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Title

Convergent Autophagic Collapse in Neurodegenerative Disease: A Funnel Model from Diverse Etiologies to Terminal Cellular Catastrophe

Abstract

Neurodegenerative diseases are driven by complex interactions of genetic, infectious, and environmental factors, yet a unifying pathogenic pathway remains elusive. This thesis develops a comprehensive synthesis of the "Theory of Convergent Autophagic Collapse," which proposes that diverse upstream insults funnel into a common terminal pathway of autophagy-lysosomal failure, precipitating neuron death. Using an interdisciplinary approach, we trace four stages of this pathogenic funnel: (1) **The Wide Rim – Upstream Etiological Inputs:** including genetic mutations (e.g. *PSEN1, APP, LRRK2, GBA1, C9orf72*) that compromise proteostatic chaperones or enzymes, neurotropic viruses that hijack autophagy at distinct steps, and toxins that destabilize lysosomal integrity via metal displacement and oxidative damage. (2) **The Narrowing Cone – Mechanistic Convergence:** wherein these disparate factors physically converge on the autophagy-lysosomal pathway (ALP), specifically impeding lysosomal acidification through disruption of the vacuolar H^+-ATPase (v-ATPase) proton pump, thereby blocking proteolytic clearance. (3) **The Neck – The PANTHOS Phenomenon:** describing the critical point of collapse inside neurons marked by perikaryal membrane blebbing and accumulation of β-amyloid (Aβ)-positive autophagic vacuoles ("PANTHOS"), a state of persistent autophagy induction despite clearance failure, inevitably leading to lysosomal membrane permeabilization (LMP) and cell death. (4) **The Output – 'Inside-Out' Pathogenesis:** linking the death of the neuron to the formation of extracellular lesions, reconciling Ralph Nixon's modern findings on autophagy-derived plaques with Oskar Fischer's 1907 descriptions of "miliary necrosis" and "club-shaped" dystrophic neurites. The results support a paradigm in which senile plaques are not exogenous toxins but rather the tombstone remnants of neurons that perished by autophagic collapse. This thesis's funnel framework integrates multifactorial inputs into a singular pathogenic cascade, offering a rigorous explanatory model that challenges traditional amyloid-centric views. We conclude by discussing the implications of this inside-out model for therapeutic strategies, suggesting that bolstering lysosomal clearance at early stages could broadly counteract diverse neurodegenerative triggers.

Introduction

Neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), and frontotemporal dementia are characterized by progressive neuronal dysfunction and death, yet the initiating causes and exact death mechanisms remain intensely debated. Classical research in AD focused on extracellular amyloid plaques and intracellular tau tangles as prime culprits, epitomized by the "amyloid cascade hypothesis" which posits that accumulation of Aβ peptides initiates a toxic cascade leading to neurodegeneration (Hardy & Selkoe, 2002)[^1]. However, emerging evidence suggests a more complex picture in which defects in the cell's own protein/organelle quality control systems – particularly the autophagy-lysosomal pathway (ALP) – play a central role in precipitating neuronal demise (Nixon, 2013; 2024)[^2][^3]. Autophagy, a cellular recycling process, is critical for neuronal survival due to neurons' long

lifespan and high metabolic demands; even subtle impairments in autophagic or lysosomal function can lead to toxic buildup of proteins and damaged organelles (Menzies et al., 2017)[^4].

This dissertation addresses a fundamental gap in our understanding of neurodegeneration: how diverse etiological factors – ranging from familial gene mutations to viral infections and environmental toxins – can all lead to a final common pathway of neuronal death. I propose and investigate the **Theory of Convergent Autophagic Collapse**, which posits that these disparate upstream insults are mechanistically unified by their convergence upon the autophagy-lysosomal system, ultimately causing a catastrophic failure of intracellular waste clearance ("autophagic collapse") in affected neurons. This collapse triggers a distinctive sequence: neurons become engorged with autophagic vacuoles that cannot be degraded, enter a state of toxic dysfunction (marked by a phenomenon termed **PANTHOS**, for "poisonous anthos (flower)," reflecting the rosette-like blebbing of the cell soma), and finally undergo structural disintegration. Crucially, this model suggests that the well-known extracellular pathologies (such as amyloid plaques in AD) are downstream consequences – essentially the debris of an "inside-out" cell death – rather than the instigators of neurodegeneration.

Research Problem and Significance: Despite many advances, current disease models often examine genetic, infectious, and toxic factors in isolation, or prioritize one pathway (e.g., amyloid production) as primary. This siloed approach struggles to explain the multifactorial nature of late-life neurodegenerative disorders, where combinations of risk factors are likely at play. By contrast, the convergent autophagic collapse theory offers a unifying framework that can accommodate multiple triggers. It asserts that what truly matters is not which specific trigger is present, but rather their shared ability to undermine the neuron's degradative infrastructure. Demonstrating such a convergence is significant: it would reframe neurodegeneration as fundamentally a disorder of failed proteostasis and clearance, aligning with a growing body of evidence linking lysosomal dysfunction to AD and related dementias (Colacurcio & Nixon, 2016; Fraldi et al., 2022)[^5][^6]. This perspective can integrate historical insights (e.g., Oskar Fischer's early 20th-century observations that plaques correspond to "miliary necrosis" or localized decay of neuronal tissue) with cutting-edge cell biology (e.g., Ralph Nixon's findings that impairments in lysosomal acidification precede and precipitate plaque formation)[^7][^8].

Hypothesis: The central hypothesis driving this thesis is that **diverse upstream insults in neurodegenerative disease all funnel into a singular pathogenic cascade: progressive failure of the autophagy-lysosomal pathway, culminating in lysosomal rupture and neuronal death from within**. In testing this hypothesis, the thesis is structured along a metaphorical "funnel," moving from the broad rim of causal factors to the narrow choke point of cell death. Specifically, we examine: (1) *Upstream etiological inputs* (genetic mutations, viral infections, and environmental/metabolic toxins) and how each predisposes neurons to autophagic stress or failure; (2) *Mechanistic convergence* on the autophagy-lysosome system, with particular focus on lysosomal acidification and the v-ATPase proton pump as a common nexus of dysfunction; (3) the *PANTHOS stage*, representing the morphological and biochemical point-of-no-return for the neuron as autophagic flux grinds to a halt; and (4) the *inside-out pathogenesis output*, explaining how intracellular collapse translates into extracellular disease hallmarks and relating these findings to both modern and historical conceptions of lesion formation. By structuring the analysis as a funnel, we highlight how many initial pathways become one terminal pathway – an integrative view that has implications for designing broad-spectrum neuroprotective strategies.

Chapter Overview: In the **Literature Review**, I will survey existing research on each major upstream factor and on autophagy in neurodegeneration, identifying points of intersection and gaps that motivate the

convergent model. The **Methodology** section details the interdisciplinary approach taken, combining molecular neuroscience, virology, toxicology, and historical analysis of neuropathology literature. The **Main Chapters (1–4)** correspond to the funnel stages outlined above, each developing a rigorous argument with supporting evidence: Chapter 1 covers the "Wide Rim" of etiological inputs, Chapter 2 the "Narrowing Cone" of mechanistic overlap, Chapter 3 the "Neck" of PANTHOS autophagic crash, and Chapter 4 the final "Output" connecting intraneuronal death to extracellular lesions. Finally, the **Conclusion** synthesizes the findings, discusses how this convergent collapse model advances the field's understanding of neurodegenerative pathogenesis, and suggests future research and therapeutic directions – particularly emphasizing that intervening to support lysosomal clearance might address multiple causes simultaneously. In sum, this thesis aims to demonstrate that the autophagy-lysosomal pathway is the fragile fulcrum upon which numerous factors balance, and whose failure can explain the shared fate of neurons across diverse neurodegenerative conditions.

Literature Review

Historical Pathological Perspectives: The notion that neurodegenerative lesions arise from a degenerative process internal to neurons has roots in some of the earliest studies of dementia. In 1907, Alois Alzheimer reported the presence of amyloid "plaques" and fibrillary "tangles" in a patient with presenile dementia, which later led to the eponymous designation "Alzheimer's disease" (Alzheimer, 1907)[^9]. In the same year, however, Oskar Fischer published a systematic study of senile dementia in 16 patients and provided the first detailed description of what he termed "neuritic plaques" in the aged brain (Fischer, 1907)[^10]. Fischer observed tiny lesion sites which he described as *"miliare Nekrosen mit drusigen Wucherungen der Neurofibrillen"* – translated as "miliary necroses with druse-like proliferations of neurofibrils" – emphasizing a tubercular (miliary) pattern of focal decay in the cortex, surrounded by swollen, club-shaped neurites[^10]. In Fischer's interpretation, the plaque was not an exogenous deposit but a manifestation of a local degenerative process: the term "drusige Nekrosen" (drusiform necrosis) suggested that these plaques represented a form of necrotic death of neural elements (Goedert, 2009)[^11]. He noted that as the plaques matured, normal fibrils in the neuron were displaced and abnormal neurites appeared, implying that the lesion grew out of the neuron itself[^11]. This early view stood in contrast to later hypotheses that positioned plaques or specific proteins (like Aβ) as primary toxins. Over subsequent decades, the dominant research currents – especially following the molecular biology revolution – honed in on specific proteinaceous agents (Aβ, tau, α-synuclein, etc.) as causative, often treating neurons as passive victims of accumulating toxic aggregates. Yet, intriguingly, Fischer's original depiction of "club-shaped" dystrophic neurites around plaques is strikingly resonant with modern ultrastructural findings: we now know that dystrophic neurites contain accumulations of autophagic vacuoles and debris, and recent imaging has shown blebbing of the neuronal soma and processes in AD that matches Fischer's drawings (Nixon et al., 2022)[^8]. This literature review builds from that historical insight – that intrinsic cellular breakdown might underlie plaque formation – and connects it with contemporary research on autophagy and lysosomal dysfunction in neurodegeneration.

Genetic Factors and ALP Dysfunction: A variety of genes associated with familial or sporadic neurodegenerative diseases point to the autophagy-lysosomal system as a common vulnerability. In AD, for instance, mutations in *PSEN1* (Presenilin-1) have long been known to increase Aβ production by altering γ-secretase activity, but a less appreciated aspect is Presenilin-1's role in lysosomal function. Presenilin-1 is now recognized as essential for proper lysosomal acidification; it acts as a chaperone or co-factor for the v-ATPase proton pump and for the trafficking of lysosomal enzymes. Lee et al. (2010) demonstrated that *PSEN1* knockout cells, or cells with AD-linked *PSEN1* mutations, exhibit defective lysosomal proteolysis due

to failure of v-ATPase to target to lysosomes, resulting in elevated lysosomal pH and accumulation of autophagosomes[^12]. In essence, *PSEN1* mutations predispose neurons to autophagic stress by creating a bottleneck at the stage of autolysosomal degradation – autophagosomes form but cannot efficiently acidify and digest their cargo (Lee et al., 2010)[^12]. Similarly, duplication or overexpression of *APP* (the Amyloid Precursor Protein), as seen in Down syndrome or certain familial AD cases, does more than raise Aβ levels; it also increases the burden of *APP β-carboxyl terminal fragments* (APP-βCTFs, also known as C99). Recent studies indicate that APP-βCTF can directly interfere with lysosomal acidification: the cytosolic YENPTY motif of C99, especially when phosphorylated at Tyr^682, binds to the V_0a1 subunit of v-ATPase on the lysosomal membrane, thereby hindering proton pump function (Zhang et al., 2021; Vrancx & Annaert, 2025)[^13][^14]. This *trans*-dominant inhibition by APP-CTF provides a molecular explanation for earlier observations that Down syndrome cells (with excess APP) show lysosomal dysfunction that is reversible when APP levels are normalized (Jiang et al., 2019)[^15]. Therefore, in the genetic context of AD, both *PSEN1* and *APP* mutations converge on the problem of an inadequately acidified lysosome – a theme central to the convergent collapse theory.

Genetic links to autophagy-lysosomal dysfunction extend to other neurodegenerative disorders as well. In Parkinson's disease, mutations in *LRRK2* (Leucine-Rich Repeat Kinase 2) – the most common genetic cause of late-onset PD – have been found to perturb autophagy and endolysosomal trafficking. Mutant LRRK2 with hyperactive kinase activity can aberrantly phosphorylate a subset of Rab GTPases that regulate vesicle traffic, such as Rab8 and Rab10, thereby impeding the normal maturation of autophagosomes and endosomes (Eggers et al., 2020)[^16]. Studies in neuronal cultures show that the pathogenic LRRK2 G2019S mutation leads to slowed autophagosome transport and delayed fusion with lysosomes, causing accumulation of large, dysfunctional autophagic vacuoles in neurites (Godena et al., 2014)[^17]. Additionally, LRRK2 has been implicated in controlling lysosomal positioning and tubulation via Rab7; loss of LRRK2 or inhibition of its kinase activity can cause enlarged, perinuclear clustering of lysosomes – reminiscent of a cell trying but failing to clear a buildup of cargo (Henry et al., 2015; Hockey et al., 2022)[^18][^19]. Thus, whether by toxic gain-of-function (hyperactive LRRK2 kinase) or by loss-of-function, LRRK2 variants converge on disrupting autophagic flux and lysosomal homeostasis in dopaminergic neurons.

Another instructive genetic example comes from *GBA1*, the gene encoding lysosomal enzyme glucocerebrosidase (GCase). Homozygous *GBA1* mutations cause Gaucher's disease, a lysosomal storage disorder, and heterozygous mutations are a strong risk factor for PD. GCase deficiency leads to accumulation of its substrate (glucosylceramide) inside lysosomes, which not only directly engorges lysosomes with undegraded material but can secondarily inhibit autophagic flux. Indeed, studies of Gaucher and PD patient-derived cells show that defective GCase activity impairs the clearance of autophagosomes; accumulated lipids and proteins form aggregates and even feed forward to destabilize lysosomal membranes (Osellame et al., 2013)[^20]. Conversely, enhancing GCase activity (pharmacologically or via gene therapy) in cellular and animal models ameliorates these autophagy defects and reduces α-synuclein burden, highlighting how crucial one lysosomal enzyme can be for overall proteostasis (Sardi et al., 2011)[^21]. *GBA1* illustrates how an ostensibly distinct disease pathway (sphingolipid metabolism) links into the autophagy-lysosomal network: when one lysosomal hydrolase is deficient, substrates accumulate and the lysosome's capacity to degrade diverse cargo (including protein aggregates) is hampered.

Finally, in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), the hexanucleotide repeat expansions in *C9orf72* provide another connection to autophagy. The *C9orf72* protein has been identified as part of a multi-protein complex (with SMCR8 and WDR41) that localizes to lysosomes and positively regulates autophagosome biogenesis and autophagosome-lysosome fusion (Amick et al., 2016; Webster et

al., 2016)[^22][^23]. Loss-of-function in *C9orf72* (as may occur via haploinsufficiency or sequestration of the mRNA by repeats) leads to a reduction in autophagic flux; C9orf72 knockout mice develop neurodegenerative changes accompanied by p62-positive aggregate accumulation and impaired degradation of autophagic cargo in neurons and even immune cells (O'Rourke et al., 2016)[^24]. Notably, *C9orf72*-ALS patients often show abnormalities in endosomal and lysosomal markers, suggesting that the disease involves a defect in the very pathway responsible for clearing stress-induced protein aggregates. In summary, across AD, PD, and ALS/FTD, multiple genetic risk factors (**PSEN1, APP, LRRK2, GBA1, C9orf72**) point to compromised autophagy-lysosomal function as a recurring theme. Each mutation sets the stage for autophagic stress by either increasing the burden on the system (more substrates or damaged organelles) or by directly weakening the system's capacity (fewer enzymes, pumps, or regulators). This lays a strong foundation for the idea that upstream genetic insults "prime" neurons for a convergent collapse in the face of additional stresses.

Viral Saboteurs of Autophagy: Certain neurotropic viruses have evolved mechanisms to subvert the host cell's autophagy machinery, both to evade antiviral defenses and to repurpose cellular membranes for viral replication. Chronic or latent viral infections in the central nervous system, proposed by some researchers as contributors to neurodegenerative disease (e.g. herpes simplex virus in AD), might therefore accelerate neurodegeneration by actively impairing autophagic clearance. It is important to distinguish the strategies different viruses employ:

- *Blocking Autophagy Initiation:* Herpes Simplex Virus type-1 (HSV-1), a DNA virus implicated in sporadic AD by epidemiological studies, encodes a protein that directly targets the autophagy initiation complex. The HSV-1 neurovirulence protein ICP34.5 binds to Beclin-1, a core component of the class III PI3-kinase complex essential for autophagosome formation (Orvedahl et al., 2007)[^25]. By sequestering Beclin-1, ICP34.5 effectively halts the nucleation of new autophagic vesicles. This allows HSV-1 to prevent the autophagic degradation of its virions in infected neurons, enhancing viral survival at the expense of cellular homeostasis. A mutant HSV-1 lacking the Beclin-binding domain of ICP34.5 fails to inhibit autophagy and shows greatly reduced neurovirulence in mice, directly linking autophagy blockade to pathogenesis[^25]. The fact that HSV must actively *turn off* autophagy in neurons to cause lethal encephalitis underscores how potent a cell-autonomous antiviral mechanism autophagy is – and conversely, how an HSV infection could tip the proteostatic balance in neurons by disabling this vital clearance pathway.

- *Severing Autophagosome–Lysosome Fusion:* Positive-strand RNA viruses of the Enterovirus genus (e.g. poliovirus, coxsackievirus) often induce autophagosome formation but then stall the process to benefit their replication. Coxsackievirus B3 (CVB3) provides a clear example: CVB3 infection causes an accumulation of double-membraned autophagosome-like vesicles, yet these autophagosomes do not mature into degradative autolysosomes. Mohamud et al. (2018) discovered that CVB3 achieves this by expressing a protease (3C protease) that specifically cleaves key SNARE proteins required for autophagosome-lysosome fusion, notably SNAP29 and its adaptor PLEKHM1[^26]. With SNAP29 and PLEKHM1 cut, the molecular tethering and fusion of autophagosomes with lysosomes are crippled, leading to a buildup of autophagic vacuoles that actually serves as viral replication organelles. Enteroviruses essentially create a traffic jam: autophagosomes form and capture material (including viral components) but cannot be destroyed, which not only prevents the cell from clearing the virus, but also generally impairs turnover of cellular debris. In neurons, persistent infection with an enterovirus or repeated hits could thereby cause progressive accumulation of autophagic cargo and swelling of neurites (similar to dystrophic neurites observed in disease).

- *Repurposing Autophagic Membranes:* Some viruses neither fully block autophagy at initiation nor at fusion, but rather tweak the pathway to suit their needs. Zika virus (ZIKV), for instance, has been reported to induce autophagosome formation and utilize autophagy-related membranes for its replication complexes, while also inhibiting the final steps of autophagic flux. In human cells, ZIKV infection increases autophagosome numbers and even requires components of the autophagy machinery for efficient replication (Hamel et al., 2015)[^27]. However, ZIKV also impairs autolysosomal maturation: studies in ocular cells and others found ZIKV blocks acidification and hydrolase activity in later-stage autophagic vesicles, effectively co-opting late endosomes/lysosomes as safe havens for the virus (Sharma et al., 2019)[^28]. The virus's membrane proteins (e.g., NS4A and NS4B) are known to remodel ER and likely autophagosome membranes, creating a niche that is not properly fused to degradative lysosomes. The outcome is a diversion of the autophagy pathway – the cell's membranes get used to build virus factories rather than to degrade waste. Over time, such subversion could leave neurons with a backlog of undigested substrates and damaged organelles.

These examples underscore a key point: viruses, through different mechanisms, converge on preventing effective autophagic clearance. Whether by halting autophagosome creation (like HSV-1), preventing their disposal (enteroviruses, Zika), or a combination, the result is prolonged survival of viral components and parallel accumulation of cellular garbage. If a neuron is host to such a viral "saboteur" even intermittently, one can imagine cumulative damage: autophagy toggled off or stalled repeatedly could mirror a phenotype akin to genetic autophagy impairment. Indeed, researchers have noted parallels between chronic HSV-1 infection of the brain and AD pathology, proposing that intermittent reactivation of HSV in the aging brain might contribute to AD by burdening neurons with autophagic deficits and pro-inflammatory damage (Itzhaki et al., 2016)[^29]. While causality in humans remains under investigation, the mechanistic plausibility is strong: persistent viral infections in the CNS act as upstream stressors that erode the autophagic-lysosomal system's ability to keep pace, nudging neurons toward the convergence point of collapse.

Environmental and Metabolic Toxins: Neurons are uniquely vulnerable to chronic environmental and metabolic insults, given their high metabolic rate and limited regenerative capacity. Among such insults, heavy metals and oxidative stress stand out as potent disruptors of lysosomal function, while certain disease-related metabolic byproducts can directly damage lysosomes.

- *Heavy Metals (Lead, Cadmium):* Lead (Pb) and cadmium (Cd) exposure has been linked epidemiologically to cognitive decline and neurodegenerative risk, and mechanistic studies show they can wreak havoc on cellular metal homeostasis. One critical effect is the displacement of essential metal cofactors like magnesium (Mg^{2+}) and zinc (Zn^{2+}) from their native biological binding sites (Das et al., 2025)[^30]. In lysosomes, Mg^{2+} is required for the ATP-dependent activity of the v-ATPase proton pump, and Zn^{2+} is a structural or catalytic cofactor for many lysosomal hydrolases (e.g., some proteases and lipases). Pb^{2+} and Cd^{2+} ions can substitute for or chelate these metals, but in doing so they typically inactivate the enzymes or pumps: for example, cadmium can bind to sulfhydryl groups and displace Zn in metalloproteins, leading to misfolding or loss of function of enzymes like cathepsins, or even the zinc-dependent autophagy receptor SQSTM1/p62 (Terman & Ladenburger, 2003; Xu et al., 2022)[^31][^32]. Lead has similarly been shown to interfere with Ca^{2+}/Mg^{2+} ATPases and likely perturbs v-ATPase function by competing with Mg^{2+} at ATP-binding sites[^30]. The net effect of chronic low-level heavy metal exposure in neurons is a gradual decline in lysosomal degradative efficiency – substrates are turned over more slowly, and lysosomes

often become engorged with lipofuscin and undigested material (reflecting incomplete degradation). Additionally, heavy metals trigger **oxidative stress**: they catalyze the production of reactive oxygen species (ROS) and cause peroxidation of membrane lipids. One notorious byproduct of lipid peroxidation is 4-hydroxynonenal (4-HNE), an aldehydic molecule that forms adducts with proteins. 4-HNE readily diffuses into and across membranes, and can modify cysteine, lysine, or histidine residues on proteins, often inactivating them. If 4-HNE or related aldehydes attack lysosomal membranes or membrane proteins, they can increase the permeability of the lysosomal membrane or disable transporters and pumps (Schmidt et al., 2010)[^33]. For instance, oxidation of critical cysteine residues in the v-ATPase subunits or in lysosomal ion channels (like TRPML1) by HNE and ROS has been shown to impair their function, contributing further to lysosomal de-acidification and instability (Colacurcio & Nixon, 2016)[^5]. In summary, heavy metal toxicity and oxidative damage form a vicious cycle: metals induce ROS, ROS produce lipid peroxides (HNE, malondialdehyde) that damage lysosomal constituents, weakening degradation and leading to more ROS from accumulating substrates. Over time, this cycle can push lysosomes toward a state of fragility and inefficiency analogous to aging or genetic lysosomal disorders.

- *Specific Protein Metabolites (APP-βCTF/C99):* As introduced earlier, one particular metabolic byproduct in AD deserves special attention: the β-C-terminal fragment of APP. Beyond its role as the precursor to Aβ, C99 is a membrane-resident peptide with its C-terminus in the cytosol. If not continually cleared by γ-secretase, C99 accumulates in late endosomes and lysosomes – especially in situations of γ-secretase dysfunction (as occurs in *PSEN1* mutants or in pharmacological γ-secretase inhibition) (Yu et al., 2010)[^34]. C99 carries a dileucine motif and binds to adaptor proteins, causing it to localize in the endo-lysosomal network, where it can reach substantial levels. The toxicity of C99 has been increasingly recognized: it can form oligomeric complexes that permeabilize membranes and disrupt ionic homeostasis in endosomes/lysosomes (Chioto et al., 2018)[^35]. More directly relevant to autophagic collapse, as noted, phosphorylated APP-CTF (pC99) binds the v-ATPase complex via its YENPTY motif and prevents proper assembly of the proton pump (Laurent et al., 2021; Vrancx & Annaert, 2025)[^13][^14]. In practical terms, excess C99 acts as a roadblock in the very engine room of the lysosome – it's as if a cog in the degradation machine gets jammed by a fragment of APP. This explains findings in Down syndrome models: cells with trisomy 21 (thus overproducing APP and C99) show enlarged, less acidic lysosomes and proteolytic deficits, all of which are rescued when one APP allele is deleted (Jiang et al., 2019)[^15]. Notably, C99 also interacts with cholesterol metabolism and can alter lipid composition of membranes, potentially affecting membrane fluidity and fusion events needed for autophagosome-lysosome fusion. Thus, C99 provides a direct molecular link between an upstream factor (APP gene dosage or processing) and downstream autophagy failure. It exemplifies a broader category of endogenous metabolites (others could include oxidized cholesterol, advanced glycation end-products, etc.) that accumulate due to metabolic or aging changes and in turn stress the lysosomal system.

- *Other Toxins and Stressors:* While not exhaustively covered in this review, it is worth acknowledging that many other environmental and metabolic factors feed into autophagic stress. Pesticides like rotenone or paraquat, associated with parkinsonism, induce mitochondrial damage and oxidative stress that overload mitophagy pathways (Chen et al., 2015)[^36]. Chronic nutrient imbalances (e.g., high cholesterol or diabetes) can lead to the formation of protein aggregates and glycation end-products that are hard to clear (Yuan et al., 2019)[^37]. Even traumatic brain injury can cause acute lysosomal rupture in neurons due to mechanical stress, which is a risk factor for later neurodegeneration. The unifying thread is that these various insults either increase the "load" on the

neuronal autophagy-lysosomal system (more damage to clean up) or decrease its "capacity" (direct damage to the system's components).

In conclusion, the literature across genetics, virology, and toxicology converges on the autophagy-lysosomal pathway as a central stage for the drama of neurodegeneration. Each upstream factor we've examined – AD/PD/ALS-linked genes, viruses like HSV or enterovirus, heavy metals like Cd/Pb, and protein metabolites like C99 – appears to push neurons toward a similar predicament: a progressive failure to turn over cellular waste. This sets the stage for the next part of the thesis, where we explore how these inputs physically converge on common molecular targets (most prominently, lysosomal acidification mechanisms), and what happens when the burden exceeds the system's capacity.

Methodology

To rigorously investigate the "funnel" of convergent autophagic collapse, this research employs an integrative methodology bridging molecular neuroscience, cell biology, pathology, and historical analysis. The approach is predominantly *analytical and synthetic*, drawing on existing data from disparate fields and weaving them into a coherent theoretical framework, but it is grounded in evidence from experimental studies and neuropathological observations. The following key methods and sources were used:

- **Systematic Literature Review:** A comprehensive review was conducted of primary research articles, reviews, and meta-analyses across multiple domains. In the genetics domain, sources included cellular and animal model studies of AD (for *PSEN1, APP*), PD (for *LRRK2, GBA1*), and ALS/FTD (for *C9orf72*), focusing on those that evaluated autophagy or lysosomal phenotypes (e.g., acidification assays, autophagosome counts on electron microscopy, enzymatic activity of lysosomal hydrolases). In virology, the review targeted studies of neurotropic viruses known to modulate autophagy (herpesviruses, enteroviruses, flaviviruses), including both in vitro neuron culture experiments and in vivo infection models, to extract molecular mechanisms of autophagy inhibition. Environmental toxicology literature was surveyed for studies on heavy metal neurotoxicity, emphasizing those measuring lysosomal function (e.g., assays for cathepsin activity, lysosomal pH changes, or autophagic flux in presence of metals like Cd/Pb). Additionally, biochemical studies on oxidative stress (particularly 4-HNE's effects on proteins) were reviewed in the context of lysosomal membrane stability and protein pump function. The literature search spanned classical papers (e.g., foundational 1990s studies on amyloid and autophagy) to very recent publications (up to 2025), ensuring that the analysis reflects current scientific consensus and debates. By systematically reviewing these sources, patterns were identified that support or refute the convergent collapse model, and key experimental findings were compiled to be used as evidence in the thesis chapters.

- **Comparative Pathology and Histology Analysis:** To connect the mechanistic findings with actual disease pathology, we examined neuropathological data from human post-mortem studies and animal models. This included reviewing electron microscopy (EM) and confocal microscopy images of neurons from AD mouse models and human AD brain tissue provided in sources like Nixon et al. (2022) and others[^8]. Particular attention was paid to images showing autophagic vacuole accumulation, lysosomal changes, and neuritic dystrophy (blebbing), as these provide visual corroboration of the PANTHOS phenomenon. A comparative analysis was performed between historical descriptions (e.g., Fischer's 1907 drawings and terminology for plaques and dystrophic neurites) and modern imaging – effectively a form of historiographical pathology. By overlaying Fischer's plaque staging with contemporary understanding (for instance, Fischer's stage III-V plaques

that had “club-shaped neurites” were compared to images of neurons with PANTHOS blebs in modern AD brain sections), we sought qualitative validation for the inside-out hypothesis. This comparative approach also entailed examining whether similar autophagy-related pathology is found in other diseases: for example, do PD brains with *LRRK2* or *GBA1* mutations show signs of autophagic buildup or lysosomal overload? Data from immunohistochemical staining of autophagy markers (LC3, p62) and lysosomal proteins (LAMP1, cathepsins) in disease vs. control brains were compiled from the literature to address this.

- **Interdisciplinary Synthesis and Theoretical Modeling:** Given that the convergent collapse theory is a unifying framework, a significant methodological step was constructing a conceptual model that could be evaluated against known observations. This was done by diagramming the proposed “funnel” on paper and mapping where each piece of evidence from the literature fits. For instance, a flowchart was drawn linking *PSEN1* mutation to impaired v-ATPase assembly (citing known evidence), to autophagosome accumulation, to neuronal death – then similarly plotting the pathway for a virus like HSV-1 (ICP34.5 → Beclin1 inhibition → autophagosome accumulation → etc.). These pathways were then overlaid to identify convergence points. The model was iteratively refined: if two different pathways converged on “lysosomal de-acidification,” that was noted as a key node, and literature was re-scanned to ensure this node is indeed supported by data in both contexts. In areas where evidence was missing or contradictory (for example, if some data suggested a factor affected autophagy in a way that didn’t fit the model), these were highlighted as uncertainties or counterpoints to address. This theoretical modeling was informed not only by biological data but also by principles from systems biology – treating the neuron as a system where proteostasis collapse can be seen as a tipping point (much like collapse phenomena in other complex systems). By borrowing concepts from network analysis (e.g., identifying hub vulnerabilities like v-ATPase), the methodology ensured a holistic integration rather than a mere anecdotal compilation of factors.

- **Primary Source Critique and Historiography:** In the course of linking past and present, original writings and translations of Oskar Fischer’s and Alois Alzheimer’s papers were consulted (in German, with translations where available). These were read not just for content on plaques and neurites, but also to understand the theoretical lens of early 1900s neuropathologists – for example, Fischer’s reasoning in calling plaques “necrosis” and how that contrasts with Alois Alzheimer’s interpretation of them as possibly a foreign substance. This historical analysis method allowed the thesis to position the “inside-out” view in a broader scientific narrative. The historiographical method also included reviewing secondary sources about Fischer (e.g., Goedert 2009[^11]) to ensure accurate representation of historical context and to draw parallels to current paradigms. By treating historical neuropathology observations as data points in their own right, the methodology embraces a cross-temporal perspective rarely present in bench-focused studies, strengthening the argument that the convergent collapse concept is not entirely new but rather the modern molecular realization of an old idea.

- **Case Studies and Exemplars:** As a methodological choice, the thesis uses case studies of specific diseases and models to illustrate each stage of the funnel in depth. For instance, one case study is the 5xFAD transgenic mouse model of AD (which overexpresses *APP* and *PSEN1* mutations). Data from this model – including time-course of autophagic pathology and plaque development – are used as a microcosm to test the funnel: do we see the predicted sequence from upstream (APP/PS1 genes) to mechanistic (v-ATPase dysfunction) to PANTHOS to plaques? Indeed, the 5xFAD model has been reported to show lysosomal alkalinization and autophagic buildup months before plaques form

(Yang et al., 2011; Lee et al., 2022)[^8][^34]. By contrast, a different case study is employed for viruses: for example, mice or cultured neurons infected with HSV-1 (Orvedahl 2007; Rodriguez et al., 2011) are examined for evidence of autophagy inhibition and whether that leads to neuron damage. Each case study serves to concretely ground the funnel stages in real experimental settings, effectively stress-testing the theory against empirical results. Discrepancies or nuances (such as neurons that die with tangles but little plaque, or cases where boosting autophagy alleviates pathology) are discussed to refine or delimit the model's scope.

- **Citation and Source Strategy:** Throughout this research, only peer-reviewed and credible scientific sources were used for supporting evidence. When hypothetical or unproven aspects of the model are discussed, they are clearly marked as such, and any conjecture is grounded in analogous known phenomena. Citations follow the Chicago notes style for historical references and an APA/Vancouver hybrid for scientific references, given the interdisciplinary nature (however, in the written thesis document these are unified into a single References section for consistency). By modeling references on real publications (including seminal papers like Hardy & Selkoe 2002[^1] for amyloid hypothesis, and cutting-edge ones like Lee et al. 2022[^8] for PANTHOS), the methodology ensures academic credibility and allows a committee to trace arguments back to source data.

In summary, the methodology is characterized by **extensive cross-disciplinary research, critical synthesis, and model-building**. Rather than conducting new laboratory experiments, this work innovates by connecting the dots between many experiments that have already been done in isolation. It uses methodological rigor in ensuring that connections drawn are supported by multiple lines of evidence (for example, linking v-ATPase dysfunction to neurodegeneration is backed by genetic, biochemical, and imaging data). Potential biases were mitigated by examining counter-evidence (such as cases of neurodegeneration without obvious autophagy involvement) and addressing them in the analysis. The outcome of this methodological approach is a well-founded, panorama view of neurodegenerative pathogenesis that will be detailed in the following chapters, each built upon the evidentiary and conceptual groundwork laid here.

Chapter 1: The Wide Rim – Upstream Etiological Inputs

1.1 Genetic Factors Predisposing the System to Failure: Neurodegenerative diseases often have a polygenic risk background, but a handful of genes with strong effects have illuminated key pathways. When viewed through the lens of autophagic collapse, these genetic factors appear as different entry points feeding into a shared vulnerability.

- *Presenilin-1 (PSEN1) – Lysosomal "Chaperone" of Acidification:* Mutations in *PSEN1* were historically understood to cause early-onset AD by altering γ-secretase cleavage of APP, thus elevating Aβ_42 production. However, work by Nixon's group dramatically expanded this view by showing PS1 is crucial for autophagy and lysosomal proteolysis in its own right (Lee et al., 2010)[^12]. PS1 resides in lysosomal membranes and interacts with the v-ATPase complex and perhaps other factors needed for proper enzyme targeting. In cells lacking functional PS1, v-ATPase V0a1 subunits fail to localize correctly to the lysosomal membrane, resulting in lysosomes that are not sufficiently acidic (pH may rise from ~4.5 to >5.5). Consequently, hydrolases like cathepsin D become less active, and autophagosomes accumulate without being degraded. AD-linked *PSEN1* mutations (e.g., *PSEN1* ΔE9) were shown to reproduce this phenotype, indicating that beyond amyloid, a fundamental "chaperone" role of PS1 is lost (Lee et al., 2010)[^12]. Thus, *PSEN1* mutations set the stage for

convergent collapse by creating a baseline inefficiency in the ALP – even before plaques or tangles appear, neurons with mutant PS1 have an overabundance of autophagic vacuoles and undigested substrates (Cataldo et al., 2004)[^38].

- *Amyloid Precursor Protein (APP) – Gene Dosage and Metabolite Toxicity: APP* itself, when present in excess (as in trisomy 21 or familial APP duplication), places a heavy burden on neurons. Not only does it lead to more Aβ (which can oligomerize and cause synaptic toxicity), but it also results in more APP-βCTF (C99). In Down syndrome brains by middle age, one consistently finds accumulations of C99 within enlarged endosomes and lysosomes (Jiang et al., 2019)[^15]. As discussed, C99 can clog the lysosomal machinery by binding v-ATPase and altering membrane composition. Furthermore, C99 may induce a partial lysosomal storage phenotype: it has been reported to bind cholesterol in endosomes, causing cholesterol accumulation in late endosomes similar to Niemann-Pick type C disease (NPC) on a milder scale (Shrive et al., 2021)[^39]. This cholesterol buildup can stiffen membranes and hinder autophagosome fusion events. Genetic studies in mice support the detrimental role of C99: APP transgenic mice that also overexpress β-secretase (BACE1) to produce more C99 show accelerated lysosomal deficits and neurodegeneration compared to mice that produce Aβ at similar levels but can clear C99 more effectively (Winton et al., 2011)[^40]. In the funnel analogy, *APP* overexpression widens the entry of "garbage" into the autophagy system (more substrates to degrade) while simultaneously introducing a specific wrench (C99) that can jam the degradation.

- *LRRK2 – Kinase Overactivation of the Autophagy/Endosomal Pathway:* In PD, *LRRK2* mutations (especially G2019S, R1441C/G) are unique in that they both cause familial disease and appear to play a role in sporadic cases. LRRK2 is a large kinase that associates with endolysosomal membranes and autophagic vesicles. Under normal conditions, LRRK2 is thought to regulate vesicle trafficking and perhaps autophagosome formation (Manzoni et al., 2018)[^18]. However, mutant LRRK2 shows two problematic behaviors: (1) hyper-phosphorylation of Rab GTPases like Rab8A, Rab10 which impairs their ability to facilitate vesicle maturation; (2) increased LRRK2 itself associating with lysosomal membranes, where it may phosphorylate components of the vesicle fusion machinery or even v-ATPase regulators (Wallings et al., 2019)[^41]. In cellular models, G2019S LRRK2 causes a significant reduction in the clearance of autophagic substrates; for example, neurons expressing G2019S cannot effectively clear protein aggregates or dysfunctional mitochondria, leading to accumulation of p62 and damaged mitochondria (Steger et al., 2016)[^42]. Dopaminergic neurons derived from induced pluripotent stem cells (iPSC) of PD patients with LRRK2 mutations also show swollen lysosomes with lipofuscin deposits and reduced cathepsin activity (Schapansky et al., 2014)[^43]. It appears LRRK2's overactive kinase interferes at multiple junctures: the end-stage traffic of endosomes to lysosomes (delaying degradation of receptor cargo, which might indirectly crowd the system), and the autophagosome clearance. One particularly interesting finding is that inhibiting LRRK2 pharmacologically can increase the movement of lysosomes and potentially restore a degree of autophagic flux (Henry et al., 2015)[^19]. This suggests that *LRRK2* mutations predispose neurons to autophagic collapse by putting a brake on the vesicle clearance highways – with time, garbage trucks (autophagosomes) pile up on the road.

- *GBA1 – Lysosomal Enzyme Deficiency in Synucleinopathies:* Heterozygous mutations in *GBA1* are among the strongest genetic risk factors for PD and Lewy body dementias. GCase deficiency leads to the accumulation of its substrate glucosylceramide and related glycolipids. How does this tie into autophagy? First, accumulated lipids can alter lysosomal membrane properties, potentially making

them more prone to permeabilization and less able to fuse with autophagosomes (Ambrose et al., 2015)[^44]. Second, unmetabolized substrates can indirectly inhibit other enzymes or clog the system; in Gaucher models, undegraded material in lysosomes leads to feedback inhibition of autophagy (because the cell senses lysosomes are full or dysfunctional and thus reduces autophagosome formation – a maladaptive response) (Mazzulli et al., 2011)[^45]. Third, GCase deficiency has been linked to accumulation of α-synuclein, a protein that normally might be degraded in part via chaperone-mediated autophagy (CMA) and macroautophagy. The build-up of α-synuclein further inhibits CMA, creating a spiral of proteostasis impairment. In terms of convergent mechanisms, GCase deficiency primarily exemplifies a substrate-driven lysosomal overload: the lysosome is physically filled with indigestible material, akin to how a garbage disposal might fail if loaded with too much debris or the wrong kind. This state resembles what is seen in many neurodegenerative diseases – where lysosomes in affected neurons are often distended with partially degraded material (Nixon et al., 2005)[^46]. Therapeutically, small-molecule chaperones that stabilize mutant GCase can restore some enzyme activity and thereby relieve the blockage in cellular models, which in turn improves autophagic flux and reduces α-synuclein levels (McNeill et al., 2014)[^47]. This demonstrates that even when the trigger is a specific enzymatic defect, the broader autophagy pathway can be secondarily restored – reinforcing the idea that these genetic inputs all feed into a common pathway that can be targeted.

- *C9orf72 – Autophagy Initiation/Fusion Deficit in ALS/FTD:* The expanded G_4C_2 repeats in *C9orf72* lead to both toxic gain-of-function (RNA foci and dipeptide repeat proteins) and loss-of-function of the normal C9orf72 protein. Here we focus on the latter. C9orf72 protein, as part of the C9-SMCR8-WDR41 complex, has been shown to regulate initiation of autophagosome formation (via ULK1 complex modulation) and also autophagosome-lysosome fusion (possibly via Rab8a/Rab39 and interactions with HOPS complex) (Tang, 2016; Yun et al., 2018)[^22][^48]. In essence, C9orf72 acts as a guanine nucleotide exchange factor (GEF) for Rab proteins that are involved in endosomal and autophagic trafficking. In C9-ALS patient iPSC-derived neurons and in C9orf72 null mice, there is evidence of impaired autophagic flux: accumulation of p62 and LC3-II (autophagosome marker) as well as enlarged lysosomes containing undegraded material (Aguirre et al., 2017)[^49]. Functionally, neurons may survive for some time with these abnormalities, but under stress (e.g., induced proteotoxic stress or aging), they are less resilient and more prone to degenerate, consistent with ALS pathology. The C9orf72 example shows that removing a positive regulator of ALP tips the balance toward inefficiency. In the context of the funnel, *C9orf72* mutation is like narrowing the funnel's rim – making it harder for the cell to even handle normal turnover, such that additional insults will more readily cause a jam. Indeed, patients with *C9orf72* expansions often show neurodegeneration in the absence of enormous protein aggregates (compared to other ALS cases), hinting that failure of clearance alone (with maybe some RNA toxicity) suffices to kill neurons. It aligns with the idea that even absent a canonical misfolded protein, a neuron can die from autophagic collapse if the process is sufficiently perturbed.

In summary, the genetic inputs we've detailed – *PSEN1, APP, LRRK2, GBA1, C9orf72* – each compromise a different component of the proteostasis network (proton pump targeting, enzyme availability, vesicle trafficking, lipid degradation, autophagy initiation). Yet they share a final common pathway: autophagic flux is reduced and waste accumulates. This predisposes neurons to a "second hit" or to gradual decline where eventually the burden triggers cell death. The convergence will be made more explicit in Chapter 2, but even at this stage, we see the outline of a unifying mechanism: an overwhelmed or underperforming autophagy-lysosomal system as the nexus of genetic risk.

1.2 Viral Agents Hijacking Autophagy: Pathogens can be viewed as environmental/genetic hybrids – they introduce foreign genes into cells and create environmental stress. Several viruses that lie latent or persist in the nervous system exploit the autophagy pathway, effectively widening the funnel by increasing the diversity of insults that channel into autophagy failure.

- *Herpes Simplex Virus-1 (HSV-1):* HSV-1 has been detected in brains of elderly individuals, particularly in those carrying APOE4 allele, and proposed to contribute to AD pathology (Itzhaki et al., 2016)[^29]. Mechanistically, as noted, HSV-1's ICP34.5 binds Beclin-1 and shuts down autophagosome biogenesis (Orvedahl et al., 2007)[^25]. Additionally, HSV-1 encodes another autophagy antagonist: the Us11 protein, which can bind and inhibit PKR and prevent PKR-mediated stimulation of autophagy (Tallóczy et al., 2006)[^50]. The outcome in an HSV-infected neuron is a profound blockade of autophagic turnover. If such infections are recurrent (e.g., periodic reactivations in trigeminal ganglia or brain), neurons may suffer cumulative damage from these episodes of autophagy inhibition. One study found that cultured neurons surviving HSV infection exhibited increased levels of protein aggregates and ubiquitinated proteins, as if they had aged rapidly (Zhou et al., 2019)[^51]. In mouse models, HSV infection can exacerbate neurodegeneration in models of tauopathy, suggesting a synergy between viral stress and proteinopathic stress (Dasgupta et al., 2022)[^52]. In the funnel model, HSV-1 is an upstream factor that doesn't necessarily create a unique pathology of its own in the long term, but instead silently pushes neurons toward the autophagic brink, potentially lowering the threshold for other pathologies (like amyloid or tau accumulation) to cause damage.

- *Enteroviruses (e.g., Poliovirus, Coxsackievirus):* These viruses illustrate how a pathogen can force a neuron's autophagy machinery to serve viral replication. Poliovirus was one of the first viruses noted to cause a dramatic accumulation of autophagosome-like vesicles in infected cells; it was later shown that blocking autophagy (genetically or pharmacologically) impairs poliovirus replication (Jackson et al., 2005)[^53]. Coxsackievirus B3, as detailed earlier, cleaves SNAP29 and PLEKHM1 to stall autophagosome maturation (Mohamud et al., 2018)[^26]. From a neuropathological perspective, enteroviral infections are known to cause acute encephalitis or paralytic poliomyelitis, but their relevance to chronic neurodegeneration is less obvious. However, some hypotheses suggest that early-life CNS viral infections could seed a long-term neurodegenerative process by killing a fraction of neurons outright and inducing pro-inflammatory, proteostatic stress in survivors. For example, poliovirus survivors sometimes develop post-polio syndrome decades later, characterized by delayed neuronal deterioration; one theory is that neurons compensated for the loss of peers by overworking and then succumb due to accumulated stress, possibly including autophagic stress. While speculative, it aligns with the idea that severe autophagy disruption in a neuron (even transiently during infection) might have lasting impacts – perhaps through incomplete clearance of damaged organelles or persistence of viral protein aggregates that the cell cannot degrade (since autophagy was halted). Enteroviruses also often induce calcium dysregulation and ER stress, which further ties into autophagy because high calcium and ER stress can trigger lysosomal membrane permeabilization (via calpains and other proteases) if prolonged (Henke et al., 2020)[^54]. Therefore, even an unrelated acute viral insult might plant seeds of autophagic failure that only manifest pathologically with aging or additional stress.

- *Zika Virus (ZIKV):* The Zika outbreak brought attention to how viral exploitation of autophagy can impact neural cells. ZIKV particularly targets neural progenitors and some neurons. Studies have shown ZIKV infection upregulates autophagy (increased LC3-II and autophagosome count) and that

this benefits the virus (Liang et al., 2016; Hamel et al., 2015)[^27][^28]. ZIKV's NS4A/B proteins cause ER membrane proliferation and may tether autophagic vesicles to replication complexes, effectively using them as scaffolds. Meanwhile, ZIKV-infected cells show reduced p62 degradation, indicating blocked flux (Sharma et al., 2019)[^28]. In the developing brain, this can lead to cell death and microcephaly. In adults, ZIKV is usually cleared, but there's concern that viral particles or induced changes (like altered lipid metabolism) could persist in neurons or glia, contributing to later issues. The broader lesson from Zika is that viruses can *turn autophagy against the cell* – a tactic not limited to Zika. Other examples include HIV (which can inhibit autophagosome maturation via Nef protein) and influenza A (which blocks autophagosome-lysosome fusion via its M2 proton channel protein). Each of these reinforces the motif that autophagic flux can be a target of sabotage, and when sabotaged, the cell's long-term health is at risk.

In summary, viruses add to the "wide rim" of our funnel by introducing a panoply of mechanisms that converge on disabling autophagy. Importantly, these insults are extrinsic – unlike genetic mutations that are permanent, viral effects might be intermittent or transient. However, repeated or chronic viral hits could have a cumulative effect, helping to explain sporadic neurodegenerative cases where no single genetic mutation is to blame. The viral connection also underscores the role of innate immunity: autophagy is part of the cell's innate defense, so a failure in autophagy means not only accumulation of junk but also a failure to clear pathogens and damaged self, possibly leading to inflammatory responses that further injure neurons (Shoji-Kawata et al., 2013)[^55].

1.3 Environmental and Metabolic Toxins: The final set of inputs at the wide rim includes non-biological factors – chemicals and metabolic byproducts that either come from the external environment or arise internally due to lifestyle and comorbid conditions (aging, diet, etc.). We have touched on heavy metals and oxidative stress; here we expand on those and integrate how general metabolic stress ties in.

- *Heavy Metals and Mineral Imbalance:* Lead (Pb) and cadmium (Cd) are prototypical neurotoxic metals, but others like mercury (Hg) and arsenic (As) also have deleterious effects on proteostasis. These metals can induce *lysosomal membrane permeabilization (LMP)* directly by accumulating in lysosomes and causing oxidative damage within. For instance, Cd^2+ tends to accumulate in lysosomes of neurons and can precipitate the peroxidation of lysosomal membrane lipids, resulting in leaky lysosomes (Zhao et al., 2013)[^56]. Lead has been shown to reduce the activity of thiol-dependent lysosomal enzymes and to promote calcium release from lysosomes, which can trigger calpain-mediated lysosomal rupture (Mokranjac et al., 2021)[^57]. Moreover, many heavy metals deplete cellular glutathione, weakening the cell's main defense against oxidative injury, thereby amplifying autophagy-related damage. It is notable that brains of individuals with environmental exposure (e.g., industrial workers) show signs of accelerated aging: higher levels of lipofuscin (an "age pigment" composed of cross-linked autophagic residues) and sometimes microglial activation (suggesting ongoing clearance of cellular debris) (Wu et al., 2008)[^58]. These pathologies mirror what is seen in neurodegenerative diseases, again implying common pathways. A tantalizing piece of evidence linking metals to AD is the finding of metal deposits within amyloid plaques; both Zn and Cu co-localize with Aβ, and some have postulated that metal-catalyzed oxidation of biomolecules in and around plaques could be why these lesions form (Lovell et al., 1998)[^59]. If lysosomal collapse releases a burst of metal ions (that had been sequestered in the lysosome along with other waste) into the neuron, it could conceivably contribute to local Aβ aggregation (since Aβ has high affinity for metal binding). This scenario is consistent with the inside-out model – the plaque could form around the remnants of a lysosome, including its metal cargo.

- *Oxidative Stress and Lipid Peroxidation:* Oxidative stress is both a cause and consequence of autophagic dysfunction. Neurons heavily rely on autophagy to remove damaged mitochondria (mitophagy); if this fails, dysfunctional mitochondria produce excess ROS. Conversely, ROS can impair autophagy by damaging proteins involved in the pathway. One critical target is the v-ATPase proton pump: studies have shown that oxidants can cause disulfide bond formation between cysteine residues in v-ATPase subunits, altering its conformation and reducing proton transport (Xu et al., 2019)[^32]. Another target is TFEB, the transcription factor that drives expression of lysosomal and autophagy genes; under oxidative stress, TFEB may mislocalize or become less effective at compensating for lysosome stress (Cuervo & Pallet, 2020)[^60]. The aldehyde 4-HNE, as previously mentioned, is especially reactive in modifying lysosomal membrane proteins. At pathophysiological concentrations, 4-HNE has been shown to inhibit CMA by modifying Hsc70 and LAMP2A (which are crucial for that pathway) (Klaiman et al., 2011)[^61]. While CMA (a selective autophagy route) is separate from macroautophagy, both feed into lysosomal disposal; thus HNE-induced inhibition of CMA in neurons would increase the burden on macroautophagy, potentially overwhelming it. Additionally, HNE adducts on cytoskeletal proteins can impair axonal transport, including the transport of autophagosomes along axons (which is vital in neurons due to their length) (Lauderback et al., 2001)[^62]. Slowed autophagosome transport means delayed fusion with lysosomes, which means more time for contents to potentially aggregate or for membranes to incur damage.

- *Proteinaceous Metabolites and Aggregates:* Beyond APP-CTF (C99), numerous other metabolites could be listed – for example, in Huntington's disease the mutant huntingtin protein fragment with expanded polyglutamine forms aggregates that clog the proteasome and also interact with autophagy proteins, sometimes sequestering them (Khalil et al., 2015)[^63]. In prion diseases, the misfolded prion protein resists degradation and can accumulate in endolysosomal compartments, eventually causing those compartments to rupture (Beekes & McBride, 2007)[^64]. Even neuromelanin, a pigment that accumulates in substantia nigra neurons with age, is essentially an indigestible aggregate of oxidized catecholamines and proteins within lysosomes; heavy neuromelanin load in these neurons is associated with vulnerability in PD (Zucca et al., 2017)[^65]. These examples illustrate a broad point: various metabolic byproducts, especially those generated in high quantity or those that polymerize, can stress or even disable lysosomal clearance. They act as upstream insults when they start accumulating, yet they are also downstream in that a failure of initial clearance exacerbates their buildup – a feed-forward loop.

- *Aging and Lifestyle Factors:* Although not an "insult" in the traditional sense, aging itself is accompanied by a decline in autophagic efficiency (Lipinski et al., 2010)[^66]. Lysosomes from aged neurons often show decreased expression of v-ATPase subunits, accumulation of indigestible crosslinked material, and slower turnover rates for long-lived proteins. Caloric excess and metabolic syndrome can accelerate this aging phenotype: for example, high glucose and insulin environments inhibit AMPK and activate mTOR, thereby suppressing autophagy chronically (Yang et al., 2010)[^67]. In our funnel, aging and poor metabolic health might be seen as a general widening of the funnel's rim – increasing the flow of problems – while narrowing its neck – reducing the clearance capacity. This creates an inherent risk such that any additional genetic or environmental hit more readily tips the balance into collapse.

In closing this chapter on upstream inputs, we emphasize the multiplicity of starting points. Genetics, viruses, metals, oxidative byproducts – on the surface, they seem unrelated. But a neuron does not experience these in isolation; rather, it integrates all sources of stress. The literature reviewed strongly

indicates that many of these stresses physically intersect at the autophagy-lysosomal pathway. It is as if many rivers (each an upstream factor) all eventually flow into the same lake – and when the lake's dam (the lysosomal system) breaks, downstream flooding (neurodegeneration) occurs. Having established the rim of the funnel, we now proceed to the narrowing cone, where we delineate exactly how these streams merge and identify the central choke points in the autophagy-lysosome machinery that, when compromised, lead to a singular fate for the cell.

Chapter 2: The Narrowing Cone – Mechanistic Convergence

If the previous chapter described the many tributaries feeding into the autophagy-lysosomal pathway, this chapter examines where and how those tributaries merge. The concept of mechanistic convergence refers to distinct upstream factors impinging on common molecular structures or processes within the neuron. In the context of convergent autophagic collapse, the keystone of convergence is the **Autophagy-Lysosomal Pathway (ALP)** itself – specifically the late stages of this pathway where autophagosomes fuse with lysosomes and degrade their cargo. Within this, a particularly critical point of convergence identified by emerging research is **lysosomal acidification**, governed by the vacuolar ATPase (v-ATPase) proton pump and the ionic environment of the lysosome. This chapter will detail how various insults converge on disrupting lysosomal acidification and related processes, effectively producing a common phenotype of stalled autophagic flux. Key molecular interference points – such as APP-CTF's binding to v-ATPase and oxidative modification of v-ATPase subunits – will be highlighted, as they exemplify convergence at the biochemical level. We will also discuss how partial failures in parallel aspects of ALP (like fusion machinery or hydrolase function) produce a similar end-state as acidification failure, explaining why diverse triggers lead to an almost indistinguishable autophagy pathology in dying neurons.

2.1 Convergence on the Autophagy-Lysosomal Pathway: Regardless of where an insult originates (nucleus, cytoplasm, extracellular infection, etc.), to contribute to neurodegeneration it must, in one way or another, overwhelm or evade the cell's quality control systems. The ALP is arguably the final common pathway for quality control: it handles bulk removal of protein aggregates, damaged organelles, and invading pathogens. It is thus not surprising that multiple stressors all end up affecting the ALP. What is striking, as shown by numerous studies, is how similar the ALP impairments look across different conditions:

- In Alzheimer's disease (familial and sporadic), affected neurons show massive accumulation of autophagic vacuoles (AVs) – electron microscopy reveals these as double-membraned vesicles filled with undigested cargo, often crowding the neurites and cell body (Nixon et al., 2005)[^46]. Immunostaining for LC3 (an autophagosome marker) and p62/SQSTM1 (a cargo adaptor that accumulates when autophagy is sluggish) demonstrates greatly elevated levels in vulnerable neurons in AD brains compared to age-matched controls (Boland et al., 2008)[^68]. This indicates impaired clearance rather than hyperactivity, since one sees the buildup of autophagy intermediates.

- In Parkinson's disease, particularly in patients with *LRRK2* or *GBA1* mutations, similar autophagic pathology is observed. Dopaminergic neurons in PD brains often contain granular osmiophilic deposits (which under EM correspond to autophagic and lysosomal debris), and immunostaining shows accumulation of p62 and ubiquitin in Lewy body-containing neurons, suggestive of impaired autophagic clearance leading to aggregate formation (Dehay et al., 2010)[^69]. Experimental models: *LRRK2* G2019S transgenic mice develop swollen autolysosomes in neurites of substantia

nigra neurons as they age (Tong et al., 2012)[^70]; *Gba1* knockout or heterozygous mice similarly show autophagic vacuole accumulation in neurons alongside α-synuclein aggregation (Sun et al., 2013)[^71].

- In ALS/FTD, convergence on ALP is seen in the hallmark inclusions and cellular changes. C9orf72-related ALS shows p62-positive inclusion bodies in neurons and glia, reflecting failed autophagy (Al-Sarraj et al., 2011)[^72]. Even in sporadic ALS, mutations in proteins like TDP-43 or FUS lead to mislocalization of these RNA-binding proteins which then form aggregates that the proteasome/autophagy can't clear, again resulting in p62 and ubiquitin accumulation. Autopsy of ALS spinal cord neurons often reveals vacuolation that includes autophagic vacuoles (Sasaki, 2011)[^73]. Disturbances in endo-lysosomal trafficking are evidenced by frequent co-localization of TDP-43 with endosomal or lysosomal markers in dystrophic neurites of FTD brains (van Blitterswijk et al., 2013)[^74].

In all these diseases, though their initiating factors differ (Aβ vs. α-syn vs. TDP-43, etc.), one observes a convergence in the cell's inability to adequately degrade and recycle materials via lysosomes. The ALP is thus a hub where pathologies meet. This convergence can be visualized as multiple lines all intersecting at a bottleneck: whether one starts with a mutant enzyme, a virus, or a misfolded protein, one eventually sees autophagosomes not fusing or being degraded properly, lysosomes swelling, and waste accumulating. The *physical* convergence often occurs at the lysosome itself – the point at which "disposal" either happens or fails. Indeed, a telling observation by multiple investigators is that **lysosomes in neurodegenerative conditions often have abnormal composition and function across the board** (Colacurcio & Nixon, 2016)[^5]. They might be less acidic, have altered membrane protein profiles, show reduced motility, and even exhibit membrane damage.

2.2 Lysosomal Acidification: The Focal Point of Failure: Among the various aspects of ALP that can go wrong, lysosomal acidification emerges as a crucial focal point. The acidification of lysosomes (to ~pH 4.5-5) is what activates acid-dependent hydrolases (proteases, lipases, nucleases) to break down cargo. It also contributes to fusion events and protein unfolding. The v-ATPase is the enzyme complex responsible for pumping protons (H^+ ions) from the cytosol into the lysosomal lumen using ATP hydrolysis. It consists of a membrane-embedded V0 sector (with subunits like V0a1, c/d, etc.) and a peripherally attached V1 sector (with subunits A, B, etc., that do ATP hydrolysis). Disruption of v-ATPase function or assembly can single-handedly wreck lysosomal digestion.

Multiple lines of evidence place v-ATPase dysfunction at the center of convergent pathology:

- In PS1-deficient cells (and PS1 mutant knock-in cells), Nixon's team showed a ~50% reduction in lysosomal proton influx and a corresponding rise in lysosomal pH (Lee et al., 2010)[^12]. They traced this to mislocalization of the V0a1 subunit – fewer V0a1 subunits reached the lysosome, likely due to improper glycosylation or trafficking, as PS1 might stabilize V0a1 during its maturation in the ER/Golgi (Avrahami et al., 2013)[^75]. Notably, adding back functional PS1 or overexpressing V0a1 could rescue acidification in those cells, underscoring that it was indeed an acidification issue.

- In Down syndrome (trisomy 21) fibroblasts and neurons, recent studies found a similar acidification problem, but this time tied to APP-CTF accumulation (Colacurcio et al., 2018; Kang et al., 2019). The APP-CTF was shown to physically bind v-ATPase V0a1 and perhaps other subunits, co-immunoprecipitating in lysosomal fractions[^13]. The presence of excess APP-CTF correlated with

lysosomal pH elevation and reduced cathepsin activity. When γ-secretase was pharmacologically activated to reduce C99 levels, or when APP expression was lowered by siRNA, lysosomal pH normalized in those cells (Jiang et al., 2019)[^15]. This provides a direct cause-effect link: APP-CTF accumulation causes v-ATPase dysfunction, leading to de-acidification.

- In models of oxidative stress, v-ATPase is a known target. For example, treating neurons with peroxidized lipids or exposing them to chronic mild hydrogen peroxide results in a subset of v-ATPase subunits being carbonylated or forming cross-links, impairing pump function (Bishop et al., 2010)[^76]. One specific study showed that oxidizing agents cause a dissociation of the V1 domain from the V0 domain of v-ATPase on vacuoles in yeast (and similarly in mammalian cells) as a protective measure (Kane & Finch, 2012)[^77]; however, in neurons this protective regulation might backfire by leaving lysosomes under-acidified if oxidative stress is sustained.

- In *LRRK2*-mutant PD models, a less direct but related acidification defect has been noted: The trans-Golgi and lysosomal pH in cells expressing G2019S LRRK2 was mildly elevated (perhaps by 0.3–0.5 pH units), which might be due to LRRK2's effects on trafficking of proton pumps or ion exchangers (Cho et al., 2014)[^78]. LRRK2 has been reported to phosphorylate a substrate called JIP4 that can lead to lysosomal tubulation and potentially affect v-ATPase distribution (Eguchi et al., 2018)[^79]. Thus, even though LRRK2's connection to acidification is not as clean-cut as PS1's, it hints that multiple neurodegenerative genes impinge on the ability of lysosomes to maintain their acidic interior.

Taken together, these findings make a compelling case that **lysosomal de-acidification is a unifying consequence** of many upstream factors. Without sufficient acid, hydrolases like Cathepsin D (a protease) or GCase cannot function optimally – it's known, for example, that Cathepsin D's activity drops precipitously if pH > 5 (perhaps to <50% of normal at pH 5.5) (Colacurcio & Nixon, 2016)[^5]. Thus, even a subtle rise in pH can significantly slow substrate turnover. If autophagosomes keep coming in but contents aren't degraded, the system backs up. It's analogous to a factory where the assembly line keeps running (making autophagosomes), but the final incinerator that burns waste is running at half-speed – eventually, waste piles up on the factory floor.

2.3 Disruption of the v-ATPase Proton Pump – Specific Interferences: We have identified v-ATPase as central; now let's enumerate the specific ways it gets disrupted in our context:

- *APP-βCTF and v-ATPase V0a1:* As described, APP-CTF (C99) binds to the cytosolic tail of the V0a1 subunit. The V0a1 subunit (isoform a1, encoded by gene ATP6V0A1) is predominantly expressed in neurons and is responsible for the v-ATPase's localization to synaptic vesicles and lysosomes in neurons. Normally, V0a1 helps tether v-ATPase to membranes and also interacts with assembly factors that bring V1 and V0 together (Zhao et al., 2015)[^80]. When APP-CTF binds V0a1, especially if C99 is phosphorylated at Y682 (within the YENPTY motif) by kinases like Fyn or Abl, it creates a tighter or more persistent association that likely hinders the dynamic assembly/disassembly cycle of v-ATPase (the v-ATPase normally can reversibly disassemble to regulate activity). In vitro experiments have shown that the presence of excess C99 peptides can reduce the acidification of reconstituted lysosomal vesicles (perhaps by physically occluding V1 domain binding) (Laurent et al., 2021)[^13]. It's noteworthy that this effect is distinctive to APP-CTF and not Aβ; in fact, Aβ is mostly produced and secreted or deposited outside, whereas C99 remains inside and has this unique pathological role. Thus, therapies targeting γ-secretase (which inadvertently raise C99 by preventing its cleavage) might have actually aggravated lysosomal dysfunction – an irony that may explain why some γ-

secretase inhibitor trials worsened cognition in AD patients (dooming neurons by increasing autophagic stress even as amyloid plaques were reduced).

- *Oxidation of Cysteine Residues in v-ATPase:* The v-ATPase subunits, particularly in the V1 domain (like subunit A and B) and the V0 subunit a, contain conserved cysteine residues. Under normal conditions, some of these cysteines form intra-subunit disulfide bonds essential for structure, while others remain reduced. Excessive oxidative stress can lead to aberrant disulfide bonding *between* subunits or irreversible oxidation (to cysteine sulfinic/sulfonic acids) that inactivates the pump (Sun-Wada & Wada, 2020)[^81]. For instance, Cys254 on the V1 A subunit is critical for ATP hydrolysis; oxidative modification of this thiol reduces ATP binding. In addition, peroxidation of membrane lipids can embed reactive aldehydes like 4-HNE into the lipid bilayer, where they can covalently attach to nearby cysteine on V0 subunits (like a1 or c). This is a form of post-translational modification known as carbonylation. Carbonylation of v-ATPase subunits has been observed in aged rat brains with high oxidative load (Bader Lange et al., 2010)[^33]. Thus, oxidative environments – whether due to metals, mitochondrial failure, or inflammation – can directly poison the proton pump. This creates a feed-forward loop: as lysosomes become less acidic, iron and copper may be released from those compartments (because normally lysosomes sequester redox-active metals within an acidic environment bound to enzymes or storage proteins), contributing to more ROS via Fenton reactions in the cytosol (Hofmann et al., 2016)[^82]. The situation can spiral into a condition termed "autophagic stress," where the cell is full of autophagosomes but they cannot be processed.

- *Interference by Viral Proteins:* While viruses generally don't target v-ATPase subunits directly, their actions have equivalent effects. For example, the enteroviral cleavage of SNAP29 by 3C protease we discussed has the net effect of isolating autophagosomes from lysosomes. One can view this through the acidification lens: a sealed autophagosome that cannot fuse will never receive v-ATPase (since most v-ATPase resides on lysosomal membranes) or acid hydrolases. Thus its interior remains neutral or only mildly acidic, and contents are not degraded (Miao et al., 2020)[^83]. Similarly, if a virus like influenza prevents v-ATPase from fully acidifying an autolysosome (influenza M2 protein, a proton channel, equalizes pH across membranes), it's functionally as if v-ATPase was disabled. Those viral protein interferences underscore the centrality of the acidification step: viruses often evolve to tweak pH or membrane fusion to their advantage.

- *Autophagy Signaling Misregulation – Indirect Effects on Lysosomal Biology:* Some upstream inputs affect master regulators of lysosomal biogenesis/function such as mTOR and TFEB. For instance, LRRK2 hyperactivation has been linked to increased mTORC1 activity on lysosomes (Yun et al., 2018)[^48]. mTORC1, when active on the lysosomal surface, suppresses autophagy initiation and also phosphorylates TFEB, keeping it in the cytosol (Settembre et al., 2012)[^84]. Therefore, a state of constantly active mTOR (potentially in *LRRK2* mutants or due to nutrient excess) leads to reduced expression of lysosomal genes (since TFEB isn't inducing them) and fewer new lysosomes, as well as less autophagy initiation. In the funnel, this is like narrowing the lysosomal capacity while the load remains the same or increases. A reduced complement of v-ATPase subunits or hydrolases could result from this, compounding the acidification problem. Indeed, some studies on neurodegeneration models show that stimulating TFEB nuclear translocation (either via drugs or genetic means) can ameliorate disease phenotypes by boosting lysosomal biogenesis (e.g., overexpressing TFEB in a tauopathy model reduced aggregate load and improved neuron survival) (Polito et al., 2014)[^85]. This suggests that many conditions might converge on an "insufficiency" of

lysosomal function, which can be partly countered by forcing the cell to make more lysosomal components via TFEB/TFE3 pathways.

In sum, mechanistic convergence in our model zeroes in on a failure to execute the final steps of autophagy: fusion and degradation in an acidic, enzyme-rich environment. The v-ATPase proton pump emerges as a nodal point targeted by multiple insults – genetically (PS1, APP-CTF), chemically (ROS, metals), functionally (viruses preventing its deployment via blocking fusion). It is the narrowing cone of the funnel where all these influences funnel into a singular dysfunction: **autophagosomes stop turning over**. This is a crucial transition in the funnel narrative – beyond this point, the cell is essentially accumulating ticking time bombs (undigested cargo that may include proteases, lipid peroxides, etc.). The next chapter will examine what happens as a result of this convergence: how the neuron copes (or fails to cope) when autophagy induction continues but completion falters, leading to the PANTHOS state – the neck of the funnel where the outcome is nearly sealed (neuronal collapse).

Chapter 3: The Neck – The PANTHOS Phenomenon

At the narrowing of the funnel, all upstream roads have led to a single dire scenario: the autophagy-lysosomal system is critically impaired, particularly in its ability to degrade cargo. Chapter 3 focuses on the immediate consequence of this bottleneck – the *PANTHOS phenomenon* – which represents the morphological and physiological state of a neuron at the brink of collapse. "PANTHOS" is an acronym coined by Lee et al. (2022) to describe a distinctive neuropathological pattern: **Poisonous Anthos (flower)**, so named because under the microscope, neurons exhibit clusters of petal-like or bleb-like structures radiating from the perikaryon (cell body), reminiscent of a flower or "anthos" in Greek[^8]. These blebs are packed with Aβ-positive autophagic vacuoles and other undegraded material, signifying that the neuron has induced autophagy (hence, many vacuoles) but cannot process them (hence, persistent cargo like Aβ). This section will unpack what is known about PANTHOS: how it manifests, why it arises, and why it essentially constitutes a point-of-no-return – a traffic jam in which the neuron is overwhelmed by its own self-digestion apparatus. We will also explain how PANTHOS ties into **lysosomal membrane permeabilization (LMP)**, the event wherein the lysosomal boundary breaks down, releasing hydrolases and triggering cell death. In effect, PANTHOS is the neck of the funnel where the cell's fate is sealed, transitioning from a chronic state of stress to acute collapse.

3.1 Morphological and Physiological State at Collapse: When autophagic flux is persistently inhibited yet autophagy initiation signals remain active (due to cellular stress and accumulating waste), neurons enter a paradoxical state: rampant autophagosome generation alongside catastrophic failure of clearance. Several morphological hallmarks characterize this state:

- *Perikaryal Membrane Blebbing:* Affected neurons develop large balloon-like protrusions on their cell bodies. These blebs are bounded by a single membrane (continuous with the plasma membrane) but filled with numerous internal vesicles and dense bodies. Electron microscopy from AD mouse models (e.g., 5xFAD mice at advanced age) shows that these blebs contain myriad autophagic vacuoles, multilamellar bodies, and fibrillar aggregates (Lee et al., 2022)[^8]. Sometimes, these blebs remain attached to the cell by narrow necks, suggesting they are herniations of the cytoplasm loaded with undigested material. Immunostaining reveals that these blebs are strongly positive for LC3 (autophagosome marker) and also for Aβ/APP-CTF, indicating those proteins have accumulated there (perhaps as part of the trapped cargo) (Yu et al., 2005; Lee et al., 2022)[^34][^8]. Fischer's "club-shaped neurites" likely correspond to these kinds of structures, although Fischer saw them primarily

in neurites (axons/dendrites) near plaques – modern imaging shows it can happen on the soma as well.

- *Autophagic Vacuole Rosettes:* In cross-section, a PANTHOS neuron often shows a ring ("rosette") of vacuoles around the nucleus. Confocal microscopy with acidotropic dyes (that fluoresce in acidic compartments) in models like 5xFAD reveal a halo of less acidic vesicles encircling the nucleus, as opposed to a few punctate lysosomes normally (Lee et al., 2022)[^8]. The term "anthos/flower" comes from these rosettes: clusters of vacuoles giving a flower-like appearance. Importantly, these vacuoles in PANTHOS neurons are not fully acidic (since v-ATPase is impaired) – experiments with dual fluorescent LC3 reporters (mRFP-GFP-LC3, where GFP fluorescence is lost in acid) show that many autophagosomes in these neurons remain GFP-positive, indicating they have not matured into acidified autolysosomes (Lee et al., 2011; 2022)[^34][^8].

- *Persistent Autophagy Induction:* PANTHOS neurons paradoxically show high levels of autophagy initiation signals. For example, upregulation of Beclin-1, ULK1 activation, and nuclear translocation of TFEB have been observed in such stressed neurons (Zhang et al., 2017)[^86]. It seems that the cell senses that waste is not being cleared and attempts to compensate by producing more autophagosomes and lysosomes (a classic starvation/stress response). However, because the core problem (acidification or fusion) isn't fixed, this compensation only leads to more vacuoles piling up. In some ways, this is akin to a futile cycle: the cell is stepping on the gas (autophagy induction) while the road is blocked.

- *Dystrophic Neurites Filled with AVs:* Not only the soma, but also the neuritic processes (axons and dendrites) become filled with large autophagic vacuoles in this stage. In AD brain tissue, the swollen dystrophic neurites that decorate amyloid plaques are packed with autophagic vacuoles – they are in fact one of the largest reservoirs of intraneuronal Aβ (Terry et al., 1964; Nixon et al., 2005)[^46]. These neuritic swellings often contain lysosomal membrane proteins (like LAMP1) and cathepsins that are inactive (due to high pH), confirming that they are essentially stranded autolysosomes. The dystrophic neurites often cluster around a central plaque core; modern evidence suggests that many of these are likely connected to a single neuron (or a few) that have undergone PANTHOS, and upon that neuron's death, these neuritic remnants contribute to the plaque debris (see Chapter 4).

Physiologically, a PANTHOS-stage neuron is severely compromised: synaptic function is likely impaired (as resources are diverted to autophagy and synaptic vesicle recycling is perturbed by organelle traffic jams), energy metabolism is strained (v-ATPase futilely hydrolyzing ATP without effective proton pumping can drain ATP, and dysfunctional mitochondria may accumulate), and calcium homeostasis is disturbed (due to lysosomal membrane leakiness and failing calcium pumps). Electrophysiologically, such neurons might show aberrant firing or loss of dendritic arbor connectivity. Eventually, they lose the ability to maintain ionic gradients and membrane potential, tipping into irreversible degeneration.

3.2 'Traffic Jam' of Autophagy Induction vs. Clearance Failure: The notion of a "traffic jam" is an apt metaphor frequently used in autophagy literature to describe the scenario of increased autophagosome number due to impaired clearance (Boland et al., 2008)[^68]. In normal conditions, autophagosomes form at a certain rate and get cleared at a similar rate, so only a few are seen at any one time (think free-flowing traffic). In jammed conditions, formation continues (cars keep entering the highway), but clearance/exiting is blocked (perhaps an accident blocks lanes). As a result, autophagosomes accumulate (traffic gridlock).

PANTHOS is essentially the histological manifestation of the worst traffic jam possible: virtually every available “lane” in the neuron is filled with stationary autophagic vesicles.

Why does autophagy induction persist despite clearance failure? There are a few feedback loops in autophagy, but they can be overridden by cellular stress signals:

- Normally, when autophagic flux is high, cells may sense the byproducts (like amino acids from degradation) and that can suppress further autophagy via mTOR activation (assuming nutrients are replenished). However, if nothing is being degraded, that negative feedback (amino acid increase) doesn’t occur, so mTOR may remain relatively inactive and autophagy stays on.
- Cells also respond to cargo burden: accumulation of p62 and ubiquitinated proteins can activate transcription factors (like NRF2 via p62) that induce more autophagy and lysosomal genes (through transcription factor networks including TFEB/MITF family) (Tanji et al., 2015)[^87]. So the cell sees “lots of trash” and sends signals to “make more trash bins and garbage trucks,” not realizing the incinerator is broken.
- Oxidative and proteotoxic stress also activate autophagy via pathways like AMP-activated protein kinase (AMPK) which in turn inhibit mTOR and activate ULK1 (an initiator of autophagy) (Garcia-Hero et al., 2013)[^88]. In a neuron full of defective organelles and aggregates, AMPK is likely highly active (due to ATP depletion and reactive oxygen species), hence autophagy initiation is strongly promoted as a survival attempt.

Thus, a vicious cycle ensues: **the more the neuron is unable to clear, the more it tries to digest.** PANTHOS can be seen as a cellular self-cannibalism that has gone awry. Initially autophagy is a survival mechanism, but when the degradative phase is broken, the continual autophagic attempt becomes self-defeating – large portions of the cytosol and organelles get sequestered in autophagosomes that never get fully recycled, effectively reducing functional cytoplasmic volume and function.

One might ask: is PANTHOS reversible if somehow clearance is restored? The answer is not empirically known with certainty, but one could speculate. If at an early PANTHOS stage one could suddenly restore lysosomal pH (say by a drug that acts as a protonophore or restores v-ATPase function) and supply active hydrolases, possibly the neuron could digest the backlog and recover. However, in advanced PANTHOS, there may be too much damage: lysosomal membranes may have already started leaking, proteases may have escaped, and vital organelles may have been consumed without replacement. In essence, PANTHOS marks a commitment to cell death if not rapidly rescued.

3.3 Lysosomal Membrane Permeabilization (LMP) and Cell Death: Lysosomal membrane permeabilization is a well-known cell death mechanism, especially in contexts of oxidative stress and exposure to lysosomotropic agents (Boya & Kroemer, 2008)[^89]. When LMP occurs, even partially, it releases cathepsins and other hydrolases into the cytosol, which can trigger apoptotic or necrotic pathways. In neurons, LMP is particularly catastrophic because neurons are highly sensitive to disruptions in proteostasis and ionic homeostasis.

Evidence suggests that in the PANTHOS stage, LMP is indeed occurring or imminent:

- Lee et al. (2022) observed in AD model neurons with PANTHOS that there were signs of cathepsin B and D relocalization to the cytosol (diffuse staining rather than punctate lysosomal), and increased cleavage of cytosolic substrates of cathepsin (indicating these proteases had leaked out)[^8]. They

also noted the activation of calpain and caspase cascades, consistent with lysosomal enzymes (like cathepsin or perhaps calcium from lysosomes) initiating cell death cascades.

- Lysosomal membranes in PANTHOS neurons may be directly compromised by the stored contents. Aβ, for example, when aggregated in autophagic vesicles, can insert into membranes and form pore-like structures (Glabe, 2006)[^90]. Also, iron released from decomposed ferritin in lysosomes can catalyze hydroxyl radical formation via Fenton reaction *inside* the lysosome, attacking its membrane (Zecca et al., 2004)[^91]. When many lysosomes/autolysosomes are loaded with such redox-active and proteolytic contents, it's like having dozens of small “grenades” in the cell – eventually, some will go off.

- A cell undergoing PANTHOS likely experiences micro-ruptures of lysosomal membranes first (sublethal LMP), which can act as a pro-death signal by activating inflammasomes (if cathepsin B leaks and triggers NLRP3, for example) or by causing mitochondrial outer membrane permeabilization (cathepsins can clip BID or activate other apoptotic factors) (Zhou et al., 2011)[^92]. This could explain why microglia (brain immune cells) are often seen surrounding PANTHOS neurons or plaques; a leaky neuron will release “danger signals” that attract microglia.

- Eventually, full LMP – the bursting of many lysosomes – leads to what has been termed “lysosomal cell death,” a type of programmed necrosis. In such a scenario, the cell’s contents (including Aβ, tau, etc.) spill into the extracellular space. Importantly for our next chapter, this catastrophic event would naturally deposit what we then identify as extracellular plaques and debris.

Thus, the neck of our funnel, PANTHOS, represents the prelude to neuronal death. It encapsulates a self-amplifying failure of autophagic clearance culminating in structural disintegration via LMP. Once a neuron reaches this stage, it is unlikely to recover; it’s analogous to an engine that has seized – too gummed up with waste to run, and now leaking oil everywhere.

We should note that PANTHOS may not occur in every neuron uniformly; it's probably a phenomenon in those neurons that are both heavily burdened and for some reason unable to upregulate compensatory pathways enough. It might occur in “bursts” – a neuron stays in a stressed state for a while, then hits PANTHOS and rapidly dies, releasing its contents. This could manifest in humans as the relatively sudden appearance of new plaques or bursts of neuroinflammation. Some imaging studies in AD patients indeed show that neurodegeneration isn’t completely linear – there may be punctuated periods of accelerated decline, possibly reflecting waves of neuron loss. PANTHOS events could underlie such episodes.

In conclusion for this chapter, we have drawn a picture of a neuron at the funnel’s neck: loaded with undigested cargo (Aβ-positive AVs), trying desperately to clear them by making more autophagosomes, and ultimately suffering lysosomal rupture and death. The PANTHOS concept synthesizes many threads – it directly connects the subcellular failure (autophagy collapse) with a visible pathological hallmark (perikaryal blebbing and plaque precursor formation) and an outcome (LMP-mediated cell death). With this understanding, we can now proceed to the final stage of the funnel: the output. We will explain how the inside-out model of pathogenesis emerges from neuronal PANTHOS death, connecting to the formation of extracellular lesions such as senile plaques, and how this reinterprets the role of those lesions in diseases like Alzheimer’s.

Chapter 4: The Output – The 'Inside-Out' Pathogenesis

Having followed the funnel from the wide rim of triggers to the narrow neck of PANTHOS collapse, we reach the output stage: the aftermath of neuronal death by autophagic failure, and how this outcome manifests as the classical lesions observed in neurodegenerative diseases. Traditional views often regarded extracellular aggregates (plaques in AD, Lewy bodies in PD (which are actually intracellular), etc.) as causative toxic agents depositing in the brain. The "inside-out" pathogenesis model flips this perspective for Alzheimer's disease and possibly other proteinopathies: it posits that the hallmark extracellular lesion – the senile plaque – is not an exogenous toxin that kills the neuron, but rather the tombstone or debris field left behind after the neuron dies from an internal catastrophe (Nixon, 2017; 2022)[^3][^8]. This chapter will connect modern findings by Ralph Nixon and colleagues to historical observations by Oskar Fischer, demonstrating a remarkable concordance: what Fischer termed "miliary necrosis" (small focal death of neural tissue) and "drusige (druse-like) neurites" corresponds to what we now identify as neurons undergoing PANTHOS and leaving behind plaques composed of their intraneuronal contents. We will synthesize these ideas and discuss how the inside-out model reframes our understanding of plaques and potentially other lesions, with implications beyond AD. In essence, this output stage of the funnel completes the journey: multiple inputs → one mechanism (autophagic collapse) → one terminal cellular event (lysosomal death) → yields the known pathological signature (plaque) as an output.

4.1 From Neuronal Collapse to Extracellular Lesion: When a neuron undergoes lysosomal membrane permeabilization and dies, what happens to the accumulated contents that were once inside it? Two primary outcomes are possible: (a) phagocytic cells (microglia in the CNS) rapidly clear the debris; or (b) if the debris is indigestible or overwhelms phagocytes, it remains as an extracellular deposit. In AD, the cores of senile plaques are largely composed of fibrillar Aβ peptide, often surrounded by other cellular remnants (lipids, metals, and proteins like apolipoprotein E, complement factors, etc.) (Selkoe, 1994)[^93]. It has been puzzling why Aβ would accumulate extracellularly in such a focal, dense manner – if Aβ were simply secreted slowly over time, one might expect more diffuse deposits. The inside-out model provides a plausible answer: **the dense core plaque is essentially the spilled contents of a single neuron (or a few neurons) that died loaded with aggregated Aβ and autophagic material**.

Ralph Nixon's recent work directly supports this. Using 5xFAD mice (an aggressive AD model) and advanced imaging, his team tracked individual "PANTHOS" neurons and observed that these neurons were the principal contributors to new plaque formation [1] [2]. In compromised neurons, Aβ (particularly Aβ_42) and APP-βCTF had accumulated in enlarged, de-acidified autolysosomes; when those neurons degenerated (often marked by LMP and then disappearance of the soma), a new extracellular Aβ deposit appeared at that location (Lee et al., 2022)[^8]. By quantitative analysis, they estimated that the vast majority of senile plaques in those mice could be accounted for by an antecedent PANTHOS neuron [2]. The release of intraneuronal Aβ in a bolus upon cell death would form a seeding core around which some extracellular polymerization might continue even after the neuron is gone. Microglia would attempt to clean up the mess – indeed, plaques are often associated with activated microglia and infiltrating astrocytes – but Aβ fibrils are partly protease-resistant and can persist in the tissue (especially if they become deposited on scaffolds like dystrophic neurites or extracellular matrix).

Another intriguing aspect is the presence of dystrophic neurites around plaques. Fischer described "club-shaped neurites" in association with his later-stage plaques (Fischer, 1907)[^10][^11]. Modern immunostaining shows that around each amyloid plaque in AD brain, there are numerous swollen, torpedo-like neuritic fragments positive for APP, Aβ, ubiquitin, and autophagy markers (Su et al., 1993; Nixon et al.,

2005)[^46]. These are interpreted as the remnants of neurites from one or more neurons that were connected through that region. The inside-out model suggests that some of these dystrophic neurites may actually belong to the dying neuron itself – as its soma breaks apart, pieces of its dendrites/axons loaded with autophagic debris might remain caught in the sticky plaque matrix. Others may belong to neighboring neurons whose processes ran through the area and got secondarily affected by inflammatory or toxic factors from the plaque (like a collateral damage). Fischer's staging of plaques (I–V) as a continuum of lesion development matches with this – early plaques might start intracellularly (stage I,II in Fischer's terms, possibly small accumulations within neuron), then stage III–V involve "drusige Wucherungen" (club-like proliferations) i.e., dystrophic neurites and expansion of the lesion as the neuron disintegrates [3] [4].

To further marry the historical with the modern: Fischer called plaques "miliary sclerosis" or necrosis because he believed they were tiny areas of degeneration, not foreign bodies. He observed glial reactions around them consistent with cleaning up debris (Fischer, 1907; Goedert, 2009)[^10][^11]. Oskar Fischer's contemporary Alois Alzheimer, on the other hand, was more cautious in interpretation; he noted the presence of plaques and tangles but did not assert their nature, though later researchers leaning on Alzheimer's prestige often treated plaques as primary lesions. One cannot help but notice that Fischer's interpretation aligns better with what Nixon et al. are now showing: plaques are sites of "necrosis" (or cell death) with neuritic debris.

4.2 Synthesizing Nixon's Findings with Fischer's Descriptions: Let's explicitly draw parallels:

- Fischer's *"miliare Nekrosen…eine regelmässige Veränderung…bei seniler Demenz"* (tiny necroses, a regular change in senile dementia) implies that these tiny areas of tissue death were consistently found in dementia [5]. He likely saw loss of neurons and local debris. We now think that widespread neuron death occurs in AD, but identifying individual neuron's death events is hard in post-mortem static tissue. Nixon's approach essentially caught neurons in the act of dying (via PANTHOS markers) and then correlated that with plaque presence [1] [2]. This is a direct vindication of Fischer's assertion that plaques are necrotic foci.

- Fischer's "drusige Wucherungen der Neurofibrillen" (druse-like excrescences of neurofibrils) refer to the swollen, beaded neurites he saw around plaques [5]. "Druse" in pathology usually refers to round deposits (like in the eye's Bruch's membrane in macular degeneration). He thought these were proliferations of neurofibrils (which we now know as accumulations of mislocalized cytoskeleton and organelles). In PANTHOS, we see neurites filled with mislocalized organelles (like autophagic vacuoles and even tau filaments). In fact, the neurofibrillary tangles of AD (which Alzheimer emphasized) are composed of tau filaments and often found in cell bodies and proximal dendrites of neurons – it's plausible Fischer's term "neurofibrillar proliferations" partially was describing early tangle-like changes or neurites stuffed with filaments. So, the inside-out model can also incorporate tau pathology: a neuron in distress might hyperphosphorylate tau (due to calcium release from lysosomes or other stress kinases) and those tau proteins misassemble into tangles within the blebs. If the neuron dies, tau filaments can persist as ghost tangles, or possibly get released and taken up by other cells (which might propagate pathology). Fischer didn't know about tau, but his visual of neuritic pathology is in line with modern tau and neuritic plaque pathology.

- Ralph Nixon's findings emphasize that fixing lysosomal pH issues genetically or pharmacologically can prevent PANTHOS and subsequent plaque formation (e.g., overexpressing TFEB to boost lysosomal function reduced plaques in an AD model) (Medina et al., 2011)[^94]. If Fischer had the

tools, he might have asked: can we prevent plaques by preventing that “necrosis”? Today, we are effectively asking that via lysosomal-targeted therapies. The convergence of thought is that it’s the degenerative process in the neuron that should be targeted, not just the extracellular plaque.

4.3 The Senile Plaque as Neuronal Tombstone: Reimagining the senile plaque as a tombstone of a neuron that perished reframes many aspects of AD research:

- *Causality:* It suggests that Aβ plaques are more of an epiphenomenon, or at least late-stage event, rather than the inciting cause of neurodegeneration. They mark where a neuron has died, rather than being the reason a neuron dies. This addresses some long-standing puzzles, such as why cognitive decline correlates poorly with plaque load (many plaques may simply indicate many neurons already lost, whereas soluble Aβ or tangles correlate better with ongoing disease severity).

- *Therapeutic Strategy:* If plaques are tombstones, then removing them (like with antibodies) might be like removing tombstones from a graveyard – it doesn’t bring the dead back, though it might clear inflammatory stimulus. This could partly explain the underwhelming results of some amyloid-clearing therapies: they may clear plaques but if the neurons died to create those plaques, the damage is done. Instead, therapies might need to protect neurons from reaching PANTHOS – e.g., by enhancing lysosomal function, reducing autophagy load, or preventing v-ATPase dysfunction. There is increasing interest in drugs that activate lysosomal biogenesis (like via TFEB) or that stabilize lysosomal pH (e.g., targeting chloride channels or proton channels to optimize the ionic environment) (Colacurcio et al., 2018)[^13]. The inside-out model provides strong rationale for those approaches.

- *Transneuronal Spread:* One of the debates in neurodegeneration is whether pathologies spread from cell to cell (prion-like) or arise cell-autonomously. Inside-out doesn’t exclude spread, but it suggests that at least for Aβ, a lot of it accumulates inside susceptible neurons and is then released. Once outside, Aβ could seed other plaques or diffuse – but interestingly, Aβ plaque distribution in AD doesn’t always follow neuroanatomical connectivity as clearly as tau pathology does. Tau tangles seem to spread along synaptic circuits (Braak stages), whereas amyloid plaques appear in a more diffused pattern possibly linked to where vulnerable neurons reside and perhaps blood flow patterns. This could align with inside-out: any neuron with enough stress might produce a plaque, not necessarily because it caught Aβ seeds from a neighbor, but because it succumbed to internal stress. That being said, extracellular Aβ could still seed deposition in nearby neurons’ endosomes if taken up, so a mix of cell-autonomous and spreading may occur.

- *Other Diseases:* Does inside-out apply beyond AD? Possibly, yes. In Parkinson’s, could Lewy bodies (rich in α-synuclein) sometimes represent an “inside-out” phenomenon? Many Lewy bodies are intracellular and cause death by crowding, but when a neuron with a Lewy body dies, the Lewy body can become an extracellular “Lewy neurite” or be partially cleared. In some PD cases, extracellular Lewy body remnants are found, which could similarly be tombstones of neurons. In ALS, the extracellular deposits of TDP-43 or SOD1 are not typical, but perhaps the glial scar after a motor neuron death contains bits of those proteins.

- *Microglial response:* Viewing plaques as debris emphasizes the role of microglia and clearance mechanisms. Microglia are the brain’s undertakers. If their function is impaired (due to age or genetic factors like TREM2 variants, which are risk factors for AD), plaques/tombstones might persist

longer or cause more collateral damage. This synergizes with the idea that boosting microglial clearance (once a neuron has died) could mitigate secondary inflammation or seeding, even if the primary goal is to prevent neuronal death in the first place.

In synthesizing Nixon and Fischer: Nixon provides the molecular cell biology evidence for what Fischer intuited by looking down a microscope 115+ years ago. The scientific community, for much of the 20th century, largely focused on the wrong "suspect" (the plaque itself) rather than the process that created it (the dying neuron). With the inside-out framework, we integrate the two: the plaque is evidence of the crime, not the perpetrator. And Fischer's somewhat forgotten legacy is rehabilitated – his description of "miliary necrosis" was prescient in emphasizing localized cell death, something modern AD research is circling back to (Saito & Saido, 2018)[^95].

4.4 Conclusion of Inside-Out Funnel: Bringing the funnel metaphor full circle: we started with a wide array of possible causes (genetic, viral, toxic) and have now ended with a unified outcome – neurons undergoing autophagic collapse and leaving behind characteristic lesions. This progression underscores a key argument of this thesis: focusing on the convergent mechanism (autophagic-lysosomal failure) provides explanatory power for both the diverse inputs and the common outputs of neurodegeneration.

To recapitulate, the funnel analysis shows that:

- Upstream factors load the gun (by stressing autophagy/lysosomes).
- Mechanistic convergence pulls the trigger (via v-ATPase/acidification failure).
- PANTHOS is the bullet in motion (the neuron fatally wounded from inside).
- The senile plaque is the exit wound (visible evidence that a neuron was shot down).

Thus, the senile plaque truly is a tombstone marking where a neuron fell. It does not typically kill neighboring neurons by itself (though sustained inflammation from many plaques might contribute to an unfavorable environment). Instead, each plaque tells a story of a single neuron's demise. And those stories collectively *are* the disease progression.

In the final **Conclusion** section to follow, we will tie together the insights gleaned from each chapter, discuss the broader significance for neurodegenerative disease research, and propose future directions – particularly how understanding convergent autophagic collapse might open new avenues for intervention, shifting the paradigm away from solely targeting extracellular aggregates and toward bolstering intracellular clearance and resilience.

Conclusion

Restating Findings: This thesis set out to rigorously answer how diverse upstream insults in neurodegenerative disease can lead to a singular terminal cellular catastrophe, encapsulated in the "Theory of Convergent Autophagic Collapse." Through a funnel-structured analysis, we demonstrated that seemingly disparate factors – genetic mutations, viral infections, and environmental/metabolic toxins – all converge on the neuron's autophagy-lysosomal pathway as the vulnerable nexus. The wide rim of the funnel encompasses specific etiological inputs: mutations in *PSEN1, APP, LRRK2, GBA1,* and *C9orf72* that each impair some aspect of proteostasis (from lysosomal acidification to cargo recognition), neurotropic viruses like HSV-1, enteroviruses, and Zika that hijack or block autophagy at various stages, and toxins such as heavy metals and lipid peroxidation products that inflict damage on lysosomal enzymes and membranes.

As we narrowed the funnel, we saw these inputs physically and functionally intersect at common machinery – notably, the autophagosome-lysosome fusion and acidification processes governed by the v-ATPase proton pump. We detailed how phosphorylated APP-CTF binding to v-ATPase V0a1 and oxidative modification of v-ATPase subunits exemplify molecular interferences that many insults share or mimic, resulting in a failure to acidify and degrade autophagic cargo. The neck of the funnel, the PANTHOS phenomenon, was characterized as a distinctive state of neuron at collapse: perikaryal blebbing with rosettes of undigested autophagic vesicles (Aβ-laden), reflecting a traffic jam where autophagy induction is unabated but clearance is paralyzed. This state inevitably progressed to lysosomal membrane permeabilization and neuronal death. Finally, the output of the funnel reframed the classical understanding of lesion formation: neuronal death from autophagic collapse leads to the expulsion of proteopathic contents, which form extracellular deposits such as senile plaques. By synthesizing modern data (Ralph Nixon's autophagy studies) with historical accounts (Oskar Fischer's early 20th-century observations), we concluded that senile plaques are tombstone-like markers of neurons that died from inside-out mechanisms, rather than external toxins that cause neurons to die.

Contributions to the Field: This work makes several contributions across conceptual, methodological, and potentially therapeutic domains:

1. *Unified Pathogenic Framework:* It provides a unified theoretical framework that links multiple previously siloed hypotheses in neurodegeneration. For instance, the amyloid hypothesis, tau hypothesis, calcium dysregulation, and lysosomal dysfunction hypotheses of AD are often debated separately. The convergent collapse model integrates them: e.g., Aβ accumulates due to lysosomal failure, tau pathology can be exacerbated by autophagic stress (since kinases and phosphatases become dysregulated in stressed neurons), and calcium may leak from lysosomes during LMP, linking to excitotoxicity. By showing how these pieces fit in a temporal sequence, the thesis advances our holistic understanding of disease progression.

2. *Historiographical Insight and Course Correction:* By revisiting Oskar Fischer's work in light of modern evidence, this research performs a historiographical reappraisal that credits an overlooked pioneer and suggests that some early interpretations that were overshadowed by the amyloid cascade dogma deserve resurrection. This is not merely historical trivia; it provides a course correction for current research emphasis. The field has been reminded that focusing on the neuron's degeneration process (which Fischer essentially pointed to) is as important as cataloguing the protein aggregates found after the fact.

3. *Methodological Integration:* The approach taken – combining literature from cell biology, neuropathology, virology, toxicology, and historical texts – underscores the value of interdisciplinary synthesis in tackling complex diseases. This thesis stands as an example of how "deep research" can connect dots across domains: for instance, by putting a virologist's discovery about autophagy sabotage in conversation with a neurologist's observation of protein aggregates. The methodological blueprint here can be applied to other diseases with multifactorial etiologies (e.g., consider how convergent failure might apply in metabolic syndrome's effect on organs, etc.).

4. *Refined Targets for Intervention:* On a practical level, the findings highlight underexploited therapeutic targets. Strengthening lysosomal acidification (for example, via small molecules that stabilize v-ATPase assembly or enhance proton conductance selectively in lysosomes) emerges as a strategy that could counteract many insults at once. There is growing interest in drugs like cyclodextrins,

histone deacetylase inhibitors, or TFEB activators that can improve lysosomal function – our analysis strongly supports prioritizing such approaches. Additionally, therapies aimed at early removal or reduction of APP-CTF (C99) might prevent the cascade of autophagic collapse, pointing to a nuance in anti-amyloid strategies (perhaps complementing Aβ-targeting with C99-targeting). For Parkinson's and related disorders, the analysis suggests that co-therapies addressing lysosomal function (e.g., increasing GCase activity in GBA1 mutation carriers, or LRRK2 kinase inhibitors to ease trafficking jams) may synergize with approaches like anti-synuclein agents.

5. *Connection to Aging Biology:* The convergence on autophagy also aligns neurodegeneration with general aging mechanisms. It implies that neurodegenerative diseases may be accelerated or exaggerated manifestations of an aging process – lysosomal decline – that in milder form affects all individuals. Thus, it lends support to the notion that enhancing autophagy/lysosomal health could extend not only healthy lifespan of neurons but perhaps overall healthspan.

Limitations: While the thesis argues strongly for the convergent autophagic collapse model, it is important to acknowledge limitations and alternative perspectives. One limitation is that much of the evidence is correlative or from animal models. Human verification of "inside-out" events is challenging, though the circumstantial evidence (like presence of intraneuronal Aβ preceding plaques in Down syndrome brains, or the correlation of neuritic plaque burden with markers of lysosomal stress in human AD) supports it. Another consideration is disease specificity: AD pathology is particularly well explained by this model, but other diseases (like pure tauopathies or prion diseases) might involve variations – e.g., prions cause direct toxicity that might not always need autophagy failure to kill cells (though even there, prion propagation could be facilitated by autophagy pathways). Thus, convergent collapse might be a primary driver in some diseases (AD, PD, ALS) and a secondary or contributing factor in others.

Additionally, not every neuron in AD undergoes PANTHOS; some die via other routes or not at all. Cognitive decline in AD also involves synaptic dysfunction from soluble Aβ oligomers, which our model only indirectly addresses (we might posit that those oligomers themselves often come from neurons that are starting to fail at clearance, but oligomers can also be secreted normally). Therefore, while inside-out covers plaque genesis, a comprehensive disease theory would integrate how early synaptic toxicity and later tangle pathology fit in – tangles, for instance, might spread independently but still could be exacerbated by autophagy issues (since impaired autophagy can increase tau aggregation and vice versa).

Future Directions: This thesis opens several avenues for further research:

- *Biomarkers:* If convergent autophagic collapse is key, can we detect it in living patients? Potential biomarkers might include elevated neuronal autophagy markers in CSF or blood exosomes (e.g., LC3-II levels, p62, LAMP1 fragments indicating LMP). Recent studies have found extracellular vesicles carrying lysosomal proteins from neurons might be increased in AD; validating such markers could allow early detection of autophagy stress before widespread neuron loss.

- *Longitudinal Imaging:* The prediction that PANTHOS leads to plaques could be tested in experimental systems. In vivo two-photon imaging in AD mouse models might catch neurons filling with autophagic vacuoles and then see if a plaque appears at that site after the neuron disappears. Some of this was done by Lee et al. (2022), but more refined real-time observation could strengthen causality. In humans, advanced PET tracers that detect lysosomal activation or autophagic vacuoles

(if developed) could potentially show hotspots of autophagy dysfunction that precede amyloid PET positivity.

- *Applicability to Other Diseases:* Each neurodegenerative disorder could be re-examined through this funnel. For example, does a similar funnel exist for synucleinopathies? Upstream hits: *LRRK2, GBA1, pesticide exposure, aging* → Converge on lysosomal dysfunction → "PANTHOS-like" event for dopaminergic neurons → release of α-synuclein leading to Lewy body remnants. Some evidence suggests neuromelanin (which accumulates in dopaminergic neurons) could be a parallel to Aβ in being an intraneuronal buildup that on neuron death becomes an extracellular marker (dopamine neurons that die leave neuromelanin granules taken up by microglia). Exploring these parallels could generalize the inside-out hypothesis.

- *Therapeutic Trials:* Perhaps the most important future direction is translating this knowledge into therapy. Drugs that enhance lysosomal acidification or autophagic flux could be tested in models of AD/PD for efficacy. For AD, combining an amyloid-lowering approach with an autophagy-boosting approach might yield synergistic benefits: the former reduces new burden, the latter helps clear existing burden and prevent collapse. Genetic therapy is another angle: could we, for example, provide a neuron with a "backup" acidification mechanism? One wild idea is targeting neurons with proton pumps from other sources (like light-activated proton pumps or Na^+/H^+ exchangers) to compensate for v-ATPase decline – essentially giving neurons an extra way to acidify compartments if v-ATPase is failing.

- *Cross-disease Comparisons:* Another line of inquiry is comparing why certain neuron types are more prone to autophagic collapse. In AD, large pyramidal neurons in cortex and hippocampus are vulnerable, whereas in PD, dopaminergic neurons of substantia nigra are key. Are there cell-type differences in basal autophagy or lysosomal resilience? For instance, substantia nigra neurons have huge axonal trees and may be especially challenged to transport autophagosomes to the soma for digestion; similarly, long corticocortical neurons have high metabolic load. Understanding these differences might explain selective vulnerability in diseases.

Closing Thoughts: In conclusion, this dissertation has argued that the end-stage pathology of neurodegeneration is best understood not as an external assault by protein aggregates, but as an internal systems failure – a collapse of the cell's recycling and waste management system under multifactorial stress. This perspective does not so much overthrow prior findings as it reorganizes them into a more coherent cause-effect timeline. By adopting an integrative funnel view, we can appreciate how a tangle of causes can result in one final common pathway, offering a strategic vantage point for both comprehension and intervention. The hope is that by targeting the convergent point (autophagic-lysosomal function), we might intervene in a way that is robust to the various causes – akin to fortifying the dam rather than chasing down every raindrop that contributes to the flood.

In bringing to light the consonance between early 20th-century neuropathology and 21st-century cell biology, this work also reminds us that progress in science is often a spiral: we return to old ideas with new tools and understanding. The theory of convergent autophagic collapse bridges past and present, providing a refined narrative for neurodegenerative disease that honors the complexity of its inputs but finds unity in its outcome. If this narrative is correct, then the neuron's most ancient defense mechanism – autophagy – might also be its Achilles' heel when overwhelmed, and saving the neuron's life may depend on shoring up this vital system against the many insults of time, genes, and environment.

Future Research Directions: (as an epilogue, optional) Based on these conclusions, future research should prioritize: - Developing imaging and fluid biomarkers for autophagy-lysosomal dysfunction in patients. - Investigating autophagy-enhancing treatments in early disease stages (possibly repurposing drugs like mTOR inhibitors or those used in lysosomal storage disorders). - Exploring gene therapy or CRISPR-based fixes for known autophagy-related mutations (*e.g.*, correcting PSEN1 processing of V0a1, or supplementing GBA in PD). - Examining other proteinopathies under the inside-out lens to see if similar patterns emerge, thereby broadening the paradigm of convergent collapse beyond AD.

By continuing in these directions, the ultimate goal would be to delay, halt, or even reverse neurodegenerative diseases by ensuring neurons do not drown in their own waste – a fitting strategy if indeed the root cause of the final collapse is an overwhelmed cellular sanitation system. In the battle against diseases of aging, enhancing the cell's housekeeping might prove to be one of the most effective ways to keep the lights on in the aging brain.

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