity within ARC neurons control

firing rates and neuropeptide release to affect feeding in response to energy status [148–151]. Thus, neurons of the ARC represent a critical central node in energy homeostasis.

As described above, autophagy is tightly regulated by the coordinated activity of AMPK and mTOR. In contrast to most of the CNS, fasting induces autophagic activity in the hypothalamus [101,102]. To define the role of autophagy in ARC neurons and central control of energy homeostasis, Kaushik et al conditionally deleted the required autophagy protein Atg7 in ARC^{AgRP} neurons using the Cre-LoxP system [101]. Mice lacking Atg7 in ARC^{AgRP} neurons (which promote feeding) had lower body weight and fat mass relative to controls suggesting a deficit in ARC^{AgRP} neuron function as these neurons promote feeding. One mechanism through which ARC^{AgRP} neurons promote feeding in response to food deprivation is to increase the expression of AgRP mRNA. Kaushik et al reported that in mice lacking Atg7 in ARC^{AgRP} neurons, fasting failed to induce an increase in AgRP expression. They concluded that intact autophagy is required for the fasting-induced mobilization of free fatty acids (FFA) from lipid droplets, a process known as lipophagy [152], and that FFA signalling normally activates AgRP mRNA expression by ARC^{AgRP} neurons. These results elegantly demonstrate a cell-type specific role for autophagy in the central control of energy homeostasis.

In contrast to ARC^{AgRP} neurons, conditional deletion of Atg7 in ARC^{POMC} neurons leads to increased body weight. Bouret and colleagues reported that this effect correlated with decreased axonal arborization of ARC^{POMC} neurons and innervation of target regions such as the PVH [153]. Both the Bouret and Lee groups demonstrated that autophagy was required in ARC^{POMC} neurons for a normal response to leptin: systemic or intracerebral administration of leptin did not suppress feeding in mice lacking autophagy in ARC^{POMC} neurons [153,154]. This lack of appetite suppression was associated with increased circulating leptin levels, further suggesting a state of relative leptin resistance. The precise mechanism, however, through which autophagy regulates ARC^{POMC} axonal outgrowth or biochemical response to leptin remains obscure. Furthermore, autophagy also contributes to the cellular response to neuropeptide Y, another key contributor of feeding within the hypothalamus [155].

AMPK also mediates fasting-induced synaptic plasticity within the ARC [156]. Whether changes in AMPK activity affect autophagic flux within the ARC and whether autophagy contributes to fasting-induced and AMPK-dependent synaptic plasticity in the ARC remains unknown. Although some AMPK targets, such as p21-associated kinase (PAK), are implicated in fasting-induced synaptic plasticity, it is not clear if PAK modulates autophagy in the ARC [156]. While lipophagy is implicated in feeding behavior, identification of additional potentially relevant autophagic targets required for fasting-induced plasticity, such as neurotransmitter receptors or mitochondria, requires further investigation.

b. Hippocampus and spatial memory—Recent reports highlight a role for autophagy in hippocampus-dependent behavioral tasks. Glatigny et al demonstrated that knockdown of the required autophagy proteins Beclin-1, FIP200, and Atg12 in the hippocampus reduced novel object recognition and contextual fear conditioning [136]. This effect was similar when the shRNA was expressed under the control of a neuron-specific promoter or a general

promoter suggesting that loss of *neuronal* autophagy led to these behavioral phenotypes. This group then used pharmacological tools to acutely manipulate autophagy and found that autophagy inhibition in the hippocampus, using Spautin-1, during but not after, the training phase of the contextual fear conditioning task reduced freezing during a probe trial while stimulation of autophagy during, but not after the training phase, increased freezing during the probe trial. These results suggest that hippocampal autophagy is required for the formation of contextual and object recognition memories.

A decrease in hippocampal autophagy also mediates hippocampal dysfunction in a mouse model of Fragile X syndrome (FXS). Suzanne Zukin and colleagues found reduced autophagy in hippocampal neurons lacking Fmr1, a model for FXS [135]. Reduction of mTOR signaling in hippocampal region CA1 rescued novel object recognition in FXS mice and this was dependent on the required autophagy protein Atg7. Thus, at least a subset of behavioral phenotypes in FXS mice arise from a deficit in hippocampal autophagy.

V. Speculation and future directions

1. Basal vs induced autophagy—One key remaining question in the study of neuronal autophagy is the distinction between ongoing basal autophagy and autophagy induced by extrinsic factors including cellular stress or synaptic input. Deletion of the required autophagy proteins Atg5 and Atg7 throughout the CNS leads to widespread changes in behavior, formation of cellular inclusions and eventual neurodegeneration [17,18]. These reports, along with the observation of relatively high basal levels of autophagosome biogenesis in neuronal cultures, supports a model in which ongoing autophagic activity is required for normal neuronal homeostasis and survival in the CNS [99,106]. This hypothesis seems particularly attractive because neurons are post-mitotic and unable to dilute toxic or damaged proteins and organelles by cell division, and may contribute to why autophagic dysfunction is found in many neurodegenerative diseases [157]. In contrast, however, recent findings that autophagic degradation can be induced on rapid (minute) timescales [131] and by neuronal activity [15] suggest that autophagy may in some cases provide a permissive or downstream role in the control of neuronal plasticity.

Defining the relative importance of basal autophagy versus activity-dependent inducible autophagy for neuronal function remains an important open issue. Furthermore, whether the targets of autophagic degradation are altered during induced autophagy, or whether the rate of autophagy alone changes is unknown. These questions are presently challenging to address, as cell-type specific manipulation of autophagic activity generally necessitates genetic approaches that occur over long timescales and lead to compensatory responses in vesicle trafficking pathways that may obscure the responses to autophagy disruption. Furthermore, while rapid pharmacological manipulation of autophagic responses can be accomplished with newly developed compounds, many of these target kinases upstream of autophagy, such as Vps34 and ULK1, that have pleiotropic effects on cellular pathways. The advent of temporally specific, genetically encoded regulators of autophagic activity will more easily permit dissection of basal versus inducible autophagy in the CNS.

2. **Substrate specificity—**While early studies of autophagy were often interpreted to demonstrate that cytosolic macromolecules were non-selectively sequestered into autophagic vacuoles [54–56], more recent work has found that specific proteins or damaged organelles can be selectively degraded by autophagy [53]. In neurons, this specificity may be particularly important as the requirements for synaptic plasticity are distinct between brain regions and across developmental stage. It seems likely that autophagy may selectively degrade a subset of proteins within particular neuronal populations or during specific developmental stages. Such specificity could be achieved through: 1) selective expression of adapter proteins that only sequester a subset of possible autophagic cargo, 2) regulated post-translational modification of autophagic cargo which directs substrates for autophagy only under specific conditions, 3) a yet-to-be described recycling pathway that removes sequestered cargo from mature autophagosomes prior to fusion with the lysosome. Identifying the list of autophagic substrates in specific cell types or during specific developmental time points could be achieved using either genetic or biochemical approaches. Eliminating autophagy with temporal specificity in distinct cell types would lead to the accumulation of cell-type specific autophagy substrates. Alternatively, new proteomics tools that label autophagosome associated proteins in distinct cell-types could be used to define autophagic cargo biochemically [158]. These approaches will provide insight into the dynamics of autophagic degradation within neurons and how autophagy contributes to synaptic function.

3. **Intersection with endolysosomal system—**Finally, it is critical to elucidate the relationship between the endosomal system and autophagy within neurons. These two cellular pathways are deeply interconnected in terms of their molecular regulation [79,159,160] and because late endosomes and mature autophagosomes fuse prior to fusion with lysosomes and cargo degradation [161–167]. Interestingly, early steps in both autophagy and endocytosis are both regulated by Vps34 [159], endophilin [132] and synaptojanin [133], suggesting that these shared regulators may coordinate a balance between these two pathways. Second, Atg9, a transmembrane protein involved in early autophagosome biogenesis, is moreover present on endocytic vesicles that fuse with nascent autophagic membranes, suggesting that endosomes potentially act as a membrane source to growing autophagosomes [40,42,52]. Later steps in the maturation of both endosomes and autophagosomes depend on the same proteins including Rab7 [160,168,169] and the same proteins have been found to be degraded by the endolysosomal system *and* autophagy (see [15]).

The connection between autophagy and endocytosis presents experimental and conceptual challenges. First, many experimental perturbations that affect autophagy may either also directly disrupt endocytosis, such as PI3K inhibition [30–32], or cause compensatory changes in the endolysosomal pathway and be responsible for the observed phenotypes. Combined pharmacological and genetic manipulations, genetic complementation and convergent phenotypes from distinct manipulations of each pathway would strengthen conclusions that one pathway is directly involved in the process of interest.

Second, implicating autophagy *per se* in the degradation of membrane proteins is difficult. As a key class of autophagy substrates that can be degraded by autophagy to control

neurotransmission [15,16], identifying the precise mechanism of their degradation by autophagy represents a key conceptual question. For example, while Shehata et al demonstrated that GluR1 degradation during chemical long-term depression (chem-LTD) depends on expression of the required autophagy protein Atg7 [15]. GluR1 degradation during chem-LTD depends on endocytosis and endolysosomal trafficking/degradation [15,170–172]. Whether GluR1 truly enters *bona fide* autophagosomes or whether Atg7 plays a distinct role in the endolysosomal degradation of GluR1 during chem-LTD remains unknown. This participation of both pathways is a strong possibility considering that endosomal fusion with autophagosomes to form *amphisomes* may provide a key final step in the degradation of endosomal contents. In contrast, $GABA_A$ receptor degradation at the NMJ in *C. elegans* depends on autophagy and these receptors are observed in LC3-positive vesicles [16]. Key experiments defining the localization putative autophagic substrates would enhance our understanding of the mechanisms of autophagy at synapses.

VI. Conclusion

Here, we have reviewed the accumulating evidence that autophagic protein degradation plays an important role in both disease-associated and non-pathogenic states within the CNS and contributes to synaptic transmission, plasticity and behavior. The reports that autophagy contributes to presynaptic homeostasis and postsynaptic function suggest that neurons have coopted an evolutionarily conserved stress response pathway to meet its needs in both synaptic compartments. We look forward to a deeper understanding of the mechanism through which autophagy acts at the synapse and contributes to synaptic function that we are sure will soon emerge.

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Highlights

- The molecular machinery of autophagy is evolutionarily conserved from yeast to mammals.
- Neuronal autophagy occurs in the pre- and post-synaptic compartments.
- Autophagy controls neurotransmitter release, receptor trafficking, and synaptic plasticity.
- Neurons have coopted a ubiquitous cellular stress response pathway to regulate neurotransmission.

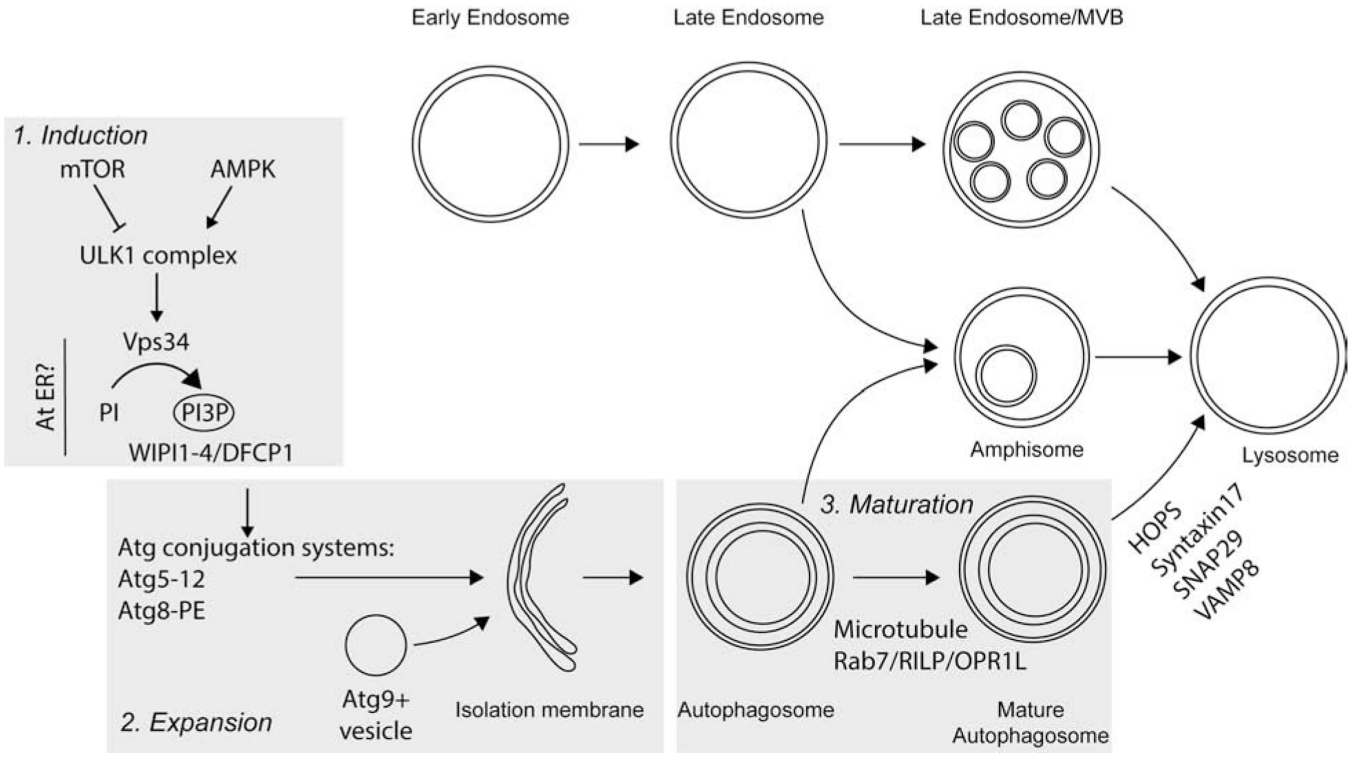

Figure 1. Schematic representation of the mechanisms of autophagy.

Autophagy induction is driven by mTOR and AMPK. These metabolic kinases stimulate PI3P synthesis which recruits the PI3P binding proteins DFCP1 and WIPI1-4 to a membrane source. The activity of the Atg conjugations systems and Atg9+ vesicles expand the autophagic membrane. Once the membrane closes, the autophagosome matures, traffics to the perinuclear area and fuses with lysosomes. Late endosomes can fuse with closed autophagosomes to form amphisomes. Cargo is not depicted for simplicity.

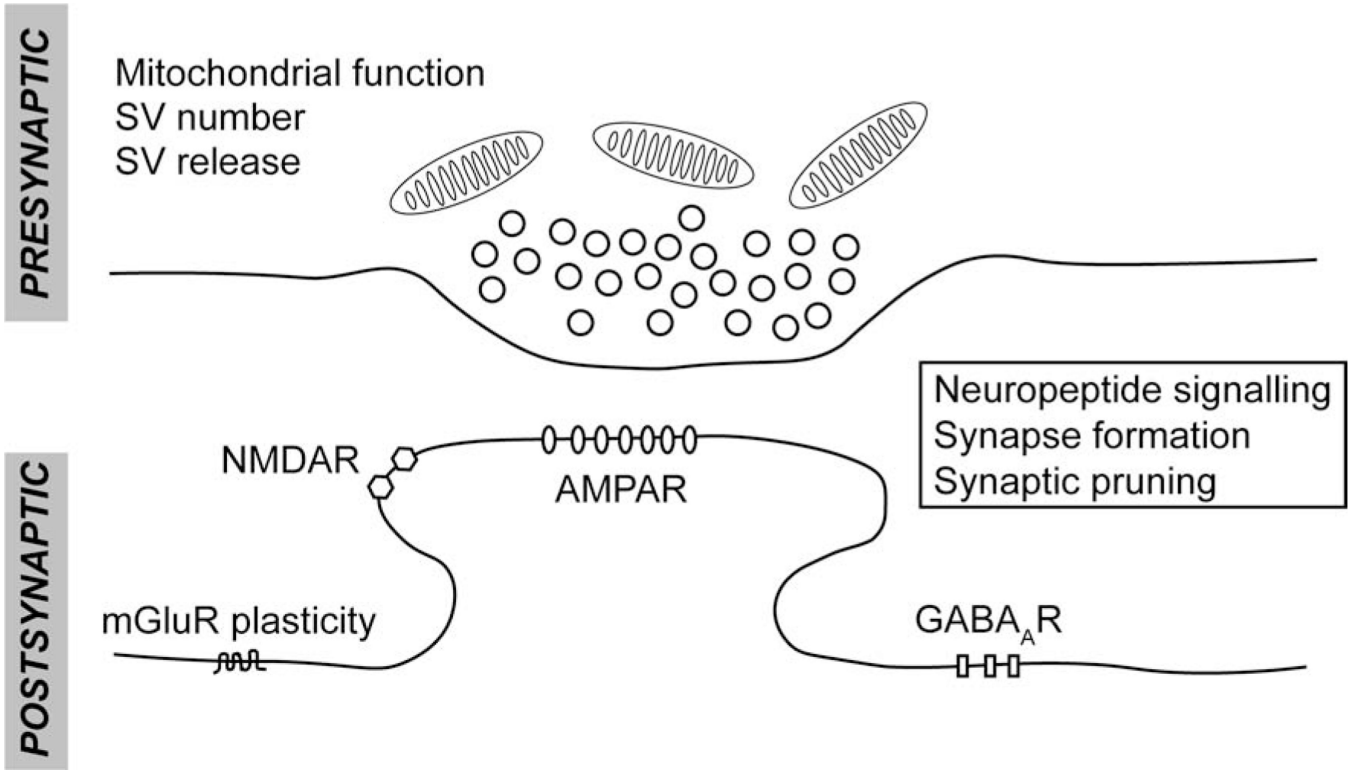

Figure 2. Aspect of neurotransmission that are regulated by autophagy.

Autophagy can control neurotransmission both pre- and postsynaptically. In the presynapse, autophagy controls synaptic vesicle (SV) homeostasis and release as well as mitochondrial function. Postsynaptically, autophagy controls excitatory neurotransmission by degrading AMPA receptors and $GABA_A$ receptors. The absence of autophagy disrupts synaptic plasticity that is dependent on metabotropic glutamate receptors. Finally, autophagy modulates neuropeptide signaling, synapse formation and synaptic pruning; the locus of action is unknown in these cases.