Lysosome dynamics in neurons, maintaining cellular homeostasis

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Abstract

Lysosomes are membrane-enclosed organelles found in nearly all eukaryotic cells. They are characterised by a highly acidic lumen which contains hydrolases. They are mainly responsible for the degradation of macromolecules and for providing cellular building blocks in times of stress using the autophagy or endolysosomal pathways. However, the function of lysosomes extends beyond its function in degradation, several novel functions have recently been ascribed to lysosomes. A lot of research in lysosome dynamics has been done in non-polarized cells. The dynamics of lysosomes is less established in polarized cells, such as neurons. Neurons are highly compartmentalized and pose spatially extended neuronal cytoplasm. The dependence of neurons on optimal lysosomal function is especially important since lysosome disfunction is linked to several neurodegenerative diseases. In this review, we describe the roles played by lysosomes in neurons and how they maintain cellular homeostasis. We will focus on transport and regulation of lysosomes and how this is special for highly polarized cells. Furthermore, we discuss non-degradative functions of lysosomes in neurons. And finally, how lysosome disfunction contributes to neurodegenerative diseases.

Chapter 1: Introduction

Lysosomes are membrane bound organelles present in eukaryotic cells. Lysosomes were first discovered by de Duve in the mid-20th century using centrifugal fractionation [1,2]. A few years later, lysosomes were recognised as the main site for degradation [2]. Lysosomes were shown to have proton-pumping ATPases, which maintain the luminal environment at a pH of 4.6 – 5.0 [2]. Lysosomes contain acid hydrolases which are activated at low pH to degrade a broad range of macromolecules. This activity allows cells to clear unwanted material and provide nutrients in times of stress. Two major pathways in non-polarised cells have been recognised in which lysosomes can degrade materials. Autophagy delivers cytoplasmic material and organelles to lysosomes for degradation [3–5]. In parallel, endocytosis delivers material taken up from outside the cell through early and late endosomes to lysosomes [6]. The delivery of material to lysosomes is mediated by fusion with autophagosomes, phagosomes or endosomes which creates hybrid organelles in which the cargo is degraded [7]. Once substrates are digested within lysosomes, the degraded material is exported into the cytoplasm and used either as energy source or as building blocks.

The function of lysosomes is not restricted to degradation of macromolecules. Lysosomes have an important role in secretion and plasma membrane repair by exocytosis [8]. Additionally, lysosomes act as regulatory hubs for cellular and organismal homeostasis. Lysosome-mediated signalling pathways and transcription programmes are able to sense the status of cellular metabolism and control cellular homeostasis [9]. Is has been realized that lysosomes can also engage with other membrane-bound organelles, such as ER, Golgi complex and mitochondria. These interactions involve the formation of membrane contact sites with important functional consequences and thought to allow non-vesicular transport of lipids and calcium[10,11].

Little is known about the dynamics and function of lysosomes in neurons. Neurons are highly polarized and have a specialized organelle composition in the soma, dendrites and axon which adds another layer of complexity. Vesicles undergo long-distance transport from the soma to the distal regions which can span up to 1 meter long in human neurons. In this review, we will discuss lysosome distribution, lysosome definition in neurons. Furthermore, lysosome transport and regulation in the different compartments will be discussed. Additionally, we will discuss interesting non-degradative functions of lysosomes. Genes regulating lysosomal function are implicated in lysosome storage diseases and in common neurodegenerative diseases, such as Alzheimer's disease (AD, Parkinson's disease (PD) or amyotrophic lateral sclerosis (ALS)[12]. This points out the importance of optimal

lysosome function for maintaining neuron health and raises fundamental questions about how the lysosomal system is adapted to the unique features of neurons.

Chapter 2: Lysosome distribution and definition

Lysosome distribution throughout the neurons is more complex than in non-polarized cells, because neurons consist of multiple compartments, each with a different morphology. Furthermore, there is not yet a conclusive definition of mature lysosomes, since many different markers have been used to identify lysosomes.

2.1. Location and maturation of lysosomes in neurons

Lysosomes are broadly distributed throughout the cytoplasm in non-polarised cells, where they are most concentrated in a central region surrounding the microtubule-organizing centre (MTOC) nearby the nucleus, but have also been found near the periphery of the cell[13]. Also, in polarized cells such as neurons, lysosomes have been reported to be present in all regions of the cytoplasm to play essential roles. The localized nature of neuronal functions require lysosomes to be precisely distributed. Lysosomes were initially thought to have a polarized distribution in neurons, wherein they are predominantly present in the soma [14]. Lysosomes have also been observed in dendrites[15] and have been observed to be relatively rare in axons [16,17]. More recently, lysosomes were found to have a more non-polarized distribution in neurons. Lysosomes were still most concentrated in the soma, but were also present along dendrites and axons, demonstrating the presence of lysosomes in all neuronal domains [18,19]. The soma is likely the most efficient site for bulk degradation of materials, since most processes requiring biosynthetic building blocks take place here.

Lysosomes are differentially distributed throughout the neurons, creating a gradient of degradative activity[20–22]. Early endosomes (EEs) provide the starting point for late endosomes (LEs) maturation and is the main sorting centre for endocytic cargos[23]. Markers commonly used to identify EEs are the small GTPase Rab5 and the rab5 effector protein early endosome antigen 1 (EEA1). The small GTPases Rab5 and Rab7 regulate maturation of endosomes[24]. Interestingly, early work showed that EEs labelled with EEA1 are present in the dendrites and soma, but absent in axons [25,26]. This may suggest that EEs are differentially regulated in dendrites and in axons.

The more mature organelles are enriched near the soma. These organelles are mature versions of LEs and contain lysosomal hydrolases. Rab7 is required for maturation from EEs to LEs and mediate transport towards the soma. In dendrites, the gradient is steeper in thinner dendrites, where mature lysosomes are rarely present [21]. A degradative gradient was also observed for autophagosomes which mature into autolysosomes when transported from distal axons towards the soma, where they fuse with LEs[27]. Once in the soma, autophagosomes are restricted to the somatodendritic domain and are unable to enter the axon again, but are able to enter dendrites [28]. Transport of autophagosomes in dendrites is however bidirectional and stationary[29]. Like EEs, these results suggest a compartment-specific regulation of autophagosomes.

2.2. Identification of lysosomes

Functionally mature lysosomes are classically defined based on four features, namely, a high degree of acidification (pH 4.5—5), high levels of lysosomal membrane proteins (LMPs) and lysosomal hydrolases, and a lack of mannose 6-phosphate receptors (MPRs)[30]. Specific labelling of degradative lysosomes is critical in understanding lysosome distribution, trafficking and functionality. There has been a lot of discussion in recent years on the identity of lysosomes. Many different markers have been used to identify lysosomes and therefore there is not yet a conclusive definition of lysosomes. The most abundant LMPs are lysosome-associated membrane proteins (LAMPs). LAMPs are the most routinely used markers for identifying lysosomes. Lamp-1 labelled lysosomes were observed in axons of rat hippocampal neurons[18]. While lysosomes uniformly express LAMP-1, these proteins are not static components of the lysosomal membrane. Expression of LAMP-1 is in equilibrium between lysosomes, endosomes and the plasma membrane[31] . LAMP1-labelled organelles can also be hybrid

organelles representing intermediates of endocytic and autophagic pathways[16,19,20]. Furthermore, lysosomes undergo reformation, suggesting that there is a lot of exchange between different endocytic compartments.

Other routinely used markers are LysoTracker probes. The probes track organelles based on their acidity, thus enzymatically active lysosomes. Using this probe, mature lysosomes were detected in dendritic spines. Inhibiting lysosomal proteolytic function altered lysosome movement and reduced spine intensity[19,22]. However, many of the reported lysotrackers are unspecific, because they may suffer from fluorescent quenching and low pH sensitivity, which also labels LEs.

Lysosomal enzymes, such as cathepsins and β-Glucocerebrosidase (GCase), are often used as markers to identify mature degradative lysosomes. It has been observed that lysosomes labelled with cathepsin B, cathepsin D and GCase were enriched in the soma, but also positioned along axons and abundant in distal tips [32]. In this study, they used microfluidic devices that physically separate axons from cell bodies and dendrites which allows better visualisation of lysosomes distributed in distal axons[32]. These results are consistent with the results obtained by using LAMP-1 as a marker as described previously [18]. The use of the marker LAMP-1 has been evaluated further by combining the markers LAMP-1 and lysosomal hydrolases. It has been observed that a significant population of LAMP-1 labelled organelles did not colocalize with organelles containing the hydrolases in axons[20,21]. Also in dendrites and the soma, LAMP-1 positive organelles partially colocalized with cathepsin-labelled organelles. Whereas all cathepsin-positive organelles contained LAMP-1[21]. Reference to LAMP1-positive organelles alone could thus lead to a misinterpretation of degradative lysosomes. Furthermore, over 80% of the LAMP-1 positive vesicles observed in axons were positive for TGN markers, that are normally not present on lysosomes. These compartments also appeared tubular and were mildly acidic, therefore they were identified as transport carriers (TC). Due to the use of many different markers, there is large disagreement in where the lysosomes are located in neurons. It makes sense to classify LAMP-1 positive, degradative compartments as lysosomes. Whether these compartments are lysosomes or transport carriers, additional markers are needed such as the TGN marker.

To make lysosome labelling more specific, other types of markers have been engineered for lysosome tracking. For example, lysosome targeting fluorescent derivatives called NIMCs [33] or nanodisc-based particles, namely dye entrapped silica nanoparticles (DSiNPs)[34]. These makers were shown to exclusively label lysosomes and suitable for long term imaging in non-polarized cells and may be good candidates for lysosome markers in neurons as well.

Chapter 3: Lysosomes dynamics

Digestion of cytoplasmic material is delivered to lysosomes via the autophagy pathway. The pathway of autophagy is activated during stress conditions and contains several routes; macroautophagy, microautophagy and chaperone-mediated autophagy[36]. Furthermore, organelle-selective autophagy has also been reported. Autophagosomes fuse with LEs (amphisomes) or mutivesicular bodies (MVBs) and finally with lysosomes (autolysosomes) [37–39]. Autophagosomes are generated at the subdomains of the ER in the axon terminals [27]. Initiation of dynein mediated microtubule depend transport brings the autophagosomes towards the soma [40] where they mature by fusion with lysosomes[41].

Digestion of material taken up from outside the cell is processed using the endolysosomal system. Extracellular materials internalized by endocytosis reach endolysosomes through highly specialized trafficking from EEs to LEs and finally to mature lysosomes. The endolysosomal system is extremely dynamic and highly organized. This system contains various membrane bound organelles, including EEs, LEs and lysosomes. The small GTPases Rab5 and Rab7 are key determinant of EEs and LEs. Cargo progression between early and late endosomes is mediated by the conversion of Rab5 to Rab7 [42]. LEs are competent to fuse with lysosomes by direct fusion or kiss and linger [43]. Endolysosomal organelles and autophagosomes move between the centre and the periphery of the

cell along microtubule tracks [44]. In non-polarized cells, microtubules are radially distributed anchored with their minus-ends at a defined MTOC and their plus- ends near the periphery. Polarized cells, such as neurons, lack a centrosome dependent MTOC and have a more complex microtubule organization. Axonal microtubules are uniformly oriented with their plus-ends positioned away from the cell body, whereas dendritic microtubules have a mixed conformation[45]. Long distance transport along microtubules is driven by motor proteins. Kinesin motors mediate anterograde (outward) transport, whereas dynein motor motors mediate retrograde (inward) transport [46]. However, because dendritic microtubules exhibit mixed polarity, kinesin and dynein motors may drive lysosome movement in either direction in dendrites. Adaptor proteins mediate coupling of organelles to motor proteins and regulate compartment specific transport.

3.2. Retrogade transport of lysosomes

The first observation of retrograde transport of lysosomes in neurons was demonstrated by measuring the pH of axonal endocytic cargos showing the movement of acidic organelles from the distal to the proximal site of the axon[40]. Later, it was reported that endocytic substances moved through EEs and LEs along retrograde transport towards the cell body. Rab5 and Rab7 act in a sequential manner in controlling axonal retrograde transport in neurons[47]. Dynein is the motor protein that drives endolysosomal transport towards the soma. Dynactin interacts with dynein and is required for linking cytoplasmic dynein to endolysosomes[48]. Dynein recruitment to endolysosomes is mediated by several mechanisms.

The adaptor protein snapin is reported to have a role in attaching dynein to LEs/lysosomes. Snapin is a subunit of biogenesis of lysosome-related organelles complex-1 (BLOC) and was first identified as a neuronal SNARE-binding protein[49]. Later, it was observed that snapin deficient neurons displayed accumulation of late endocytic organelles and impairs lysosomal function, suggesting that Snapin is crucial for late endosomal and lysosomal trafficking [50]. Snapin acts as an adaptor linking LEs to dynein motor proteins and plays a crucial role in dynein-driven retrograde transport of LEs and maturation. Snapin-dynein mediated transport is essential for delivery of late endosomal cargo from distal processes in the neuron to the soma where mature lysosomes are located.

Another important regulator of lysosome retrograde transport is JNK-interacting protein 3 (JIP3). Knockout of JIP3 in mice neurons resulted in accumulation of lysosomes and caused axonal swelling. These swellings are a major but poorly understood feature of Alzheimer's disease amyloid plaques. These results established the critical role of JIP3-dependent axonal lysosome transport [51]. Lysosomes accumulations were also observed in studies using orthologues of mammalian JIP3 deficient Caenorhabditis *elegans* and zebrafish neurons [52,53], and in human iPSC-derived neurons, suggesting JIP3 is evolutionary conserved. Furthermore, knockout of *JIP4*, resulted in enhanced accumulation of lysosomes, revealing that JIP4 has an overlapping function with JIP3 in regulating retrograde lysosomal transport in neurons[54].

An additional mechanism for dynein recruitment to LEs and lysosomes involves Rab7 and its effector Rab7-interacting lysosomal protein (RILP) [55]. The effectors of the Rab7 GTPase play multiple roles in the endosome-lysosome and autophagy-lysosome pathways. Rab7 is a GTPase that defines the identity of LE/lysosomal compartments. The Rab7 effector WDR91 promotes autophagy-lysosome degradation in neurons. While loss of WDR91 led to uncontrolled fusion between lysosomes and endosomes, the fusion of lysosomes with autophagosomes was not affected [56]. Expression of truncated RILP inhibits degradation and disperses lysosomes. Overexpression of the full-length protein increases perinuclear aggregation of LEs and lysosomes. Thus, RILP expression induces the recruitment of functional dynein-dynactin motor complexes to Rab7-containing LEs and lysosomes which are then transported to the minus-end of microtubules[57]. In non-neuronal cells, the

Rab7-RILP-dynein-dynacting complex associates with a cholesterol sensor named ORP1L [58], which means RILP dependent retrograde transport of LEs and lysosomes is regulated by cholesterol levels (next chapter, Fig. 1B).

3.3. Anterograde transport

Anterograde lysosomes transport is mediated by kinesin motor proteins. 45 genes that encode for the kinesin superfamily proteins (KIFs) have been identified in the mouse and human genome. KIFs are important molecular motors that transport cargos to the plus-end of microtubules[59].
Coupling of lysosomes to kinesin motor proteins is mediated by the multisubunit complex BLOC-1-related complex (BORC). BORC associated with the lysosomal membrane where it functions to recruit the small GTPase Arl8 [60,61]. Arl8 binds to the soluble protein SKIP, a protein which has been shown to bind to the kinesin-1 light chain in *Salmonella enterica*[62]. This promotes movement of the kinesin-1 motor towards the plus-ends of the microtubule in the peripheral cytoplasm.

In neurons, shRNA-mediated knockdown of the different components of the BORC-Arl8-SKIP-kinesin-1 machinery resulted in reduced presence of lysosomes in axons, but not in dendrites. Suggesting that this ensemble also plays a critical role in lysosome transport in neurons, specifically in the axons [18]. Members of the kinesin-3 family have also been observed to couple lysosomes to microtubules. Arl8A interacts with kinesin-3 which promotes plus-end microtubule to the periphery of the neurons[63].

Additionally, protrudin has been shown to mediate coupling between kinesin and lysosomes. Protrudin is an ER membrane protein that is involved in ER-lysosome contact sites. At these contact sites, kinesin-1 is transferred from protrudin to endolysosomal organelles via Rab7 and its effector FYCO1[64]. It has been observed recently that mitochondria can also be recruited by protrudin to form a three-way contact site together with the ER and lysosomes [65]. The functional role of these contact sites is however not fully understood, but may suggest a mechanism for intraorganellar communication.

JIP3, which has previously been shown to interact with dynein for retrograde transport, also interacts with the C-terminal tail of kinesin heavy chain[66]. This may suggest that JIP3 is important for coordination of bidirectional transport of lysosomes[67]. Future studies are needed to elucidate the underlying mechanism in regulating bidirectional transport of lysosomes.

3.4. Lysosome transport in dendrites

Transport of lysosomes in dendrites is poorly understood because they exhibit a 50:50 mixed polarity of microtubules. This means that neither dynein, not kinesin are solely responsible for retrograde and anterograde transport. Evidence has been presented that dynein is present as both a retrograde as an anterograde motor in dendrites[68]. Furthermore, it was demonstrated that maturing endosomes transport to-be-degraded cargo to the soma using Rab7-dependent retrograde transport[21]. However, in axons, Rab7-compartments move solely retrograde towards the soma, whereas in dendrites Rab7 compartments move bidirectionally. Which motors and which scaffold proteins are involved the motility of lysosomes in dendrites and how the underlying pathways are in their regulation remain exciting questions for future research.

3.5. Are lysosomes transported at all?

There has been a lot of discussion in recent years on the identity of lysosomes. As discussed in the previous chapter, many different markers have been used to identify lysosomes and therefore there is not yet a conclusive definition of lysosomes. Several studies have reported bidirectional transport op lysosomes in axons[18,21,32,69]. LAMP-1 positive compartments both encompass degradative and non-degradative organelles, but work from some labs have showed that degradative lysosomes can

be transported into the axon tips using markers against degradative sensors such as GCase or active cathepsin[32]. These studies have clarified the criteria which may be used to characterize mature lysosomes, which can be defined as acidic LAMP-1 positive organelles containing active hydrolases. These studies provides evidence that mature lysosomes are indeed transported.

In contrast, Recently a paper described that classic lysosomes are restricted from axons in intact brains, therefore not lysosomes, but transport carriers (TCs) from the TGN deliver lysosomal components to axonal organelles[70]. They did provide evidence for transport of degradative LAMP-1 vesicles (marked by cathepsin D). However, these structures were either non-motile or restricted to retrograde transport with only a few anterograde outliers. The difference between these observation might be caused by the use of different cathepsins as markers. For example, cathepsin D is most stable by a pH of 3.5-4.0[71], whereas cathepsin B has an optimum pH of 4.5 – 5.5[72]. Since mature lysosomes are more acidic than other acidic compartments, such as LEs, there might also be variety in the activation of cathepsins between organelles, thus a variety in visualisation of these markers. Rigorous criteria is therefore needed to distinguish between fully mature lysosomes and their precursors, such as LEs, and golgi-derived vesicles.

Chapter 4: Lysosomes Regulation

Lysosomes carry out local protein degradation to supply local environments with the necessary building blocks. We previously discussed the different transport machineries responsible for retrograde and anterograde transport of lysosomes in neurons. Next, we will discuss how lysosomes positioning and transport is regulated.

4.1. ER-lysosome contacts regulate lysosome positioning

The endoplasmic reticulum (ER) is one of the largest organelles in the cell and forms extensive contacts with other organelles, including LEs and lysosomes. The ER is highly dynamic and undergoes fast remodelling in a matter of seconds. The ER is a network of tubules and cisternae. In neurons, the ER cisternae are restricted to the soma and the ER tubules are distributed along the dendritic and axonal domains[74]. In non-polarized cells, ER-lysosomal contacts have roles in cholesterol exchange and endosome positioning[75]. Furthermore, collision of lysosome carried along microtubules split ER tubules [76]. For a long time, it was unclear how ER organization regulates lysosomes and how this is linked to motor transfer and microtubule interactions at contact sites in neurons. Recently, it was demonstrated that knockdown of ER tubule-shaping proteins caused drastic reduction of lysosomes in the axon, but not in the soma. Disruption of somatic ER tubules caused accumulation of enlarged and less motile mature lysosomes in the soma, due to drastic reduction of fission events. ER shape thus regulates lysosome size and availability in the axon and somatic ER tubules promote lysosome translocation into the axon [69]. The same study showed that ER-lysosome contacts are enriched in the a pre-axonal region together with the microtubule- and kinesin-1-binding ER protein P180. Suggesting that this is the location where lysosome motility, fission and axonal translocation is promoted [69].

4.2. Lysosomes regulation by sensing the metabolic environment

As discussed earlier, protrudin is an integral ER membrane protein that interacts with the GTPase Rab7 and transfers kinesin-1 to the lysosomes to mediate anterograde transport. Anterogade transport of lysosome can be regulated by alterations in the metabolic environment. For example, the enzyme carnitine palmitoyltransferase 1C (CPT1C) regulates lysosome abundance at axon terminals depending on the nutrient status of the cell. Under sufficient nutrient conditions, sensing of the fatty acid precursor malonyl CoA by CPT1C enhances protrudin transfer of kinesin-1 to LEs,

thereby promoting lysosome redistribution to the cell periphery to support axon growth. However, under metabolic stress, this process is prevented[77].

On the other hand, high endosomal cholesterol levels promote minus-end transport of lysosomes. High cholesterol concentration in the lysosomal membrane is a hallmark for lysosomal storage diseases with characteristic LE clustering at the minus end of microtubules. Under low-endosomal cholesterol levels, the lysosomal membrane protein ORP1L changes its conformation and induces the formation of ER-lysosome contact sites via interaction of the ER protein VAP. VAP subsequently interacts with the Rab7-RILP receptor.[78]. Together, these regulated transport mechanisms allow neurons to adapt to changing cellular conditions by effectively trafficking and positioning lysosomes throughout the cell.

4.3. AP-4 regulates lysosome composition and transport

The adaptor protein complex-4 (AP-4) is a member of the adaptor proteins family which are involved in sorting and trafficking of cargo in cells[83]. AP-4 has been shown to colocalise with the TGN, suggesting AP-4 is involved in sorting at the TGN[84]. In neurons, depletion of AP-4 caused strong retention of the protein ATG9A, which is involved in autophagy, leading to defects in autophagosome maturation and accumulation of axonal aggregates[85]. Recently, a study found that the composition of lysosomes in AP-4 depleted human iPSC-derived neurons was altered. Upon loss of AP-4, the distribution of the protein Sortilin 1, which is involved in cargo transport between the TGN and lysosomes, was retained in the TGN and cathepsin L delivery to lysosomes was reduced [86].

Furthermore, loss of AP-4 caused axonal swellings that were enriched in acidic lysosomal marked by LAMP-1 and lysotracker, suggesting that the retrograde transport is altered upon loss of AP-4. The distribution levels of the adaptor protein JIP3 were also evaluated in AP-4 depleted neurons. In these neurons, multiple JIP3-positive axonal swellings were observed compared to control neurons [86]. AP-4 is thus an important regulator in the composition and transport of (endo)lysosomes.

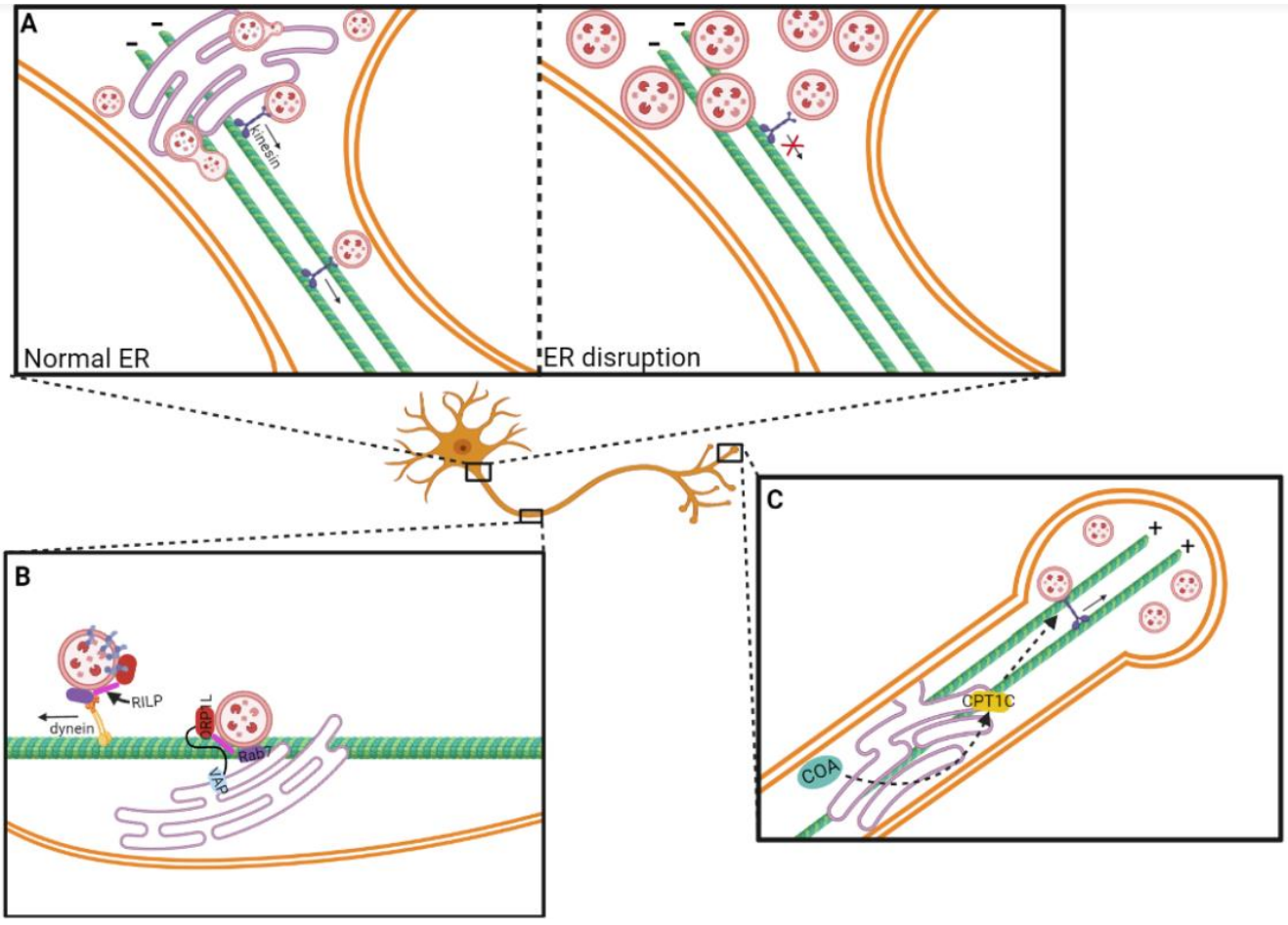

Fig. 1. **Regulation of lysosomes in neurons**
(A) Schema shows the distribution of neuronal lysosomes in presence of the ER (left) and disruption of the ER (right). The ER regulates lysosomal transport using kinesin into the axon along microtubules and the size of the lysosomes by fission. When the ER is disrupted, transport into the axon is prevented and enlarged lysosomes are restricted to the soma. (B) The amount of cholesterol regulates transport of lysosomes using dynein. The protein ORP1L (red) senses cholesterol concentrations in the lysosomes. High cholesterol concentrations mediate protein transport using dynein. Low cholesterol concentrations induce lysosome-ER contact sites via the protein VAP (blue) and transport is prevented. (C) sensing of the fatty acid precursor malonyl CoA by CPT1C enhances protrudin transfer of kinesin-1 to LEs, thereby promoting lysosome redistribution to the cell periphery to support axon growth

Chapter 5: Non-degradative functions of lysosomes in neurons

The function of lysosomes extends beyond its function in degradation. For example, lysosomes function as calcium storages, lysosome exocytosis is important for synaptic plasticity and membrane repair. Furthermore, lysosomes serve as important platforms for RNA-granule transport.

5.1. Calcium release

Lysosomes are believed to be equipped with ion channels and transporters to control Ca^{2+} release and signalling[87,88]. For example, transient receptor potential mucolipin 1 (TRPML1) is a lysosome ion channel which mediates calcium efflux[89] and mediates the transfer of calcium into mitochondria in non-polarized cells regulating calcium homeostasis [90] (Fig. 1A). Mitochondria are spread around the cell in a relatively uniform manner. In human dopaminergic neurons, mitochondria-lysosome contact sites are dynamically formed in the soma, axon and dendrites [91]. These contact sites have been visualised using outer mitochondrial membrane protein TOM20 and lysosomal membrane protein LAMP-1. It was previously discussed that LAMP-1 positive structures includes both mature lysosomes and other endosomal compartments[20]. To characterize the identity of the endosomal structures, GCase activity was inhibited, which is a lysosomal hydrolase. It was observed that GCase inhibition disrupted mitochondria-lysosome untethering [91], suggesting the presence of contacts between degradative lysosomes and mitochondria. [91]TRPML1 has also been observed in hippocampal neurons[92], suggesting the mechanism in which Ca^{2+} is transferred from lysosomes to mitochondria might be the same in neurons.

Furthermore, the transient receptor potential A1 (TRPA1) has been shown to be highly expressed in sensory neurons. In dorsal root ganglion (DRG) neurons, TRPA1 was found both in the periphery and intracellularly and colocalized with the LAMP-1 lysosome marker, suggesting the localization of TRPA1 in lysosome-like organelles. To determine whether acidic lysosomes are critical for Ca^{2+} release, the neurons were treated with a cathepsin C substrate which selectively lyses lysosomes[93]. As a result, this deactivated TRPA1 induced release of Ca^{2+} in DGN neurons (Fig. 1B). The activation of TRPA1 triggered endosomal Ca^{2+} release which may contribute to vesicle exocytosis and neuropeptide release[94] or regulate autophagy[95].

5.2. Signalling

Previously it was described how the availability of nutrients regulate lysosome transport. Nutrient sensing can also activate signalling pathways involving lysosomes. For example, in nutrient rich environments, mTORC1 (mechanistic target of rapamycin complex) is recruited to the lysosomal surface where it is activated by the small GTPase Rheb. Lysosomes do not only serve as a platform for mTORC1, but recruitment of mTOR to lysosomes is regulated by a complex of Ras-regulated GTPases which are regulated by lysosomal transmembrane proteins, and recruitment by the level of amino acids inside the lumen of lysosomes is modulated through the V-ATPase[79]. The mTORC1 pathway in low-nutrient environments leads to the activation of autophagy, allowing recycling of intracellular components. In non-neuronal cells, mTORC1 is recruited to promote cell growth and cell division. Control of mTOR activity is therefore critical for the cell since its dysregulation may lead to diseases such as cancer, metabolic diseases and diabetes. Mature, differentiated neurons do not divide at all, so mTOR recruitment for cell proliferation is unnecessary in these cells. Inactivating mTOR resulted in the identification of several neuron-specific signalling pathways, such as regulating neuron growth, neuronal complexity and synaptic plasticity[80–82].

5.3. Transport of RNA

Neurons rely on local translation for cell-specific function. Local translation requires long distance transport of RNA from the nucleus to distal parts of the neuron. RNAs interact with RNA-binding proteins (RBPs), which assemble into phase separation structures called RNA granules[107]. A recent study showed that RNA granules hitchhike on lysosomes in order to be transported bidirectionally over a long distance in axons[108]. Hitchhiking is a transport mechanism where cargos achieve motility by docking into other membrane-bound organelles, such as lysosomes, which have already recruited motor proteins[109]. It was observed that RNA granules co-transport with LAMP-1 positive organelles [107]. Using mass-spectrometry, the protein annexin A11 (ANXA11) was identified as a molecular tether between RNA granules and lysosomes. ANXA11 only interacts with lysosomes in the presence of calcium and the phosphoinositide $PI(3,5)P_2$ [107]. Importantly, this study referred to all LAMP-1 positive structures as lysosomes, which include both mature lysosomes and other endosomal compartments[20]. Future research is therefore needed, which uses multiple lysosomal markers to determine if these RNA granules hitchhike on mature lysosomes, other endosomal compartments or both.

More recently, live imaging revealed that pre-miRNAs (precursors for mature miRNA) were able to hitchhike on LEs/lysosomes for transport in the axons of cultured *Xenopus* retinal ganglion cells. Pre-miRNA was shown to be delivered to axonal growth cones, where they then could be processed into mature miRNAs [110]. In this study, multiple markers, such as Rab7, Rab5, LAMP1, CD63 and lysotracker, were used to characterize the vesicles on which pre-miRNAs hitchhikes more specifically. Using these markers, it was observed that pre-miRNAs are primarily transported by LEs and lysosomes [110].

5.4. Remodelling of the extracellular matrix

Exocytosis of lysosomes was first observed in cells which were invaded with the parasite *Trypanosoma cruzi,* which caused a calcium efflux. Lysosomes mobilized to the cell periphery and fused with the plasma membrane in response to Ca^{2+} [96]. Lysosome exocytosis has since then emerged as an important mechanism in diverse processes, such as remodelling of the extra-cellular matrix. Lysosomes contain more than 60 acid hydrolases, including cathepsins which are activated in the acidic environment of the lysosome and are responsible for degradation of various cellular macromolecules[97]. Certain cathepsins were already found at a neutral pH in the extracellular space decades ago[98], suggesting this could lead to remodelling of the extra cellular matrix (ECM). SNAREs directly regulate the fusion between lysosomes and the plasma membrane. Action potentials induced the release of Ca^{2+} which triggered lysosomes to fuse with the plasma membrane resulting in the release of the hydrolase cathepsin B which is involved in ECM remodelling and synaptic plasticity by degrading the ECM[73]. Lysosomes also release Cbln1, a synaptic organizer belonging to the C1q family[99], which binds to its surface receptor Nrx at axon boutons along the axonal surface. Synaptic organizers are molecules that regulate formation, differentiation and maintenance of synapses. These findings suggest a new mechanism of activity-dependent coordinates synapse modification[100]. Treatment with glycyl-l-phenylalanine 2-naphthylamide (GPN), a cell-permeable cathepsin C substrate known to disrupt the lysosomal membrane, inhibited activity-induced exocytosis of both cathepsin B and Cbln1 in axons (Ibata et al., 2019). These observations indicate that Cbln1 was mostly targeted to mature lysosomes with a degradative capacity which are anterogradely transported towards axon terminals [100].

5.5. Membrane repair

The fusion of lysosomes with the plasma membrane during lysosomal exocytosis occurs in many cells. In response to plasma membrane damage, the elevation of intracellular Ca^{2+} levels is triggered, which induces anterograde trafficking of lysosomes[101,102]. The mechanism is critical for the survival of eukaryotic cells, which are frequently wounded by mechanical stress or pathogens. It was initially thought that lysosome exocytosis generates a patch through the addition of intracellular membrane

to the cytoplasmic side of the injured membrane [103] . However, the mechanism of membrane repair is much more complicated. In addition to lysosome exocytosis, massive endocytosis was observed and the marker LAMP-1 was detected on a few Ca^{2+}-induced endosomes. More extensive colocalization was seen with the early endosome marker EEA1 [104]. This mechanism may represent a compensatory response to lysosomal exocytosis. Furthermore, lysosomal exocytosis releases factors that can remodel the external surface of wounded cells, promoting repair. Some enzymes are active extracellularly shortly after wounding and participate in the regulation of plasma membrane repair[105]. Although lysosome exocytosis has been previously observed in neurons[106], it is unclear if these mechanisms are involved in neuronal membrane repair.

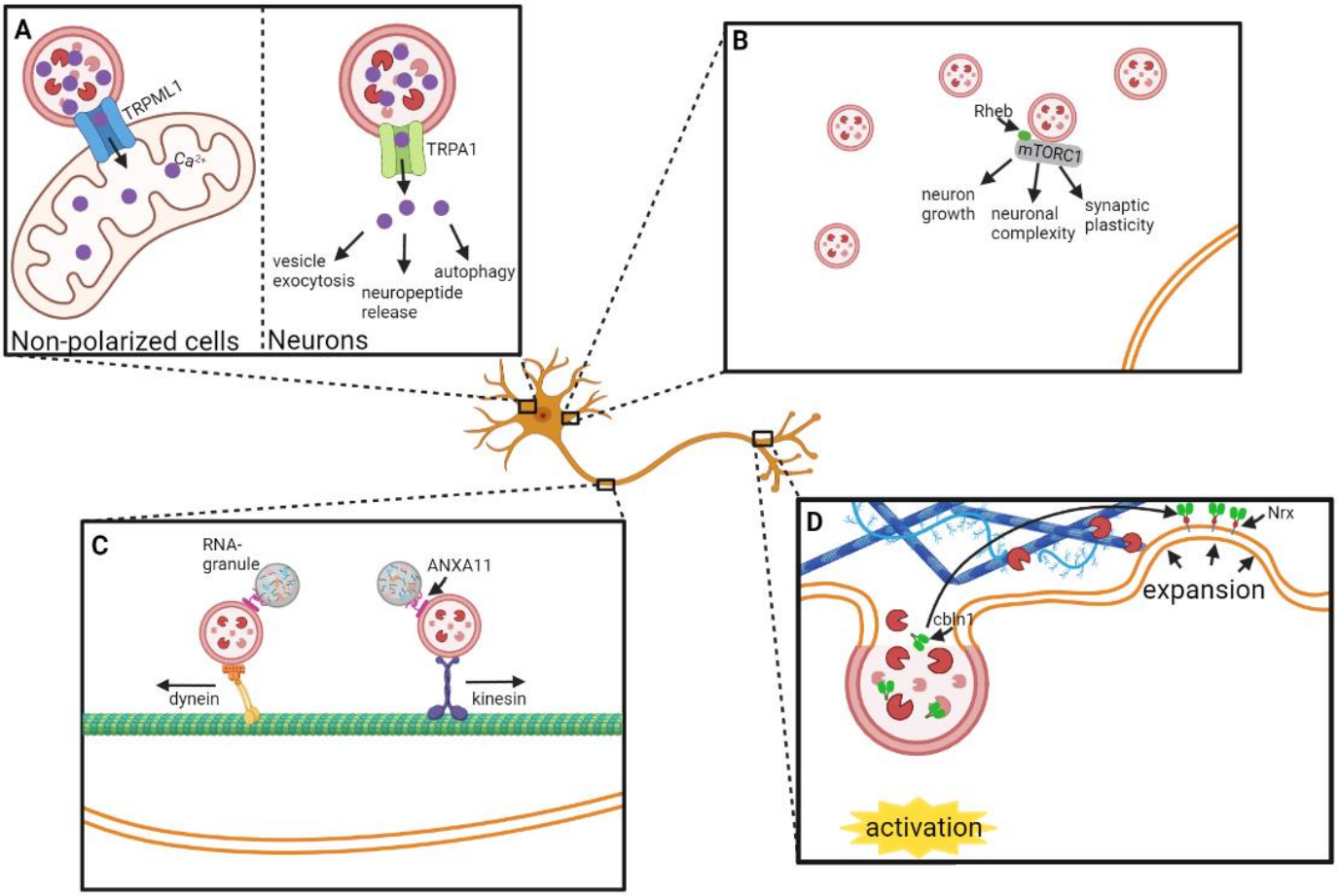

Fig. 2. **Non degradative functions of lysosomes**
(A) lysosomes deliver calcium to mitochondria via the protein TRPML1 (left) in non-polarized cells. In neurons, calcium ions are released from lysosomes via TRPA1 (right). (B) Neuronal lysosomes serve as a platform for mTORC1. recruitment of mTOR to lysosomes is regulated by the small GTPase Rheb (green). (C) RNA granules hitchhike onto the lysosomal membrane which are transported retrogradely or anterogradely using the motor proteins dynein or kinesin respectively. The protein ANXA11 tethers RNA-granules to the lysosomal membrane. (D) Upon neuronal activation, lysosomes fuse with the plasma membrane and co-release cathepsin B and cbln1. Cathepsin B degrades the extracellular matrix where axonal boutons are able to expand. Cbln1 binds to its receptor Nrx and this complex is involved in synaptic signalling.

Chapter 6: Lysosomes in neurodegenerative diseases

During aging, a wide range of insults detrimentally affect various aspects of lysosome function, including lysosome acidification, hydrolase activity and chaperone-mediated autophagy. The already declining autophagy-lysosomal function in aging is worsened by the increased need for clearance of damaged proteins and organelles in aged cells. Misfunctioning of lysosomal function during aging can thus cause severe neurodegenerative diseases.

6.1. Lysosome storage disease

Defective functioning of lysosomes cause accumulation of undegraded material in neurons. These large intracellular deposits alter the cellular function. Lysosome storage diseases (LSDs) are inherited diseases of the metabolism caused by mutations in proteins and enzymes involved in the function of lysosomes[113]. It was previously discussed that loss of function of lysosomes cause many dysregulations of processes lysosomes are involved in. Gaucher disease and Niemann-Pick disease are one of the most common LSDs. New therapeutic strategies have been developed to treat LSDs. Enzyme replacement treatment is the most frequently used therapy, where defective lysosomal hydrolases are replaced with functional enzymes [113], however crossing the blood-brain-barriers to reach to neurons remains a challenge. The development of genome edition has offered new tools to improve safety and efficiency of gene therapy. For example, CRISPR/Cas9 has emerged as one of the most versatile tools to be adapted.

6.2. Neurodegenerative diseases

Alzheimer's disease (AD) is the most common neurodegenerative disease. Aggregation of the proteins amyloid beta (Aβ) and microtubule associated protein tau (MAPT/tau) in neurons are hallmark of this disease. Earlier studies proposed that the enzyme γ-secretase cleaves the amyloid precursor protein (APP) and generates Aβ in the endolysosomal pathway[64,114]. Using Förster resonance energy transfer (FRET) γ-secretase activity was monitored. It was observed that γ-secretase cleaves APP in low-pH organelles, such as lysosomes and LEs in live neurons and that Aβ significantly accumulated in these compartments[115].

Furthermore, there is emerging evidence that deficiencies in the autophagy-lysosome pathway are linked to AD pathogenesis. The enhancement of the autophagy-lysosome pathway to remove protein aggregates has been proposed as a promising therapeutic strategy for AD. It has been shown that the autophagic pathology seen in AD most likely arises from impaired clearance of autophagic vacuoles by the lysosomes[116]. The underlying mechanism still needs to be elucidated in future studies.

Another very common neurodegenerative disease is Parkinson's disease(PD). Mutations in the *PARK2* gene encoding parkin, are associated with Parkinson's disease(PD). Parking is a E3 ligase involved in ubiquitination of mitochondrial proteins and triggers degradation of mitochondria through the autophagy-lysosomal pathway. Several studies indicated that mitochondrial dysfunction alters the expression of lysosomal genes. Also parking deficiency resulted in altered lysosomal content, morphology and function as well as autophagic changes[119].

A similar mechanism was observed in Huntington's disease (HD). Wildtype Huntingtin (HTT) participates as a scaffold protein in different steps of the mitophagy mechanism. Presence of the mutant polyQ tract impairs this mitophagy pathway and also impaired formation of the autophagy initiation complexes[120]. Interestingly, UB-labelling of the mitochondria did not seem to be affected in mutant HTT [120], suggesting that this step in mitophagy does not contribute to the global mitophagy impairment observed in HD which is different from what is observed in PD. Taken together, lysosomal-mitochondria contacts may serve as important targets for therapeutic strategies for Parkinson's and Huntington's disease and need to be elucidated in future studies.

Conclusions and perspectives

In the past few years, more attention is focussed on the function of lysosomes and their underlying mechanisms to match the unique demands of neurons. Many labs have shown that lysosomes are dynamically distributed throughout the neuronal compartments. However, the identity of lysosomes remains a topic of discussion. This is caused by the use of different markers, such as LAMP-1 or lysosomal hydrolases, to investigate lysosomes in neurons. Not all markers label the same organelles and therefore it is important for future research to have rigorous criteria on the identity of mature lysosomes. For example, what classifies the difference between late endosomes and lysosomes?

Furthermore, the importance of lysosome transport and regulation was highlighted. Lysosomes require the motor proteins kinesin and dynein for retrograde and anterograde transport respectively. Several adaptor proteins have been identified to couple lysosomes to the motor proteins. A few of these adaptor proteins have shown to be regulated differently in response to different environments. The variety of adaptor proteins could also function in selective targeting endosomes at different maturation states which rises new interesting question. For example, what are the signatures that differs endosomes from lysosomes to be targeted to motor proteins?

Also in neurons, the function of lysosomes extends beyond its degradation pathways, such as calcium release, RNA-transport and regulating synaptic plasticity. More and more novel functions of lysosomes are emerging which could be important in regulating homeostasis in neurons.
There is a large gap of knowledge in what we know about lysosome contacts with other organelles. For example, ER-lysosome and mitochondrial contact sites. These contact sites may serve in important signalling pathways required for maintaining healthy neurons, or serve as important therapeutic targets for neurodegenerative diseases. Future studies focussed on membrane contact sites involving lysosomes could provide us with useful new information in the dynamics of lysosomes and other organelles.

Moreover, lysosome disfunction is linked to several neurodegenerative diseases. In the future, lysosomes, or lysosome-related proteins, may serve as a target in finding therapeutics for neurodegenerative diseases. Therefore, there is an urgent need to investigate the dynamics and functions of lysosomes in neurons even more, since there is still a lot of aspects that need to be discovered.

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Layman's abstract

All cells contain organelles that perform specialized jobs in the same way organs, such as the liver and stomach, perform specific functions in an organism. Lysosomes are acidic membrane bound organelles that are responsible for breaking down biological material. Lysosomes are involved in various important processes. For example, they are able to destroy and recycle cellular debris and invading viruses or bacteria. A lot of research about lysosome dynamics has been done in normal cells. Little is known about the dynamics and function of lysosomes in neurons. Neurons are highly polarized and contain highly specialized compartments which makes studying lysosome dynamics much more difficult. For example, the length of a neuron can span up to 1 meter long in human neurons. The dependence of neurons on optimal lysosomal function is especially important since lysosome disfunction is linked to several brain diseases, such as Alzheimer's disease or Parkinson's disease.

Many labs have shown that lysosomes are distributed throughout the entire neuron. However, the identity of lysosomes remains a topic of discussion. This is caused by the use of many different markers to investigate lysosomes in neurons. The markers that are most commonly used are unspecific for lysosomes or label lysosome precursors. Therefore it is important for future research to have rigorous criteria on the identity of mature lysosomes in order to determine their precise location in the neurons.

Lysosomes are highly dynamic organelles which move to different locations of the cell. Neurons require coordinated transport mechanisms to maintain a steady-state distribution of lysosomes in the different neuronal compartments. Motor proteins are able to walk across cells and are important for transporting lysosomes to different parts of the neurons. Coupling of lysosomes to motor proteins is performed by specialized adaptor proteins. Lysosomal transport is regulated by other organelles or environmental cues.

Furthermore, some interesting non-degradative functions of lysosomes are highlighted which contribute to neuronal function, such as signalling, transport of other molecules or regulating neuronal morphology. Finally, how lysosome disfunction is linked to several brain diseases is discussed. In the future, lysosomes, or lysosome-related proteins, may serve as a target in finding therapeutics for brain diseases. Therefore, there is an urgent need to investigate the dynamics and functions of lysosomes in neurons even more, since there is still a lot of aspects that need to be discovered.