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Investigating Potential Synergistic Effects of LM11A-31 and DCP-LA to Protect Neurons Under an Oxidative Stress Model of the Aging Brain

A Thesis in Neuroscience

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Abstract

Aging, traditionally considered a natural developmental process, shares critical biological hallmarks with pathological conditions (for example, Alzheimer's disease). Through an exploration of what exactly these mechanisms are emerges a compelling case for aging's reconsideration as a treatable condition. The effects of contemporary treatment methods for neurodegenerative diseases are modest at best, delaying only symptom progression. Either reversing damage already done or targeting the root causes before they develop requires a restructuring of the discourse surrounding how these deficits occur in the first place. If aging is considered as a process driven by modifiable cellular events, such as dysregulated oxidative stress or dendritic atrophy, they can be therapeutically addressed before cognitive deficits manifest. Highlighting the roles of two drugs that have only ever been analyzed independently of one another, DCP-LA and LM11A-31, this study surveys their potential synergy on cell viability and microtubule stability in neuronal cell culture under varying degrees of oxidative stress. This study followed a Ferrous-Amyloid-Buthionine-NMDA (FABN) model of oxidative stress, measuring viability via MTS assay and stability through immunocytochemistry. LM11A-31, by modulating p75 neurotrophin receptor signaling, may mitigate apoptosis triggered by oxidative stress and amyloid-beta toxicity. Similarly, DCP-LA is known to enhance PKC-ε's activity, critical for synaptic plasticity, neurotransmitter release, and cytoskeletal stability. There is an urgency to develop preventative interventions to offset both the projected economic burden of neurodegenerative diseases as well as society's apprehension towards them, and these two drugs working in tandem may be a step in the right direction in doing so.

Contents

Introduction

Aging's Neural Odyssey: Unveiling the Complications of Aging

This paper aims to conduct a comprehensive exploration of the aging process and its impact on cognitive health, honing in primarily on how the definition of aging should be reevaluated. While often considered a natural and inevitable process, aging entails rather distinct biological mechanisms and consequences that blur the line between normal development and pathological decline. It will be considered that aging is not simply a passive accumulation of time, but an active series of interactions between cellular events like oxidative stress, inflammation, and mitochondrial dysfunction. These processes establish an environment conducive for cognitive decline, especially in the context of neurodegenerative diseases like Alzheimer's disease (AD). The argument at hand is one that juxtaposes the shared biological markers of Alzheimer's disease and aging, such as amyloid-beta (Aβ) plaques and tau tangles. Aging does not guarantee the development of dementia, but given the similarities between "normal aging" and dementia, the variability seen in cognitive outcomes are emphasized by the profound influence of environmental, genetic, and lifestyle factors corroborated by contemporary research. Current therapeutic strategies for Alzheimer's disease and pathologies alike focus predominantly on symptom management, and are often critiqued as being insufficient in treating the cause before the disease develops. Addressing the underlying aging processes that predispose the brain to neurodegeneration by reconsidering what defines "normal aging," and going further as to inquire if aging was ever truly "normal" in the first place, may catalyze a preventative treatment approach that targets these deficits before they begin to sequester. The unfolding case is one that calls for a paradigm shift toward targeting aging itself as a modifiable phenomenon, and suggests emerging neuroprotective interventions, particularly ones involving the

neurotrophin p75 receptor and protein kinase C-ε , may offer novel opportunities to delay or mitigate cognitive decline to improve quality of life in older populations.

Social and Financial Implications of Aging

Within the realm of neuroscience, few subjects evoke as profound a sense of apprehension and trepidation as the process of aging. While the passage of time brings with it experience and wisdom, it simultaneously presents a host of obstacles, not the least of which is the fear of losing one's independence. A 2024 survey of over 2,000 U.S. adults revealed that 63% worry most about the potential of declining health, where specifically 80% of individuals 77 years and older dread cognitive decline in particular (Hall, A., 2024). This fear, passionately embedded in the collective consciousness, speaks to a fundamental constituent of human existence — the value of autonomy and self-efficacy. As individuals progress towards the later stages of their lives, the specter of dependence grows darker, casting a shadow over what should otherwise ideally represent a period of introspection and fulfillment. On average, it costs $9,000 a month for a single room in a nursing home alone, not to mention the additional fees and services if memory care is necessitated for those living with dementia (Care Patrol). Overall, it is estimated that the cost associated with caring for and treating those with dementia will reach 368 billion dollars by 2040 (Super, N., Ahuja, R., & Proff, K., 2019).

The Physiological Costs of Growing Older

Aside from financial worry, contributing also to this apprehension are none other than the notorious physical burdens associated with aging, as managing one's health proves increasingly complicated. Aging and the changes it brings contributes to the pathogenesis of several diseases,

including cardiovascular, neurodegenerative, biliary, and kidney disease, as well as macular degeneration and even cancer (Liguori, I., et al., 2018). The cellular events that are most prevalent as one ages may exacerbate tissue damage, inflammation, and dysfunction, ultimately leading to the development and progression of these illnesses. Of the cohort of individuals aged 50 and older, the population of those living with at least one chronic disease is projected to increase 99.5%, meaning that of the 71.5 million adults in the U.S. living with a disease, that number is expected to reach 142.6 million by 2050 (Ansah, J. P. & Chiu, C. T., 2023). The national population is aging, with a larger proportion reaching older age brackets. As people grow older, they are more likely to develop chronic health conditions due to cumulative exposure to risk factors, genetic predispositions, and age-related changes in physiology (Fabbri, E., et al., 2015; Lloyd-Jones, D. M., et al., 2006; Manolio, T. A., et al., 2009). Chronic illnesses are often multifactorial and complex, requiring long-term management and care. The prospect of assuming age-dependent conditions forges a landscape that brings with it the need for reassurance amidst a time that should be characterized by the free pursuit to undergo novel experiences and create new memories.

There are many different cellular changes that take place over time. Oxidative stress and cellular senescence, in which cells cease to divide and enter a state of irreversible growth arrest, are natural processes that occur as cells age and respond to stressors in their environment. Senescent cells often exhibit altered gene expression and secrete senescence-associated secretory phenotype (SASP) factors, which can have both beneficial and detrimental effects on surrounding tissues (Cuollo, L., et al., 2020). Although their role in bringing about cell-cycle arrest in senescent cells allegedly supports their tumor-suppressive abilities, SASP factors also

have the power to ignite inflammation and even tumor formation (Krtolica, A., et al., 2001; Rodier, F., et al., 2009; Watanabe, S., et al., 2017).

Should Aging Be Treated as a Medical Condition

In the current climate, there are mainly two sides encompassing the debate of what exactly aging is. There are scientists who regard aging as a continuation of the normal developmental process — something that does not need to be treated. Others consider it to be a distinct phenomenon altogether, one which should be treated as a medical condition. To determine whether aging is something to "treat," it is crucial to compare how scientists approach diseases. Consider hiccups versus chronic illnesses. A case of hiccups is a generally benign, mildly troublesome condition, rarely requiring medical intervention. In contrast, chronic illnesses like diabetes or heart disease involve significant health risks and the implementation of long-term management strategies in order to reduce mortality and improve one's quality of life. This distinction between conditions warranting treatment and those that perhaps do not is made based upon the severity, impact on daily functioning, and the underlying pathology characterizing them. Applying this framework to aging, some researchers contend that aging results from the deterioration of the regulatory processes governing morphogenesis, or development, and morphostasis, or maintenance/stability of an organism's form (Walker, R. F., 2022). This idea posits that the same regulatory mechanisms responsible for growth and development during early life also guide maintenance and repair in adulthood. As these regulatory processes deteriorate over time, the result is aging. This perspective emphasizes that the decline in these processes is consistent with the notion that aging is simply an extension of the development trajectory, not a separate event. Those who view aging as such might argue that

it is a natural part of life, akin to the hiccups scenario, where the focus should be on managing symptoms rather than treating it as a disease. It may be presumed that aging encompasses a series of normal biological changes that, while challenging, do not necessarily meet the criteria for being a pathology needing treatment.

On the other hand, others suggest that the aging process, including mitochondrial dysfunction and loss of proteostasis (protein homeostasis), actually diverges from developmental pathways (Mallilankaraman, K. B., Kennedy, B. K., Sorrentino, V., & Luciani, A., 2024). From this viewpoint, aging involves specific degenerative processes that could be considered analogous to chronic illness, suggesting that interventions targeting these processes could mitigate the negative impacts of aging. This is similar to how diseases like diabetes are treated today, by identifying their underlying mechanisms. How, then, might society decide which viewpoint is correct? It may simply boil down to the very definition of "disease:" "a disorder of structure or function in a human, animal, or plant, especially one that has a known cause and a distinctive group of symptoms, signs, or anatomical changes" (Oxford University Press., 2024). Aging is not this vague, inevitable decline, but rather a process driven by specific biological mechanisms that do bring about certain typical symptoms. Accordingly, mitochondrial dysfunction and loss of proteostasis are distinct events and could potentially be targeted to produce therapeutic effects. Viewing aging through the lens of these specific mechanistic events would allow scientists to pinpoint precise biological processes that drive aging. Once aging is understood as a process made up of distinct mechanisms, it becomes possible to develop treatments specifically targeting these mechanisms. Shifting the focus from merely managing the symptoms to their root causes, aging's biological pathways can be better understood, just like how chronic illnesses are targeted via their molecular mechanisms.

One's Youth, Habits, and Environment: The Foundations of Cognitive Aging

Understanding aging as a series of events to be therapeutically targeted is one piece of the puzzle. These cellular checkpoints are not met at the same time for everyone nor do they come with the same degree of change as a consequence of the infinite confounding factors of every single person's own environment and habits. It is contemporarily theorized that maturation of the brain is typically achieved between one's mid-to-late 20s. Research has demonstrated time and time again that adolescents and young adults are exceptionally vulnerable to social impulses wielding the power to wreak havoc on neurodevelopment. The use and abuse of alcohol and drugs during this critical period of myelinogenesis, among other integral neurological processes occurring at this stage, may culminate in white matter dysfunction (Arain, M., et al., 2013). Myelinogenesis, a developmental era crucial to effectively insulate neurons allowing for proper nervous system communication, has been demonstrated to continue into adulthood. It has long been argued that a human's "prime time" is their youth, but statements like these ignore that even in one's younger years, detrimental cognitive deficits can occur in a different kind of way — deficits based on choices that one may very well not have much control over. Previously discussed were "the root causes" of aging, and these root causes can begin in one's youth as a testament to their actions, habits, and environment. All this to say, because myelinogenesis has been shown to continue into adulthood and can be disrupted by one's environment, perhaps it can also be positively influenced too, further corroborating just how indispensable it is to engage in behaviors promoting brain health during this time. The cognitive reserve hypothesis presupposes that interacting with and maintaining a cognitively stimulating career/array of hobbies hinders the onset of cognitive impairment (such as mild cognitive impairment, Alzheimer's disease, etc.). Instituting a routine task intensity (RTI) index, where lower scores indicate an occupation that is

more cognitively demanding, those in a high RTI cohort (part of a less cognitively challenging occupation) are predicted to have a greater risk of cognitive decline after 70 years of age than those in more mentally engaging fields (Edwin, T., et al., 2024). The idea of "non-use" of the brain during one's 50s, 40s, 30s, and even 20s appears to lead to higher risk of dementia later on that may be palliated by self-intervention. Emanating from this principle is an infamous study evaluating over 600 nuns aged 75 to 102 at the time of enrollment, which has provided even more of a reason to advocate for sustaining intellectual activity as much as possible. Beginning in 1986, David Snowdon of the University of Kentucky has finely combed through the lives of the School Sisters of Notre Dame who had elected to participate (Lemonick, M. D. & Park, A., 2001). They led similar lifestyles in terms of diet, physical activity, healthcare access, and routines, and also represented a highly educated population of adults. Many in their older age possessed an impressive mental sharpness even in the presence of senile plaques and neurofibrillary tangles almost indistinguishable from that of a brain expected to be cognitively declining. Via assessments of their autobiographies, those who consistently produced more positive emotion tended to see a lower onset of dementia. Those who did go on to experience cognitive decline were more often than not beginning to use less and less positive emotion in their writing as time progressed. Furthermore, the complexity and richness of the content of these autobiographies appeared to be strong predictors of dementia decades later (Snowdon, D. A., et al., 1996). Publications written in early life with low idea density (the rate in which new ideas are presented) and grammatical complexity persistently correlated with lower cognitive test scores years later. Of the 14 nuns in the study who had passed away and were categorized as having written with low idea density in their early life writings, all were later found to have neuropathy confirming Alzheimer's disease. Correspondingly, none of those with high density were affected.

By fostering activities that augment thought and provoke academic rumination, one can potentially bolster cognitive resilience. Or it is possible that those who are predisposed to AD may struggle with higher cognitive abilities even at a younger age. Such may very well be a reason why not everyone will see the same mental changes as they age, even when living in comparable environments.

Is Age Just a Number or is it a Few

Biological Age

Nonetheless, after internal and external factors have taken their toll, influenced both by one's genetics and the environmental decisions they have decided to partake in — or the ones thrust upon them as one does not always have agency over their environment — the resulting consequence is a brain that will no longer be undergoing the same genre of development it once had. Instead of rapid periods of restructuring and enhancement, the brain, after one's 20s, will embark on the gradual journey of aging with expected changes and declines. In other words, it has been hypothesized that the prefrontal lobe, limbic system, neurotransmitter pathways, and other related structures will emerge from this early life experience to begin undergoing the gradual changes aging brings. This hypothesis is also one that lends researchers to begin contemplating why in one's later years does the brain not work as effectively. If maturation has been reached and these systems are left vulnerable to decline in some way, is that decline, or aging, the same for everyone? Evidently, present-day data would say no. For many years, scientists studying aging have largely assessed changes in genes, metabolism, and how these genes are activated or inactivated through the examination of epigenetics. Or, they have looked at how cardiac muscles and other tissues transform over time. Much of recent discourse has now

begun to consider what has been occurring at the cellular level, or more specifically, the biophysical properties of cells — how they move, how they are shaped, their mechanical traits, and other like processes. (Phillip, J. M., et al., 2017). Age is no longer limited to how many years an organism has been alive. Doctors can determine one's biological age using measurements like cholesterol levels, blood pressure, and lung capacity. However, these measurements, although most commonly utilized by physicians to assess one's biological age, vary in terms of their predictive accuracy and can be clouded by other variables of influence. A study sampling physiological data from over 55,000 individuals employed a mathematical model using measurements like cholesterol levels and blood pressure to predict the participants' biological age (Libert, S., Chekholko, A., & Kenyon, C., 2022). In doing so, they noted that their predicted value had changed with respect to various age-influencing environmental and genetic factors. Wealth, smoking/exposure to smoke, and alcohol consumption are just a few factors they found to influence their study's "Δage" variable (the deviation of one's biological age from his or her chronological age) and "youthfulness" variable (participants were believed to be more youthful if their biological age was lower than their chronological age). Alcoholic beverages, such as beer, negatively impacted one's predicted biological age, whereas champagne and wine did not, most likely because refreshments of this kind tend to be associated with higher socioeconomic status. For both men and women, their "qualifications" (years of schooling achieved) also impacted "youthfulness" in a statistically significantly positive manner: each year of additional schooling tended to correlate to greater "youthfulness." Wealth and education are non-biological variables that, in this study, appeared to possess the power to bring about physiological changes contributing to one's biological age (Libert, S., Chekholko, A., & Kenyon, C., 2022). Although it is possible for doctors to calculate biological age and take that value into account when devising

a treatment plan, there is often plenty of background noise that must be acknowledged when drawing comparisons between one's chronological age and this perceived calculation.

Cellular Age

Likewise, cellular age deviates from solely considering one's physical age. It surveys the functionality of the cell as opposed to the calendar year in which it is formed, and that measurement is not the same for everybody nor does it always directly coincide with one's physical age either. Understanding cellular age would not only permit scientists to better understand the aging process, but would also allow for more timely interventions of age-related diseases and personalized treatment plans. But like biological age, the process of measuring one's cellular age is not without its confounding variables. These measurements are commonly based on telomere length, which decreases as cells replicate, as well as the presence of cellular senescence markers (Vaiserman, A., & Krasnienkov, D., 2021). While telomere length can be a useful indicator, it too, is influenced by many factors. It has been demonstrated that genetic variants associated with shorter telomere length (certain single nucleotide polymorphisms) are linked to one's development of Grave's disease, for example (Ye, M., Wang, Y., & Zhan, Y., 2022). In sum, genetic predispositions can impact telomere length, swaying one's probability of developing disease, ultimately altering the trajectory of one's calculated cellular age. Lifestyle choices, including diet, physical activity, and smoking can also significantly affect telomere length (Chen, M., et al., 2024). Regular physical activity is associated with longer telomeres whereas smoking and poor diet are linked to accelerated telomere shortening.

Although biological and cellular age measurements can provide valuable insight into the quality of one's life, they can be blurred by both biological and non-biological influences.

Additionally, measurements like these are best compared between populations, due to the fact that there are stark contrasts from person to person. Nonetheless, because of how simple deviations in these values indicate an individual's risk for certain diseases and other health complications, there is still merit in utilizing them if one understands that these measurements can also be determined by genetics, environment, and lifestyle choices. This versatile influence reinforces how aging is tailored to the individual and the endeavors they partake in — it is not a cookie-cutter experience. Ultimately, there are compelling observations to suggest aging is not a monolithic experience. Aging does not appear to be solely under developmental control, and these environmental variations can further complicate aging's hallmark cellular events for better or for worse. How exactly and why exactly it occurs, and the speed in which it takes place, are data points that do not graph the same from one individual to the next. If medicine begins to treat aging as a variable phenomenon impacted by a myriad of different factors, it may be through the manipulation of these components that comprise aging that true peace of mind and confidence can be achieved for those approaching this stage of life.

Aging: Brain Mass versus Brain Volume

The brain undergoes significant transformations with age, marked by architectural changes that occur even in the absence of the pathological disturbances commonly associated with aging. Among the most popularized is the notion that the volume of the brain as well as its weight decreases approximately 5% each year after one has reached 40 years of age (Peters, R., 2006). However, recent studies indicate that the annual decrease in brain volume is actually closer to 0.2% to 0.5% each year, depending on the age group (Yamada, S., et al., 2023). Specifically, from the age of 40, brain volume is said to decrease, instead, 0.2% annually, and

this rate can increase to about 0.5% for those 60 years and older. Even though brain volume does seemingly decrease each year, brain volume and brain mass is not always directly correlated with cognitive health. During adolescence, for instance, brain mass decreases due to the reduction of gray matter approximately 1% each year, a process linked to synaptic pruning (University of Pennsylvania School of Medicine, 2017; Psychology Today). This pruning helps refine neural connections, enhancing synaptic efficiency. Hence, a reduction in mass or volume can be part of healthy brain development rather than an indication of decline. As exemplified, less does not always mean worse, but it is generally understood that brain mass declines tend to be more significant than volume loss in the sense that mass loss directly reflects a reduction in the neurons and synapses, leading to impairments in cognitive function and memory (Marhounová, L., et al., 2019). In contrast, brain volume loss might include changes in fluid spaces or the extracellular matrices, which are alterations that do not tend to be as directly linked to cognitive decline.

But if it is known that variations in lifestyles can induce radically different cognitive outcomes from one person to the next, is this annual volume loss truly the sole fault of aging? Donald Hebb's 1949 publication, *The Organization of Behavior: A Neurological Theory*, has partly addressed this inquiry. In investigating the stimulating quality of cage environments, Hebb discovered that rats raised in an environmentally complex (EC) condition demonstrated a specific disparity. When exposed to learning a novel task that was not only new, but also complex (tasks requiring the rats to hold on to bits of information to complete them correctly), waves of differences were illustrated (Hebb, D. O., 1949). It was not as if the EC rats learned things that the other rats could not learn, it was simply that they acquired the knowledge faster with less trials and reinforcement. Many subsequent studies replicated Hebb's experiment. One

study in particular involved raising the rats in varying conditions like Hebb (individual cages with no toys or friends, social cages with friends but no toys, and environmentally complex cages with toys and friends) and the researchers later analyzed their brains (Faherty, C. J., Kerley, D., & Smeyne, R. J., 2003). Neurons were stained to assess their morphology across varying regions of the nervous system. One region in particular of the hippocampus known as the CA1 region, involved with visual-spatial information and oftentimes thought to be associated with memory-tracking, displayed neurons most distinct for that of the EC group (Faherty, C. J., Kerley, D., & Smeyne, R. J., 2003). The intensity and the amount of branching increased dramatically. Moreover, the soma of the cells appeared much larger. One possibility is that the increased quantity of dendrites and the size of the soma are linked to one another. The golgi apparatus and the endoplasmic reticulum extend their membranes into dendrites and they are structures that are going to need to expand to aid in the generation of the physical components of those dendrites (Kennedy, M. J., & Ehlers, M. D., 2011). This process perhaps requires an enlarged soma to compensate. It is also possible that enhanced dendritic branching leads to the accentuation of the cellular process of signal summation for opening up the NMDA (N-methyl-D-aspartate) receptors (Kim, M. H., et al., 2009). What drives the release of the magnesium block for these receptors is the amplification of excitatory signals that are converging on the membrane (Vargas-Caballero, M. & Robinson, H. P., 2004). Highly dense dendrites would also imply that the signals are occurring close together. Long-term potentiation allows for calcium to enter the cell, modifying the synapses, and this is made both faster and easier in the EC condition as opposed to to the other conditions (Malenka, R. C., & Nicoll, A. R. A., 1999). And accordingly, adjusting the strength of the synapses allows for behavioral alterations to take place, permitting plasticity to occur.

Rethinking Cognitive Enrichment: From Lab Models to Real-Life Outcomes

Although these results, taken at face value, project mental stimulation as a promising and intuitive solution to cognitive stagnation, there are other considerations that must first be deliberated. In this experiment, which is the experimental condition and which is the control? It would not be implausible to suggest that the EC condition itself is much like a control, as rodents in their natural habitats live and travel in groups, engaging with and manipulating their environment all the time. One could argue that the other two conditions are abnormal developmental patterns. Unfortunately, the vast majority of research delving into the variations in the brains of animals has been conducted with animals living under IC conditions. In collecting data and interpreting it, it is possible that too much of the background of neurobiological research has been leveraging an artificial model system that may be abnormal in the context of brain development. Therefore, in contemplation of the infamous 5% brain volume loss statistic, such may not be the case for everyone alike nor the consequence of biology alone. Hence, a major conclusion drawn from Hebb's work could very well be that regression into more isolated and socially elementary environments may potentially predispose the brain to cognitive decline. This raises a compelling question about societal attitudes toward aging and how they may inadvertently contribute to cognitive decline. The treatment of the elderly, often characterized by social isolation and limited environmental enrichment, stands in stark contrast to research emphasizing the importance of cognitive stimulation and social engagement for maintaining brain health and thus brain mass. Environments like these, deprived of mental and social complexity, could exacerbate the natural loss of brain mass and function associated with aging. However, dementia has persisted in individuals who maintain mentally active and enriched lifestyles, raising important questions about the aging process. A longitudinal study following

over 1,900 individuals revealed that reports of a higher frequency of participation in mentally-stimulating tasks (a higher cognitive baseline) tended to correlate to AD-onset delays of upwards to 5 years (Wilson, R. S., et al., 2021). Regardless, after a 6.8 year follow-up, nearly a quarter of participants still developed the disease, despite citing regular involvement in cognitively-enriching tasks. Studies like these indicate that while environmental complexity seems to reinforce cognitive fortitude, it may not completely offset the underlying biological mechanisms of aging. It is also possible that early cognitive impairment is an obstacle to the potential benefits mentally-stimulating task involvement appears to bring about. The fact that a significant portion of participants still developed AD despite regular cognitive engagement accentuates the limitations of environmental interventions in offsetting the disease's biological progression. Early cognitive impairment refers to the initial stages of cognitive decline that manifest prior to the appearance of more serious conditions like AD. It often takes on the form of mild memory lapses or issues with problem-solving that are more pronounced than expected for "normal aging," yet are still short of any diagnostic criteria for a specific pathology. In the context of this particular study, individuals experiencing early cognitive impairment are engaging in mentally-stimulating activities, but perhaps the presence of underlying neurodegenerative processes could diminish the protective effects of these kinds of activities. Early cognitive impairment may very well reflect the onset of neurodegenerative diseases, predisposing the brain to amyloid-beta plaque deposition and tau tangles that are resistant to this kind of cognitive stimulation alone. This study illustrates that despite how mental engagement in these tasks can build cognitive reserve, on its own, it may not be commanding enough to counteract the pathology taking place once these biological mechanisms are set in motion. This discussion aims

to emphasize the multifaceted nature of aging, where both genetic and environmental factors interplay to influence brain health.

The Impact of Aging on Dendrites

While it has been noted cerebral atrophy may not predictably occur in a stepwise fashion for all human beings due to contrasting lifestyles, occupations, genetics, etc., it is a form of volume loss known to be affiliated with reductions in dendritic branching (Blinkouskaya Y. & Weickenmeier, J., 2021). Immunohistochemistry of the hippocampal region has demonstrated the considerably potent concentration of RNA in and around dendrites (Bartlett, W. P., & Banker, G. A., 1984). In inquiring why exactly this is the case, it is vital to consider the difference between axons and dendrites — an axon is typically going to have the same class of neurotransmitter at all of its terminals whereas dendrites possess greater variability. One major component of the morphology of dendrites is its branching that amplifies the amount of both surface and localized area driving stronger inputs and better associating different inputs together. It has long been postulated that dendritic configuration revises rapidly, innately promoting neuroplasticity. It may be proposed that the need for restructuring the branching pattern may be partly regulated by dendrites having localized protein-synthesizing machinery. An *in vivo* study utilizing a rat model proclaims that despite being raised in EC conditions, once rats reach the period of cellular senescence (around 24 months), the arborization of their dendrites decreases significantly (Kolb, B., Gibb, R., & Gorny, G., 2003). Upon further scrutiny, the figures within this paper demonstrate modest effects at best, and it is the current sentiment that “normal aging” changes in dendritic arbor are mild and region-specific (Burke, S. N., & Barnes, C. A., 2006). There are other changes, however, associated with aging that can enable dendritic arbor decreases such as

oxidative stress and hormone loss (Wang, X., & Michaelis, E. K., 2010; Castillo-Fernández, S., & Silva-Gómez, A. B., 2022). If the changes that aging is often accompanied with directly affects the manner in which dendrites arborize, the extent to which someone can engage in plasticity is not only complicated, but synaptic density and transmission are also consequently decreased (Dickstein D. L., et al., 2007). It is also possible, however, that because rats are short-lived creatures, changes in dendritic arbor and other factors may appear in humans due to the greater length of time in which the human brain has to undergo them. Notwithstanding that this does not guarantee the development of a neurodegenerative disorder, synaptic loss tends to be a hallmark of these diseases. Regardless, impaired synaptic function may lead to deficits in learning and memory, as the brain's ability to encode and retrieve information is effectively compromised. Equally important, when synaptic transmission is hindered, the speed and efficiency by which such information may be retrieved is also negatively impacted. In short, aging has the potential to create an environment in which the data stored in the human brain is not just more difficult to access, it may also be slower to find and add to.

Hallmarks of Alzheimer's Disease

AD diagnosis depends upon not just cognitive deficits but also on the appearance of amyloid-beta plaques and tau tangles. Amyloid-beta plaques are extracellular deposits formed from amyloid-beta peptides, or fragments of the amyloid precursor protein also known as APP (Hampel, H., et al., 2021). When these plaques abnormally accumulate within the brain, they interfere with neuronal communication and trigger inflammatory responses that can exacerbate neurodegeneration. Neurofibrillary tangles (NFTs), on the other hand, are intracellular aggregates of hyperphosphorylated tau protein (Iqbal, K., et al., 2010). Tau normally serves to stabilize

microtubules within neurons, but in AD, it becomes abnormally modified, leading to the formation of tangles that disrupt cellular transport, resulting in neuronal death. The diagnosis of AD typically begins with the clinical observation of cognitive deficits, such as impairments in memory, reasoning, and problem-solving. These deficits are noted to progressively interfere with daily functioning and behavior. Despite advancements in positron emission tomography (PET) and cerebrospinal fluid (CSF) tests to detect these proteins, the only way to definitively identify AD is through post-mortem analysis.

It is also not uncommon to uncover neurofibrillary tangles and amyloid-beta plaques (which will be explored in greater depth) in an otherwise unremarkable elderly brain (Guillozet A. L., et al., 2003). The preclinical stage of AD is posited to last anywhere between 10-20 years, and the presence of these proteins without cognitive deficits may not always indicate an "unremarkable" brain, given this rather long incubation period (Vermunt, L., et al., 2019). In AD, these abnormalities tend to be specific to this disease according to their presentation patterns. The deposition trend for amyloid-beta occurs primarily in the hippocampus and cerebral cortex for AD as opposed to other neurodegenerative diseases (Murphy, M. P. & LeVine, H., 2010). Similarly, while tau abnormalities are seen in other neurodegenerative diseases known as tauopathies, regions like the medial temporal cortex and neocortex, where tangles can take place, is known to be a hallmark for AD (Burkett, B. J., Johnson, D. R., & Lowe, V. J., 2024).

Neurofibrillary Tangles

Neurofibrillary tangles are abnormal structures found in the brain, primarily composed of tau protein. Tau is a neuronal protein with a high affinity for microtubules, and is enriched in axons where it wears many hats, including axonal stability/transport and polarity maintenance

(Duan, A., et al., 2017). Individual microtubules are relatively short in comparison to the whole axon, so a relatively stable network of microtubules is intrinsic to utmost axon stability. Microtubules facilitate diverse cellular processes, including but not limited to intracellular transport as well as neuronal growth (Cooper, G. M., 2000). The long scaffolds forged by microtubules extend from one end of a neuron to another, contributing to the development and maintenance of cell shape. The dynamic state of microtubules and microfilaments permits a mature neuron to retract old axons and dendrites, and instead, extend new ones. That very growth and shrinkage is predicated upon the needs of the brain to be able to process and modulate how it is responding to incoming stimuli day by day. When a kinase phosphorylates tau, this event allows for the microtubules to shift into a more dynamic state due to the fact that in the presence of an additional phosphate group, tau undergoes a conformational change, reducing its affinity for microtubules (Padmanabhan, P., et al., 2024). When tau is later hydrolyzed, it will go on to stabilize that same microtubule in its new configuration. This mechanism demonstrates just how swiftly neurons are able to modify their shape, furthering the concept that such takes place as one is actively engaging in knowledge acquisition. In the event hyperphosphorlyation occurs, the microtubules are assembled and destabilized to such a degree that they come to possess the potential to reside in the brain as insoluble clumps interfering with neuronal communication, normative cell functions, and may even yield cell death.

Amyloid-Beta Plaques

In the same way NFTs have been discovered within neuropsychologically sound brains of older adults, so have amyloid-beta plaques. Their aggregation disrupts cellular communication and their presence is posited to ignite inflammatory responses counterproductive to neuronal

survival (Hughes, C., et al., 2020). The presence of amyloid-beta oligomers has been correlated with the production of TNF-alpha, a cytokine serving as a signaling molecule that first induces inflammation, in both BV2 microglial cells and astrocytes. However preliminary, there is an argument to be made that inflammatory responses may be playing a bigger role than what is understood in both aging and dementia pathology. In older age, the body is believed to enter a state referred to as inflammaging, a period of mild, yet persistent chronic inflammation, suggested to be driven by cellular damage and immune system alterations (Baylis, D., et al., 2013). Because of this immune system decline, it is believed that older individuals are more susceptible to dementia pathology through the production of these immune-associated cytokines. TNF-alpha, although neuroprotective against amyloid-beta, when excessively produced, can actually exacerbate chronic inflammation, leading to tissue damage and worsening neurodegeneration as a result. Following the creation of radiotracers proficient in detecting amyloid-beta, initial research conducted over a decade ago began to simply scratch the surface of just how prolific the accumulation of this protein truly is. Emerging research indicates that amyloid-beta plaques are either forming in a very long-preclinical phase or they are, instead, benign (Iddi, S., et al., 2019). Either way, they accumulate without yielding immediate cognitive impairment, suggesting that their mere presence does not definitively signify immediate neurodegeneration or cognitive decline. One study in particular led its pioneers to predict that as much as a third of the older adult population has significant amyloid-beta deposition in their brains, but high amounts does not always mean *bad* amounts (Rodrigue, K. M., Kennedy, K. M., & Park, D. C., 2009). Although much of the sentiment surrounding aging from the 1990s to the early 2000s hyper-fixated on the “amyloid-beta cascade” hypothesis, conjecturing that a “cascade” of events beginning with the abnormal processing of amyloid precursor protein

avalanches into dementia pathology, these plaques were later unearthed in the brains of individuals living free of cognitive ailments.

The Glymphatic System: Sleep and Waste Clearance in the Brain

In young adults, amyloid-beta does indeed accumulate; however, there are mechanisms of waste removal that encapsulate this protein into that "waste" category, alleviating the brain of its burden distinctively during sleep. The glymphatic system is a rather recently discovered waste clearance system in the brain operating primarily during sleep. It is akin to the lymphatic system in other parts of the body, though it retains characteristics tailored to the brain's unique environment. Functioning via a network of channels, the glymphatic system harnesses the flow of cerebrospinal fluid into the brain to flush out waste products from the interstitial spaces between brain cells — amyloid-beta included. It is possible that amyloid-beta is produced at high quantities even at an early age, but the glymphatic system is efficient at preventing its accumulation (Zhou, Y., 2020). The activity of this system is heightened during sleep, when the space between brain cells increases, optimizing waste removal. Slow wave sleep in particular is associated with greater glymphatic activity, elucidating the importance of adequate sleep (Boespflug, E. L., & Iliff, J. J., 2018). It is no surprise this process is disturbed by aging given the sleep difficulties this cohort of individuals face. A study evaluating over 9,000 adults aged 65 and older revealed that over half claim to be struggling with at least one major chronic sleep complaint (Foley, D. J., et al., 1995). Incidences of insomnia are highest among this population than young adults and tends to be a most repeated grievance (Ancoli-Israel, S., 2009). It is estimated that between 40-70% of older adults experience chronic sleep disturbances and upwards to a half may be undiagnosed (Miner, B. & Kryger, M. H., 2020). It is also during this

stage of life that the sleep cycle is experienced differently, with more time being spent in the earlier and lighter stages of sleep, which can lead to night time awakenings (Newsom, R. & DeBanto, J., 2020). Understanding all of the compounding variables that impact sleep in older adults, one can fathom how perhaps they may experience impaired clearance of amyloid-beta deposits during sleep. Such can possibly contribute to the gradual accumulation of this protein over time.

Reactive Oxygen Species

The surfacing of reactive oxygen species (ROS) is another recognized milestone of aging. The Free Radical Theory of Aging (FRTA) has long posited that aging occurs simply due to ROS accumulation over time. First outlined by Denham Harman in the 1950s, there are many publications that exemplify ROS levels increase with age (Harraan, D., 1955). There are even some publications that go as far as to demonstrate increased levels of ROS induces shorter lifespans in various animal models (Shields, H. J., Traa, A., & Van Raamsdonk, J. M., 2021). Many articles of late, however, have begun to challenge this long-standing theory, highlighting how in worm and fly models, ROS is positively correlated with lifespan — increase one and the other increases as well (Sanz, A., 2016). It is also beginning to unfold that there may be other beneficial roles that ROS fill. Studies have been conducted that suggest increases in ROS ignites downstream pathways that activate in response to stress (Ristow, M., & Schmeisser, S., 2011). The argument made is that igniting these responses not only strengthens them, but also contributes to greater metabolic health in the long run. The reality of the matter is no one knows for sure, and the contexts in which ROS may be beneficial is not applicable to everyone. How, when, and where these responses begin, as well as the molecular arena in which they take place,

likely dictate a lot of the variability surrounding the effects of ROS. Research still must further isolate the features of ROS that make them either beneficial or harmful. Nonetheless, there is still a very real world in which ROS bring about harm and their surfacing should still be considered a trademark of aging because of the known disturbances they cause during this particular stage of life — as repair mechanisms slow down, ROS accumulates. (Kumar, H., et al., 2012).

Sources of Reactive Oxygen Species

ROS themselves arise from both intrinsic and extrinsic sources. Exposure to UV/radiation is one example of an external repository of oxidative stress. Internally, most ROS escalation takes place through the mitochondrial production of ATP via oxidative phosphorylation (Warraich U. E., Hussain F., & Kayani H., 2020). The mitochondrial electron transfer chain system (ETC) is active during the process of oxidative phosphorylation, and its components are capable of generating ROS that must be cleared via antioxidant mechanisms. Failure to do so has been linked to neuronal degradation (Bhatia V. & Sharma S., 2021). These highly reactive molecules, in the form of gasses, traverse the intracellular domain, interacting with biomolecules within brain cells including lipids, proteins, and DNA. Lipid peroxidation, caused by ROS attacking lipid membranes, interferes with the integrity of cellular membranes and hence cellular function (Halliwell, B. & Chirico, S., 1993). Oxidative modification of proteins can alter their structure and function, segueing the dysfunction of enzymes, receptors, and other proteins inherent for neuronal signaling and homeostasis (Chakravarti, B. & Chakravarti, D. N., 2007). Furthermore, DNA damage fostered by ROS can lead to mutations and impair the integrity of the genome, potentially contributing to neurodegeneration and the development of diseases like

cancer (Renaudin, X., 2021). Exhibited are simply a few of the many fashions in which ROS affect the quality of life of older adults, even in the absence of neurodegenerative disease.

Amyloid-Beta and Reactive Oxygen Species

Microglia are the fundamental immune cells of the brain, playing an indispensable role in maintaining brain health. As the brain's first line of defense, they consistently survey the neural environment for indications of infection or damage, and in response to these threats, they become activated (Wolf, S. A., Boddeke, H. W. G. M., & Kettenmann, H., 2017). In this active state, microglia are capable of engulfing and clearing harmful substances like pathogens, debris, or detrimental proteins like amyloid-beta. Microglia are also capable of becoming overactivated, and in this state, they can release inflammatory cytokines and ROS, damaging nearby neurons. An exacerbation of this process produces a domino-effect that can contribute to the pathology of many neurodegenerative diseases. Amyloid-beta in particular is recognized by microglia via specific receptors, like the CD36 receptor, toll-like receptors (TLRs), and the RAGE receptor (Doens, D. & Fernández, P. L., 2014). When amyloid-beta interacts with these receptors, it can ignite processes that facilitate its removal, but also yields inflammation and oxidative stress. It has been shown that the CD36 receptor works hand in hand with TLRs to release signals that produce ROS, damaging neurons. The RAGE receptor operates similarly by increasing inflammation in the brain. Considering this line of research, it would be beneficial to aid microglia in reducing the levels of amyloid-beta in the brain without setting off a harmful inflammatory cascade.

Present-day inquiries into the relationship between amyloid-beta, ROS, and Alzheimer's disease at large have begun to proclaim that it is the accumulation of ROS, not amyloid-beta, that

serves as the pathological cascade leading to decline. It has been proposed that oxidative stress precedes amyloid-beta accumulation and tau hyperphosphorylation, where ROS first disrupt normative cellular functions (Roy, R. G., Mandal, P. K. & Maroon, J. C., 2023.) The emergence of resulting DNA damage, lipid peroxidation, and mitochondrial dysfunction creates an inviting environment for amyloid-beta sequestering and tau alterations that go on to create the complications seen in AD. Normally, the brain balances the production of ROS with antioxidant defenses to protect cells. However, when oxidative stress occurs, this balance is disrupted, and the high levels of ROS interfere with how cells communicate and function. This damage occurring in the entorhinal cortex is one of the first signs of AD, potentially taking place even before amyloid-beta plaques or tau tangles form (Juan, C. A., et al., 2021). Additionally, reduced levels of glutathione (GSH) have been observed in early stages of AD. GSH, a critical antioxidant, when depleted, limits the brain's ability to counteract oxidative stress, unfortunately leaving neurons vulnerable to enduring further stress (Roy, R. G., Mandal, P. K., & Maroon, J. C., 2023). Ultimately conveyed is an alternative manner by which AD and dementia alike may begin: through disrupting cellular balance, ROS fosters an environment conducive to amyloid-beta accumulation and tau hyperphosphorylation, accelerating neurodegeneration.

The Debate on ROS: Contributors to Aging or Indicators of Dysfunction

At the very least, the rise of ROS, overall, can cause more harm than good, yet it is still only one of many types of damage that can occur in the aging brain. Although the FRTA has been popularized, the exact degree of damage imposed on any single cell as a result of ROS remains ambiguous, and even more vague is the explanation for why the brain is unable to maintain a healthy balance of these molecules through innate clearance mechanisms (Gladyshev,

V. N., 2014). Reconsidering the debate of whether aging is a normal developmental process or something mechanistically different altogether, perhaps it must be first accepted that biological processes are imperfect. Now is this imperfection of the brain the result of a "normal" aging journey? If garbage collection trucks, over time, began to collect less and less garbage from each neighborhood, surely this would not be acceptable. If the brain, over time, began to assume greater and greater levels of ROS with a decreased clearance efficiency, why then would such not be considered abnormal? Extending what works in one's youth to their older age seems like the intuitive approach in mediating ailments that would not be medically acceptable if they presented years prior. It is important to consider that accumulating ROS may be "normal" for the aging brain, but it is equally possible that its accumulation is abnormal and causes ailments. Questions like these are further challenging the "normal aging process," inspiring the necessary ventures into how to make this period of life healthier.

Where Contemporary Treatments Fall Short

Cholinesterase Inhibitors: Donepezil, Rivastigmine, and Galantamine

Perhaps the most insidious of all these challenges is the prospect of cognitive decline as aforementioned, most notably epitomized by the onset of Alzheimer's disease. As of today, there is no solution or straightforward treatment plan that can alleviate one fully of AD symptomatology once the signs begin to show. Even after over a century since the disease had been discovered in 1906, there are only a few approaches formally approved by the Federal Drug Administration in the United States (Alzheimer, A., 1906). Cholinesterase inhibitors are one classification of these drugs that have been produced. Specifically, donepezil, rivastigmine, and galantamine are the three FDA approved cholinesterase inhibitors (Grossberg G. T., 2003).

Donepezil functions by inhibiting the enzyme acetylcholinesterase, whose role is to break down the neurotransmitter acetylcholine (Seltzer, B., 2005). As AD progresses, ACh levels in the brain decrease, which is thought to lead to difficulty forming and retrieving memories (Sam, C., & Bordoni, B., 2020). By preventing the breakdown of ACh, donepezil is able to increase the levels that would otherwise be present in the brain, believed to help improve communication between neurons. While these drugs can produce statistically significant improvements in cognitive function, activities of daily living, and overall clinical state, the effects are generally modest at best and short-lived. Meta-analyses indicate that improvements on cognitive scales like the MMSE (Mini-Mental State Examination) and ADAS-Cog (Alzheimer's Disease Assessment Scale - Cognitive Subscale) are rather small, often translating to minimal clinical benefit in real-world settings (Schneider, L. S., 2012). It is also important to consider that many of these claims have been deduced from studies comparing individuals on a continued, regular course of one of these drugs to others who are either untreated, cannot tolerate the medication, or are declining at rapid rates. Many of the effects reported in the literature derive from studies built upon personal biases and it can prove difficult to distinguish between objectivity and skewed perspectives. Long-term analyses of the effects of these drugs are scant. Most interpretations are gathered from assessments made over a period of just a few months. Among the few studies conducted over the span of a few years (1-4), it tends to be the case that effects for those being treated with any one of these medications are indiscernible from that of the control group (Petersen, R. C., et al., 2005; Feldman, H. H., et al., 2007). Likewise, this kind of relationship also persists for the other categories of approved treatments.

NMDA Receptor Antagonist: Memantine

There is only one approved NMDA receptor antagonist for AD treatment: memantine. It works by blocking NMDA receptors, which are involved in glutamate signaling in the brain (Dingledine, R., 1986). It is hypothesized that one of the mechanisms for neural degeneration may be the overactivation of these receptors (Wang, R. & Reddy, P. H., 2017). It is known that their prolonged activation can lead to complications perhaps fostered by the influx of calcium when these receptors are opened, activating the second messenger systems that are ignited by this influx, among a host of other possibilities (Rothman, S. M. & Olney, J. W., 1987). Memantine is known to be an uncompetitive antagonist, a type of inhibition where memantine will only bind to a receptor that has already been previously activated by the neurotransmitter glutamate, in this case (Robinson, D. M. & Keating, G. M., 2006). Therefore, it will only bind to active NMDA receptors, and when it does so, it physically blocks the receptors' ion channels. By blocking these channels, memantine circumvents excessive calcium influx. A meta-analysis evaluation of 18 randomized clinical trials involving over 5,004 participants has deduced that memantine only showed minor improvements in cognitive function (Blanco-Silvente, L., et al., 2018). In other words, its efficacy may not be that far off from placebo long-term.

Amyloid-Beta Targeting Drugs: Aducanumab, Lecanemab, and Donanemab

Up until this point, the drugs discussed represent attempts to evade complications through manipulating synaptic transmission, which is simply one piece of the very large and convoluted puzzle AD truly is. In the present-day, there are two main pathologies characterizing the disease: amyloid-beta plaques and tau-induced neuronal tangles. Initiatives aiming to tackle these complications hone in on the former via monoclonal antibodies. Aducanumab, lecanemab, and

donanemab have all been approved amyloid-beta targeting drugs. They each bind to specific forms of amyloid-beta: aggregated, soluble/insoluble, etc. (Söderberg, L., et al., 2023). Their binding events promote the clearance of these plaques; however, it is often not until the later stages of the disease that treatment often begins. It is possible that some accumulation threshold of amyloid-beta is important, but it is often difficult to address it after it has been reached. By that point, it may be seemingly unhelpful to begin amyloid-beta clearance therapy. On top of this, there is much controversy surrounding the efficacy of these drugs. In fact, when aducanumab received accelerated approval from the FDA in 2021, many were opposed to this decision considering the agency's own scientific analysis of the drug failed to demonstrate that its ability to lower amyloid-beta levels correlated with improved cognitive function (Maulden, A., 2022). Lecanemab, although clinically shown to reduce amyloid-beta levels as well, has only been correlated with modest cognitive improvements and, unfortunately, is also linked to serious adverse effects like ARIA or amyloid-related imaging abnormalities (Van Dyck, C. H., et al., 2023). ARIA is a term that is used to describe a host of abnormalities seen in brain scans most commonly linked to drugs of this kind and are typically broken up into two types: ARIA-E (edema/effusion) referring to swelling or fluid accumulation in the brain and ARIA-H (hemosiderin) referring to areas of bleeding in the brain (Agarwal, A., et al., 2023). Headaches, seizures, confusion, and dizziness, and are just a few of the consequences ARIA is known to be associated with. Last but not least, a trial including 1,736 participants, where 76% completed the study, convey results that are rather weak in favor of donanemab — a common outcome (Iacobucci, G., 2023). Those treated with the drug saw a 10 point decline in a 144 point scale that is utilized to assess progressing AD symptoms, whereas the control group saw a comparable 13 point decline. The progression of the disease was estimated to be hindered by just 4 months with

donanemab use, a minimal improvement. For far too long has the "amyloid-beta cascade" been treated as the most promising avenue to tackle for symptom relief. If it were true, the data from these studies would instead show more improvements than they otherwise demonstrate; therefore, there must be other explanations.

Turning the Page on Contemporary Treatment: Aging and Alzheimer's Disease Overlap

These classes of drugs have typically been analyzed in the context of AD, but perhaps it has come time to consider them as well as upcoming initiatives outside of this framework and begin asking how their effects influence the phenomenon of aging in general. ACh depletion can also take place through normative aging (Gibson, G. E. & Peterson, C., 1981). It has also been shown that aging induces hypofunction of NMDA receptors, and it is hypothesized that this may be a "compensatory neuroprotective mechanism" to avoid inappropriate signaling or influences from excessive calcium levels (Kumar, A., 2015). However, this hypoactivity can still be overcome by oxidative stress which can produce unmanageable calcium levels, and even inflammation, generating more glutamate than usual. Such can lead to the overactivation of NMDA receptors, even without a neurodegenerative disease serving as a specific trigger. Again, amyloid-beta plaques and neurofibrillary tangles are no stranger to the aging brain either.
Age is one of the greatest known risk factors for developing Alzheimer's disease. Less than 10% of all AD cases occur before the age of 65 (National Institute on Aging). After 65, one's risk doubles every 5 years (Alzheimer's Association). It is estimated that around 1/3 of the population 85 and older is living with the disease. The previously discussed approaches fail to consider the issue of aging as a mechanism for developing AD, which is why they may be falling short. There is power in preparation and it may be wise to begin untangling the web of aging to discern

whether the public can take measures prior to the uncertain onset of cognitive complications. Although scientists are not yet aware of what the turning point may be to transform a functionally sound brain into one that begins to undergo cognitive decline, it is possible there is still the choice of taking action prior to ever crossing that bridge lying somewhere in manipulating the aging process.

Neurotrophin Receptor p75 and Protein Kinase C-ε

Neurotrophin Receptor p75

Neurotrophins are a family of proteins essential for developing and maintaining neurons, as well as for coordinating their survival and apoptosis (Barbacid, M., 1994). There are two main interactions by which they can exert their effects: the Trk receptor family and the p75 neurotrophin receptor (p75NTR). The Trk receptors (TrkA, TrkB, and TrkC) occupy the forefront of the survival, growth, and differentiation of neurotrophins, with each binding to a specific neurotrophin. TrkA binds to nerve growth factor (NGF). TrkB binds to brain-derived neurotrophic factor (BDNF) and neurotrophin-4/5 (NT-4/5). TrkC binds to neurotrophin-3 (NT-3). When neurotrophins bind to their respective Trk receptors, that event triggers a series of intracellular signaling pathways that can promote neuronal survival. In contrast, p75NTR is a membrane of the tumor necrosis factor receptor (TNFR) family that takes on more versatile roles than Trk receptors (Roux, P. P. & Barker, P. A., 2002). Its purpose can be analyzed through the lens of two main tasks:

1. It can act as a co-receptor with Trk receptors, either enhancing or suppressing signaling occurring through them.

2. It can independently activate signaling pathways that either encourage cell survival or cell death and the exact outcome depends upon the cellular context and the presence of varying adaptor proteins.

Focusing on the latter, pro-neurotrophins, such as proNGF and proBDNF, are known to bind to p75NTR and induce apoptotic signaling (Guo, J., et al., 2013; Teng, H. K., et al., 2005). This is a crucial mechanism in early development that shapes the nervous system. Apoptosis, or programmed cell death, occurs in early development in a controlled manner, necessary for major life functions. For example, in the womb, apoptosis facilitates the elimination of cells residing between a baby's digits, and permits him or her to be born without webbed hands or feet (Gilbert, S. F., 2000). Also, as previously discussed, in neuronal development, apoptosis takes on the form of synaptic pruning to streamline neuronal networks. Apoptosis is also a key factor in maintaining a proper balance between cell proliferation and cell death, crucial to the homeostasis and function of a variety of tissues (Pellettieri, J., & Alvarado, A. S., 2007).

Neurotrophin p75 Receptor: Where it Goes Wrong in Aging

Considering p75NTR in particular, it is possible that this receptor may not be working to its maximum potential during the aging process. Many of the infamous pathological conditions that can develop position oxidative stress as a major culprit to disordered p75NTR signaling (Kraemer, B. R., et al., 2014). An investigation seeking to discover whether these receptors can be directly impacted by reactive oxygen species revealed they can: 4-hydroxynonenal (HNE), a natural by-product of the process producing ROS, can interact with p75NTRs, negatively influencing them to elect apoptosis (Kraemer, B. R., et al., 2014). It has also been found that when this receptor is overproduced in primary cortical neurons and glioma cells (cancerous cells

that spawn from glial cells in the brain), a fatal chain reaction takes place (Bhakar, A. L., et al., 2003). Mitochondria are influenced by this chain reaction to begin releasing cytochrome C, a signaling molecule alerting enzymes to begin to dismantle the cell, or in other words, commence apoptosis. Although widely expressed during development, the p75NTR is not largely seen throughout the brain in adulthood and is typically upregulated in the event of injury (Cragnolini, A. B. & Friedman, W. J., 2008). As a result, recent discourse has postulated that neurological conditions affecting the integrity of neurons may induce the p75NTR to ignite the process of apoptosis. Although possible, many questions remain on whether this occurs for certain. What has been shown, however, is that aside from binding to other Trk receptors and neurotrophins, the p75NTR is also able to interact with amyloid-beta (Yaar, M., et al., 1997). Cholinergic neurons located in the basal forebrain express p75NTR at the highest levels and are conversely the most susceptible to amyloid-beta binding (Woolf, N. J., Gould, E., & Butcher, L. L., 1989). This toxic pairing is one that permits amyloid-beta to guide the p75NTR to initiate apoptosis inappropriately, contributing further to the pathology of the world's leading form of dementia.

Protein Kinase C-ε

Aside from p75NTR, there is another critical team player for development belonging to the Protein Kinase C family. A kinase is an enzyme that catalyzes the transfer of a phosphate group from ATP to a specific molecule (Oxford University Press., 2024). Protein Kinase C-ε (PKC-ε) is one of the isoforms of PKC that is involved in many cellular processes (Aksoy, E., Goldman, M., & Willems, F., 2004). When neurons are activated, PKC-ε's activity is upregulated, permitting it to coordinate neuronal survival, neuroplasticity, and cell differentiation (Akita, Y., 2002). Unlike traditional PKCs, PKC-ε does not require calcium in order to become

active and is instead activated by diacylglycerol (DAG) and phosphatidylserine (Duquesnes, N., Lezoualc'h, F., & Crozatier, B., 2011). Recent findings have implicated PKC-ε's participation in a particular signaling cascade as a necessary feature for synaptic plasticity and communication (Wang, X. T., et al., 2023). Looking at presynaptic terminals in the cerebellum, researchers identified a signaling cascade known as the cAMP-EPAC-PKC-ε cascade (cyclic adenosine monophosphate-exchange protein activated by cAMP-protein kinase C-ε) that ultimately induces the phosphorylation of Rab3-interacting molecule 1 alpha (RIM1A). RIM1A is a protein that will go on to form a complex responsible for preparing and releasing neurotransmitters from synaptic vesicles, indispensable to neuronal communication. Utilizing live mice bred without the gene coding for PKC-ε, there were statistically significant reductions in the levels of RIM1A that were produced, indicating PKC-ε's critical importance for synaptic plasticity and neuronal function (Wang, X. T., et al., 2023). Without it, issues with coordinating basic movements and learning new motor skills would occur. Extending beyond the context of the cerebellum, it is also believed that PKC-ε embodies similar responsibilities for executive functioning. While in this experimental system the consequence of knocking out PKC-ε led to motor deficits, a loss of PKC-ε function in the forebrain would presumably lead to higher order cognitive deficits. Furthermore, it has been shown that PKC-ε promotes cytoskeletal stability by phosphorylating specific microtubule-associated proteins or MAPs (Callender, J. A. & Newton, A. C., 2017). These proteins, such as tau, regulate the assembly and stability of microtubules. When phosphorylated at sites that enhance their ability to bind microtubules or regulate their interactions in a productive and dynamic manner, PKC-ε can promote neuronal health.

PKC-ε: *Where it Goes Wrong in Aging*

As for the case of PKC-ε, although very few studies have thoroughly assessed the quantity of various PKC isoforms and their respective functioning in normatively aged brains, it is typically accepted that all forms decrease in both regards through the aging process (Pastore, D., et al. 2019). Of the studies that have identified PKC decline to some degree across its isoforms, memory loss and cognitive decline emerge as repeated consequences (Pastore, D., et al. 2019). Further assessments using mouse models of aging found that older mice (18 months and older) scored lower on assessments of cognition that younger mice (3 months), and these effects were exacerbated by surgery not directly linked to the brain (Lu, W., et al., 2022). In real-world applications, individuals aged 60 and older who underwent hip replacement surgery were assessed for postoperative cognitive dysfunction (POCD) using Aβ1-42 cerebrospinal fluid levels before and after (Evered, L., et al., 2016). 27.3% of patients with low Aβ1-42 levels, below the cut-point for AD neuropathology, went on to develop POCD. Only 4.3% of patients with levels higher than the cut-point developed POCD. The cut-point is a value denoting the difference between low and high Aβ accumulation. Being below the cut-point refers to a high amount of accumulation. A greater quantity of Aβ plaques can contribute to a lower neurological resilience to POCD, which is potentially linked to increased neuroinflammation, mitochondrial dysfunction, defective autophagy (cellular cleanup), and apoptosis. All of these variables have been attributed to the aging process (Evered, L., et al., 2016). So when researchers of the aforementioned mice study conducted laparotomies and partial hepatectomies (surgeries concerning the abdomen and liver) and found that doing so impacted cognition particularly for older mice, it suggests that aging further complicates the maintenance of brain health. And it complicates such even in the event of ailments not directly linked to the brain; however,

diminished PKC-ε pathway function may be to blame. With diminished PKC-ε functioning, the brain may be less adaptable to secondary insults like surgery. Other animal models have demonstrated that aged rats have lower PKC-ε activity in hippocampal neurons (Hongpaisan, J., et al., 2013). Treatments seeking to increase PKC-ε activity, coupled with spatial memory training, enabled aged rats to begin performing at levels statistically insignificantly different from young mice. In other words, performance appeared comparable after treatment. It may not come as a surprise then, that PKC-ε deficits are even more pronounced in a brain undergoing pathological cognitive decline like Alzheimer's disease, with expression and function lower than that in the aging brain (Matsushima, H., et al., 1996). Both p75NTR and PKC-ε represent pivotal players in early neuronal development and function. However, much like current cognitive decline interventions, studies pertaining to these players have been limited to observing their behavior during distinguished conditions: Parkinson's disease, stroke, Alzheimer's disease, etc. The treatments of today focus predominantly on the pathologies of cognitive decline rather than trying to transform the aging process preceding it that brings with it the same ailments, but at varying degrees.

DCP-LA and LM11A-31

DCP-LA

As briefly touched upon, there are methods by which PKC-ε may be augmented via "activation," and one of the most selective activators *in vitro* is DCP-LA with a 7-fold potency over other PKC isozymes (Kanno, T., et al., 2006). It is hypothesized that DCP-LA is able to bind to PKC-ε on its phosphatidylserine binding site (Kanno, T., et al., 2006). This study investigating various fat molecules found that unsaturated fats like dioleoyl-phosphatidylserine

and linoleic acid are able to activate PKC-ε unlike saturated fats. DCP-LA, a derivative of linoleic acid, is able to do without the need of phosphatidylserine for help. Aside from upregulating PKC-ε activity, DCP-LA is also known to be an inhibitor of glycogen synthase kinase-3β or GSK-3β (Nishizaki, T., 2017). GSK-3β is a central enzyme in the phosphorylation of tau, a key component in the formation of neurofibrillary tangles. Amyloid-beta wields the capacity to activate GSK-3β, ramping up the process of hyperphosphorylation. DCP-LA is able to mediate this in a few identified ways (Nishizaki, T., 2017). It can directly inhibit the activity of GSK-3β, it can activate Akt, an intermediary kinase that can later inactivate GSK-3β via cellular signaling, or it can inhibit protein tyrosine phosphatase 1B (PTP1B), an enzyme that negatively regulates insulin receptor signaling. This signaling, when enhanced, can lead to Akt activation and downstream GSK-3β inactivation. DCP-LA, by activating PKC-ε, will upregulate its ability to also mediate these effects downstream. Together, supporting PKC-ε function and GSK-3β inhibition, DCP-LA fosters an environment conducive to resisting cognitive disrepair.

LM11A-31

Likewise, a molecule known as LM11A-31 works similarly to rectify issues concerning the p75NTR. LM11A-31 has been shown to interfere with the receptor's degenerative signaling (inappropriate commencement of apoptosis) by directly blocking the binding site by which AB or proBDNF may associate themselves and exert this undesired effect (Tep, C., et al., 2006; Yang, T., et al., 2008). Mouse models taking a look at the impact of this molecule in the middle to late stages of Alzheimer's disease identified that, following treatment, neuron and associated neurites increased in density, even in areas of the basal forebrain where p75NTR expression was great (Knowles, J. K., et al., 2013).

DCP-LA and LM11A-31: Why Are They So Promising

What matters most is not simply the mechanics by which each of these molecules associate with either PKC-ε or p75NTR. It is that they can and do, but their utility has been limited to the confines of the treatment of cases that can be classified as moderate to severe. These compounds represent the ability to shift the medical focus of therapeutic strategies from treating advanced pathologies to that of preventing conditions like Alzheimer's disease or lessening the impact of its onset. DCP-LA's dual action as a PKC-ε activator and GSK-3β inhibitor showcases its potential as an early intervention in the disease process by reducing tau phosphorylation and enhancing synaptic plasticity in the human brain that becomes more susceptible to these issues with age. LM11A-31 holds promise for its capability to shield neurons and diminish neuroinflammation, benefits that should not be reserved for treating an illness only when it appears. Preserving neuronal function before significant neurodegeneration takes place may happen with this molecule, making it a vital tool in aging research. After all, it is a field where the goal is often to extend the period of cognitive health as opposed to merely delaying the inevitable decline associated with the beginning of neurodegenerative disease.

Current Experiment

The purpose of this research is to gain an understanding of whether or not there is a synergistic neuroprotective effect of a combinational DCP-LA and LM11A-31 treatment on measures of both cell viability and stability. The oxidative stress model is one that represents a preclinical AD stage as well as an aging brain that may very well not go on to develop the disease. Different concentrations of stress will be explored to model the varying stages of aging (mild to severe cellular disturbances). Two hypotheses will be tested:

1) The combination of DCP-LA and LM11A-31 will lead to an increase in cell viability for stress conditions treated with them versus stress conditions there were not by promoting the cell to not negatively select the cell death pathway in the presence of amyloid-beta as well as mitigating resulting ROS-induced inflammation and neurodegeneration.
2) This combination DCP-LA and LM11A-31 will lead to an increase in microtubule stability for stress conditions treated with them versus stress conditions there were not through PKC-ε's promotion of microtubule stability via tau phosphorylation as well as its role in reducing inflammation, coupled with enhanced selection of the neuronal survival pathway.

If hypothesis 1 is true, then there will be detectable increases of cell viability in mean absorbance scores via MTS assay for the treated stress conditions than the non-treated stress conditions. If hypothesis 2 is true, the average fluorescence of the microtubules measured through analyzing the potency of microtubule fluorescent marking will be greater for the treated stress conditions as well.

Methods

Primary Cortical Neuron Culture

This experiment utilized 96 and 24 well plates for neuronal culture. For each plate, a border was left around the inner collection of wells, leaving one row on the top, bottom, left, and right meticulously empty. This was done to avoid potential contamination of cells due to their proximity to the outside of the plate as well as to account for enhanced evaporation of the media that takes place at this outer edge. To prepare the plates for culture, Poly-L-Lysine was added to each well (100μL and 500μL respectively). This is a solution that creates an adhesive-like

coating on the bottom of the wells to allow the neurons to grow in a more fixed fashion as opposed to being loose within the well itself. The Poly-L-Lysine was allowed to sit for 24 hours before removal that was followed by three water washes lasting 15 minutes each. Plating media was then added to the wells (100µL and 600µL). It is created from one full bottle of Gibco's Neurobasal™-A Medium, which is a basal solution commonly used to support the long-term maintenance of neurons, and one 50mL tube of filtered fetal bovine serum (FBS), a serum supplement often utilized to enhance neuronal growth. This solution serves as a fourth wash that is permitted to incubate at 37°C, establishing an environment conducive to neuronal survival and development prior to plating.

The process of harnessing neurons for assessment began with the collection of embryonic rat brains from a Sprague-Dawley rat that has reached an 18-day gestation period. This time frame is crucial as the brains are underdeveloped enough that the neurons have not yet matured and are able to be mechanically manipulated via dissection without compromising their integrity or capacity to grow later on. Essentially, they are less susceptible to degradation via these processes as opposed to more advanced neurons. Upon collection, the cerebellum was removed, and the two hemispheres were isolated for experimentation. The cerebellum was discarded as the primary deficits associated with cognitive decline are not relevant to this region of the brain governing motor movement. The left and right hemispheres were separated from one another and the meninges (blood vessels) were carefully removed with forceps from the cortical tissue. Failure to do so adequately may result in the cells being unable to mature appropriately or even mature at all due to the inhibitory growth molecules meninges are able to secrete into the cellular environment.

After the hemispheres were clear of meninges, they were physically pulled apart with forceps and pipetted into a 15mL conical tube solution of trypsin in order to further separate the tissue. It stayed in this solution for 5 minutes in a 37°C water bath. Immediately afterwards, the tissue was transferred to another tube containing Hank's Balanced Salt Solution (HBSS) for a total of two times in order to thoroughly wash the trypsin away. The final tube consisted of plating media and when transferred, the cortical cells were mechanically dissociated with a flame-tipped glass pipet. A small drop of the solution was applied to a hemocytometer in order to count the concentration of cells and dilute it to a concentration of $1x10^6$cells/mL. The final 15mL tube contained the appropriate solution of cells diluted with plating media. For the 96 well plates, 100µL of cells were pipetted into each well. The 24 well plates were divided into two categories, a high and low concentration with cell amounts of 100µL and 50µL accordingly. After the cells were allowed to sit in the incubator for at least an hour at 37°C, the solution in the wells was then replaced with growth media (1 full bottle of Gibco's Neurobasal™-A Medium and 1mL of primocin, an antibiotic sensitive to both gram-positive and gram-negative bacteria).

Cultures were maintained and fed every 2 days by replacing half the concentration of solution within each well with a fresh half (growth media). The cells were allowed to incubate at 37°C and were fed on this schedule for 2 weeks. This timeframe allowed them to grow and develop prior to experimentation. The stimulation portion of this experiment took place after the 14-day mark for a period of 5 days.

Stimulation

FABN Stressor

FABN stands for ferrous sulfate, amyloid beta, buthionine, and NMDA. To synthesize a 3X stock solution, $FeSO_4$ was added to yield a concentration of 53.0µM in order to catalyze oxidative stress. Next, a 6.0mM of buthionine was established in the solution, as this compound inhibits endogenous oxidative stress responses in the cell. As neurons age, their abilities to alleviate the burden of oxidative stress weakens, and so buthionine was utilized to simulate this aspect seen in the aging human brain as closely as possible. Amyloid-beta was diluted to 1mM. Although this solution does not lead to the formation of plaques, it does expose the neurons to elevated levels of amyloid-beta. Lastly, an NMDA solution was added, establishing a 300µM concentration of NMDA in the stress solution. NMDA is an agonist for classes of glutamate receptors that allow calcium to enter the cell. In neurodegenerative diseases, it has been shown that calcium concentrations increase inside of the cell, so the addition of NMDA is thought to elicit a similar effect.

DCP-LA and LM11A-31

DCP-LA was prepared for stimulation via dilution in dimethyl sulfoxide (DMSO) and kept frozen in 100mM stock solution at -20°C. A 20mM stock solution of LM11A-31, diluted in sterile water, was also frozen for preservation. When diluted in media for experimental manipulations, the final working concentrations were 100µM for DCP-LA and 10nM for LM11A-31.

Experimental Design

See **Table 1** for the experimental timeline. For the 96 well plates, there were 6 rows by which experimental manipulation could take place (**Figure 1**). The first row was always the control row — no addition of the drugs together nor stress solution. Next was the positive control, or the row that would be treated with a growth media solution housing DCP-LA and LM11A-31. The stress solution was diluted to varying concentrations: 0.0625X, 0.125X, 0.25X, and 0.5X. The next four rows included two of these concentrations where they were plated alone or with the treatment in solution. A similar pattern followed for the 24 well plate, except instead of being horizontal, the design was organized by columns (See **Figure 1**). The first column was the control, with the top well being a high concentration of cells and the bottom well a low concentration. Then DCP-LA and LM11A-31 were added to the control media solution for assessment in the next column. Lastly, a stress concentration was selected and plated on its own or with the treatment for the last two columns.

Table 1: Cell Culture, MTS Assay, and Immunocytochemistry Timeline

Day 1: Dissection	**Days 3, 5, 7, 9, 11, and 13**: Feeding	**Days 14, 15, 16, 17, and 18**: Experiment	**Day 19**: MTS Assay and Immunocytochemistry	**Day 20**: Dark Room Imaging
The primary cortical brain cell collection occurred and the cells were cultured	Every two days, the neurons were fed by replacing half of the media with fresh Neurobasal Growth Media	The varying DCP-LA and LM11A-31 as well as the FAB conditions were administered for 5 days	An MTS assay was conducted on the 96 well plates the immunocytochemistry process begun for the 24 well plates	Dark room imaging took place

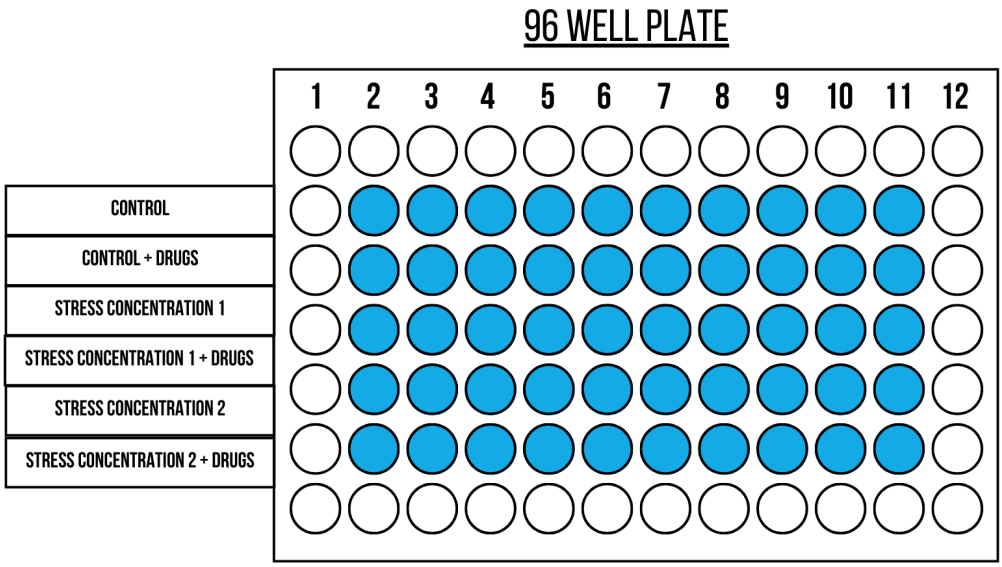


Figure 1: 96 Well Plate Experimental Design

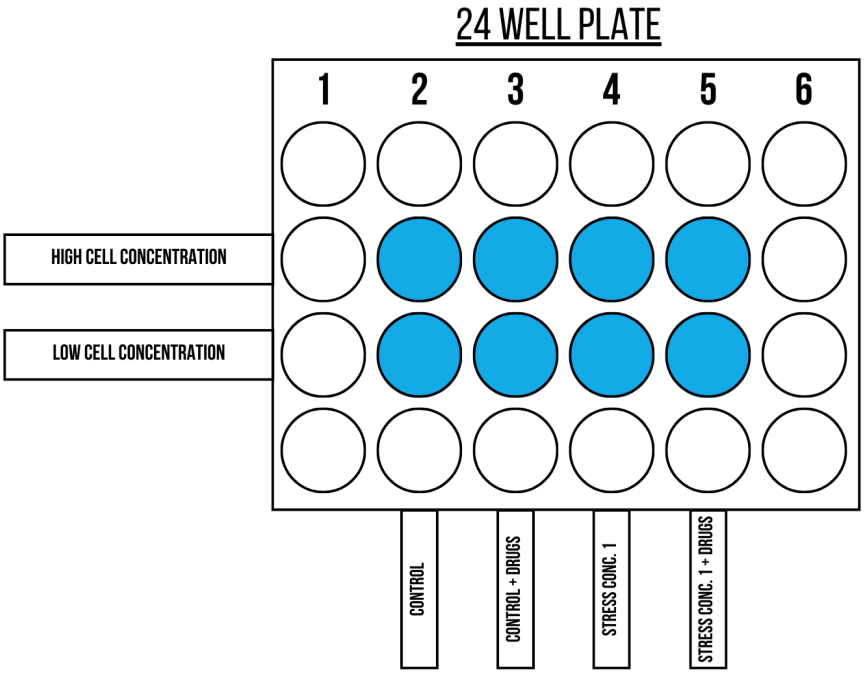


Figure 2: 24 Well Plate Experimental Design

MTS Assay: Assessment of Cell Viability

The 96 well plates were utilized to conduct an MTS assay, a colorimetric assessment commonly utilized in neuronal cell culture to quickly gather information regarding cell viability and cytotoxicity. This procedure makes use of a tetrazolium compound that is usually colorless until reduced by active cellular enzymes, transforming it into a highly colored product or formazan. The compound in this case is MTS or 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium. It is mainly reduced by the mitochondria of metabolically active cells, producing a dark brown/purple color indicating the presence of active cells. This process is particularly useful as it does not require lysing or manipulating the cells in a way that can destroy them. Measuring the absorbance of light at 490nm is done via a spectrophotometer, and it is at this value that the absorbance of the formazan product can be assessed. A higher absorbance value (a darker well) depicts a well that is undergoing a higher rate of metabolic activity, or in other words, has more viable cells (the assay cannot distinguish between neurons and non-neurons).

Immunocytochemistry: The Shape of Neurons

Fixation

In order to evaluate the structural integrity of the neurons, they were frozen in time and stained with a selective antibody for microtubules. A key feature of tau tangles is the consequence of unstable microtubules. To prepare for dark room imaging, all media was removed from the 24 well plate and the cells were immediately fixed in 4% paraformaldehyde for 20 minutes. The cells were then incubated with 0.5% Triton for 10 minutes (1mL of Triton to 200mL of Phosphate-Buffered Saline or PBS). It is a solution that enables the cell membranes to

become more permeable (creates holes) allowing the antibody to penetrate and bind to the tubulin (the building blocks) of the microtubules. The cells were then washed in PBS, a buffer solution mimicking the natural conditions of the cells, so that diluting substances and washing cells does not cause osmotic shock. The cells were then incubated for 1 hour on a shaker with the primary antibody (anti-acetylated tubulin). Anti-acetylated tubulin is an antibody that specifically binds to tubulin proteins that have undergone acetylation, a post-translational modification. Acetylation refers to the addition of an acetyl group to a protein, and in the context of microtubules, acetylation at lysine 40 stabilizes the microtubules, making them more resistant to depolymerization (or breaking down) (Janke, C., & Montagnac, G., 2017). After 3 washes with PBS, the cells were incubated for another hour on the shaker with a fluorescent secondary antibody that would permit the microtubules to be visible under the microscope.

Imaging

In the dark room, 15 images per well were captured using a Nikon Inverted Fluorescent Microscope and uploaded to NIS Elements. On this software, data for each image for the whole image (average fluorescence and minimum fluorescence) was recorded. These values were subtracted from one another to ascertain how much the overall fluorescence signal deviates above the background or baseline signal. A higher fluorescence value indicates a healthier, stable microtubule. Individual neurites and their average fluorescence were captured adhering to a systematic collection system. A overlaying circular grid (**Figure 2**) was fixed at the center of a cell body. Skipping the layer closest to the center, approximately 5 neurite data points were collected from each ring until 20 total data points were recorded for the entire image. Using the Region of Interest (ROI) statistics polygon tool, the outline of each neuron was traced as long as

it was contained within the borders of the circle it resided in. These borders marked the beginning and ends of the neurite. This circular grid organization ensured that the full span of the image was being analyzed without skewing the data with collection from neurites particularly close to the cell body and thus brighter in appearance.

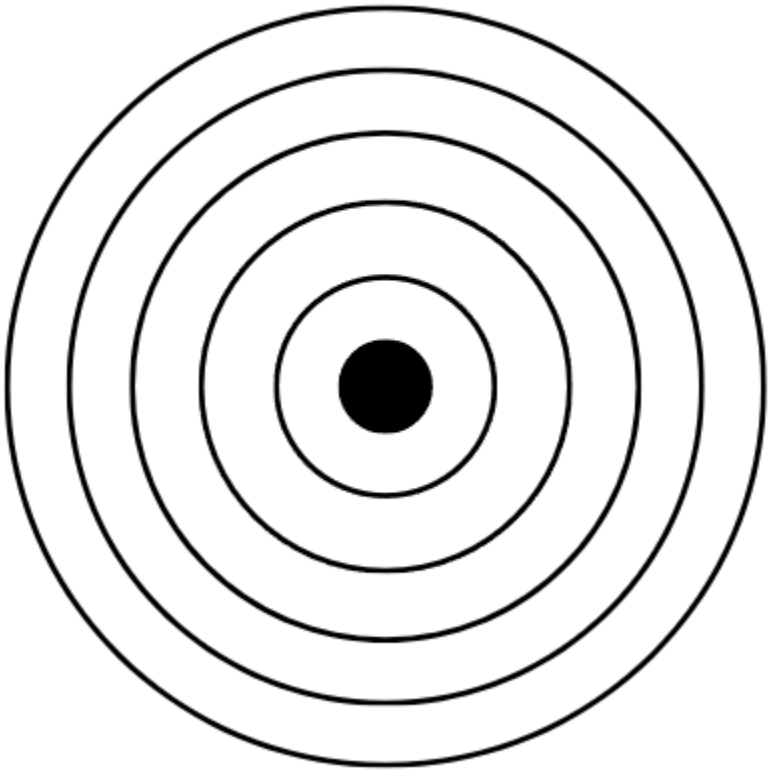


Figure 3: Neurite Collection Grid

Results

DCP-LA and LM11A-31: Effects on Cell Viability

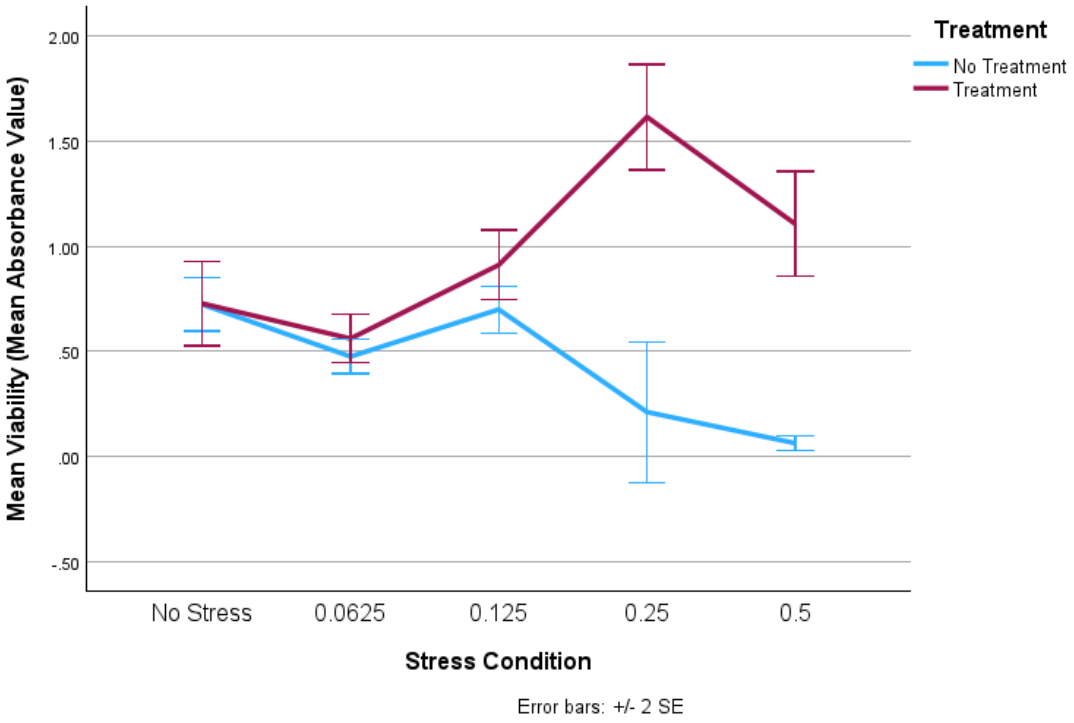


Figure 4: Mean Cell Viability by Stress Condition and the Presence of DCP-LA and LM11A-31. Cells were stimulated with varying degrees of FAB stress (no stress, 0.0625X, 0.125X, 0.25X, and 0.5X) for five days either treated with or without the DCP-LA and LM11A-31 treatment. Data was collected from three separate experiments.

Two-week old primary neuron cultures were exposed for five days to varying concentrations of FAB stress conditions with and without DCP-LA and LM11A-31 treatment. They were tested for viability under the following conditions: no stress, 0.0625X FAB, 0.125X FAB, 0.25X FAB, and 0.5X FAB. A line graph (**Figure 4**) was created in order to illustrate the interaction between treatment (0 = no treatment and 1 = treatment administered) and FAB stress condition (0 = no stress, 1 = 0.0625X, 2 = 0.125X, 3 = 0.25X, 4 = 0.5X) on mean cell viability measured by MTS assay. Two lines are plotted: one for the no-treatment condition (blue line) and one for the treatment-administered condition (red line). Analysis of the data demonstrated an

interaction between treatment and the stress conditions. In the no-treatment condition, the highest mean cell viability is observed under no stress and the lowest at 0.5X stress. In contrast, in the treatment-administered condition, mean cell viability increases with stress levels up to 0.25X, and begins to decrease slightly at 0.5X. Importantly, under treatment, cell viability is consistently higher compared to the no-treatment condition across all stress levels, and this gap between the two conditions is most pronounced at higher levels (0.25X and 0.5X).

In order to corroborate the findings of the graph, a 2x5 two-way analysis of variance (ANOVA) was conducted to examine the effects of the drugs (0 = no treatment, 1 = treatment administered) and FAB stress condition (0 = no stress, 1 = 0.0625X, 2 = 0.125X, 3 = 0.25X, 4 = 0.5X) on cell viability. Results revealed a significant interaction between treatment and stress conditions, $F(4, 150) = 19.43$, $p < 0.001$, indicating that the relationship between stress condition and viability differed based on treatment presence. However, the main effect of stress condition is also statistically significant, $F(4, 150) = 5.05$, $p < 0.001$, as is treatment presence, $F(1, 150) = 37.28$, $p < 0.001$. Thus, there are differences in mean cell viability depending upon the particular stress condition as well as the presence or non-presence of the DCP-LA and LM11A-31 treatment, without an interaction between the two variables. According to partial-eta squared, 32.6% of the variance in cell viability can be explained by treatment presence after controlling for stress condition. Likewise, 11.9% of the variance in cell viability can be explained by the stress condition after controlling for treatment presence. Overall, approximately 34.1% of cell viability variance can be attributed to the interaction between these two variables.

For the no-treatment condition, mean cell viability was lowest for 0.5X, followed by 0.25X, 0.0625X, 0.125X, and no stress. The no stress condition did not statistically significantly differ from 0.125X, but was statistically higher than all other conditions ($p < 0.001$). Similarly,

the 0.0625X stress condition did not differ from the 0.125X condition, but did have a significantly higher cell viability average than the higher stress conditions ($p < 0.004$) and a lower cell viability than the no stress condition ($p < 0.02$). The highest stress conditions (0.25X and 0.5X) were statistically different from all other groups except from each other, performing the worst ($p < 0.001$).

For the treatment-administered condition, mean cell viability was lowest for 0.0625X, followed by no stress, 0.125X, 0.5X, and 0.25X. With treatment, the no stress condition did not differ from 0.0625X and 0.125X, but did perform statistically worse than only the highest stress conditions: 0.25X and 0.5X: ($p < 0.001$ and 0.006, respectively). For 0.0625X stress, this condition performed worse than 0.125X ($p < 0.015$), 0.25X, and 0.5X ($p < 0.001$). The 0.125X stress condition performed better than 0.0625X, but worse than 0.25X ($p < 0.001$). The 0.25X and 0.5X conditions performed better than all other groups, except the 0.25X condition performed better than 0.5X ($p < 0.002$). See **Figure 5** for a graphical representation of these findings.

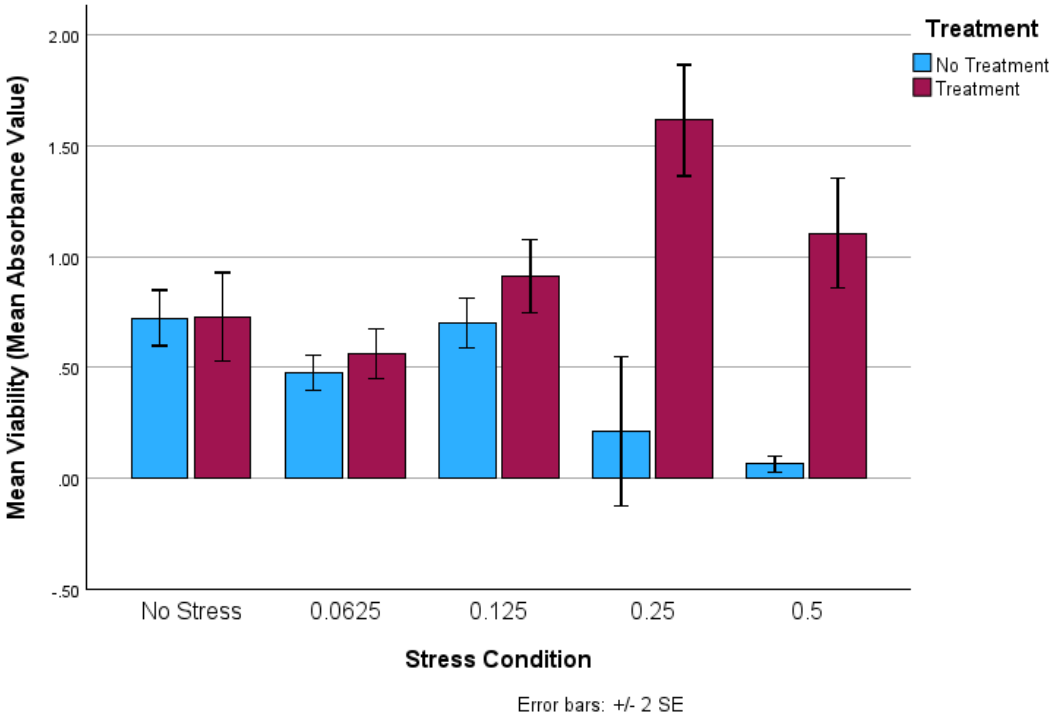


Figure 5: Cell Viability via MTS Assay of Neurons Treated With and Without DCP-LA and LM11A-31 Under Oxidative Stress Conditions. Cells were stimulated with varying degrees of FAB stress (no stress, 0.0625X, 0.125X, 0.25X, and 0.5X) for five days either treated with or without the DCP-LA and LM11A-31 treatment. Depicted is the relationship between each stress condition's no-treatment versus treatment-administered cell viability scores. Data was collected from three separate experiments.

DCP-LA and LM11A-31: Effects on Microtubule Stability

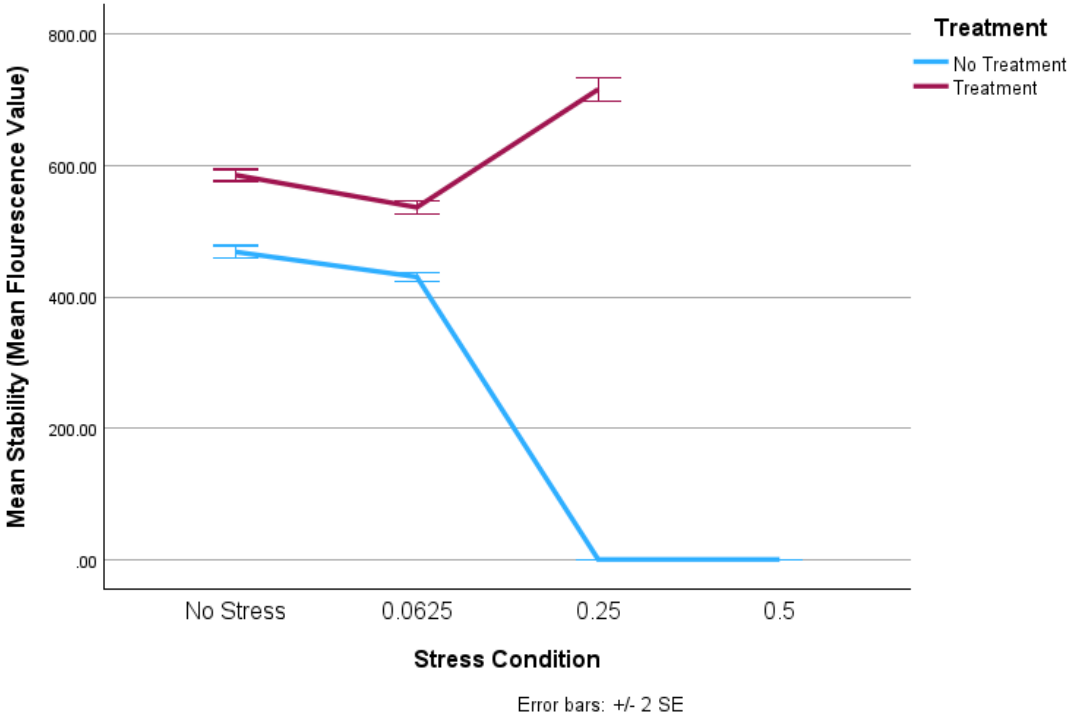


Figure 6: Mean Microtubule Stability by Stress Condition and the Presence of DCP-LA and LM11A-31. Cells were stimulated with varying degrees of FAB stress (no stress, 0.0625X, 0.125X, 0.25X, and 0.5X) for five days either treated with or without the DCP-LA and LM11A-31 treatment.

Parallel cultures to the previous MTS studies were made at the same time and treated in the exact same way to undergo fixation and staining for microtubule stability. A line graph (**Figure 6**) was created in order to illustrate the interaction between DCP-LA/LM11A-31 treatment (0 = no treatment and 1 = treatment administered) and FAB stress condition (0 = no stress, 1 = 0.0625X, 2 = 0.125X, 3 = 0.25X, 4 = 0.5X) on mean microtubule stability measured through fluorescent marking tagging microtubules. Two lines are plotted: one for the no-treatment condition (blue line) and one for the treatment-administered condition (red line). Although a 0.25X and 0.5X stress condition was experimentally conducted and intended for

immunocytochemical analysis, both conditions did not yield any viable cells, and thus microtubules, for analysis in the no-treatment condition. They are denoted as 0. Additionally, technological difficulties with the device running NIS Elements resulted in the software being offline, and did not permit data for the 0.5X condition that has undergone treatment to be captured. See **Figure 7** for sample images of what these conditions looked like under the microscope. In the two highest stress conditions, stable microtubules were not seen in dark room imaging. Faint white orbs appear intermittently in the photos. Some staining reagents such as the antibodies used in this experiment can aggregate and nonspecifically bind to cellular debris or floating particles, producing these glowing orbs. As per the 0.5X FABN condition that underwent treatment, imaging revealed mild microtubule staining that was not as bright as that of the control group nor the 0.25X condition that underwent treatment. Nonetheless, there is a significant qualitative difference between the absence of staining altogether in the non-treated 0.5X condition versus the treated one, where staining is much more prominent. Normally there is uniformed branching of dendrites and high-quality staining for the control group and the lower stress conditions, so for these three conditions overall, it does appear that unstable microtubules characterize them.

In the no-treatment condition, microtubule stability is essentially non-existent after the 0.0625X condition, given the inability to gather any data from immunocytochemical analysis (for the 0.25X and 0.5X conditions). Conversely, in the treatment-administered condition, stability remains higher than the no-treatment condition across the stress levels; however, the effect is most prominent at higher stress levels (0.25X and 0.5X). There appears to be an interaction between the presence of the treatment and the stress condition, suggesting that treatment administration may be able to moderate the relationship between stress and microtubule stability.

To evaluate the findings suggested by the graph, a 2x4 two-way ANOVA was conducted to analyze the effects of the treatment (0 = no treatment, 1 = treatment administered) and stress condition (0 = no stress, 1 = 0.0625X, 3 = 0.25X, and 4 = 0.5X) on microtubule stability. Results indicate a statistically significant interaction between treatment presence and stress condition, $F(2, 4113) = 253.50$, $p < 0.001$, highlighting how the effect of treatment administration on stability most likely depends on the stress condition level. Moreover, there were significant main effects of stress condition on stability, $F(3, 4113) = 148.12$, $p < 0.001$, as well as treatment administration, $F(1, 4113) = 1{,}120.63$, $p < 0.001$. These main effects suggest that the differences in mean microtubule stability are dependent upon both the stress condition and the treatment administration or non-administration, irrespective of any interaction of the two. According to partial-eta squared, 11% of the variance in microtubule stability can be explained by the statistically significant interaction between the two. 9.8% can be explained alone by the stress condition, controlling for treatment administration, and 21.4% can be explained by treatment administration controlling for the stress condition.

Post-hoc testing (LSD) revealed that across all stress conditions, the lowest microtubule stability was recorded consistently for the no-treatment group. For the no-treatment condition, mean microtubule stability was lowest for 0.5X/0.25X followed by 0.0625X, and no stress ($p < 0.001$, also consider that the 0.25X and 0.5X conditions did not yield observable neurites). The no stress condition outperformed all others. The 0.0625X stress condition performed better than both the 0.25X and 0.5X conditions ($p < 0.001$). The 0.25X and 0.5X stress conditions did not differ from one another in the no-treatment condition, as they both yielded very similar results: a lack of staining of microtubules in their neurons.

For the treatment-administered condition, mean microtubule stability was lowest for 0.0625X, followed by no stress, and 0.25X ($p < 0.001$, data from the 0.5X stress condition treated with the drugs could not be gathered due to technological error). With treatment, the no stress condition performed better than the 0.0625X condition ($p < 0.001$). The 0.25X condition outperformed all others ($p < 0.001$). See **Figure 8** for a graphical representation of these findings.

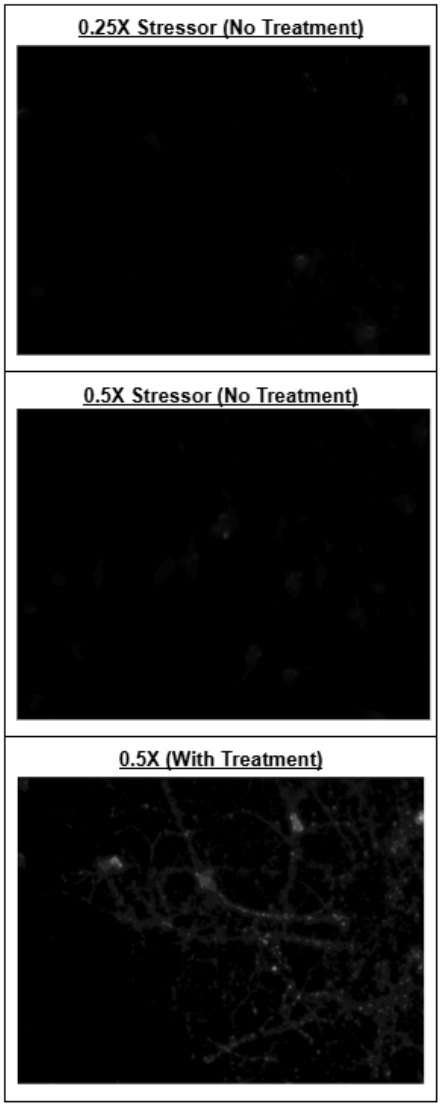
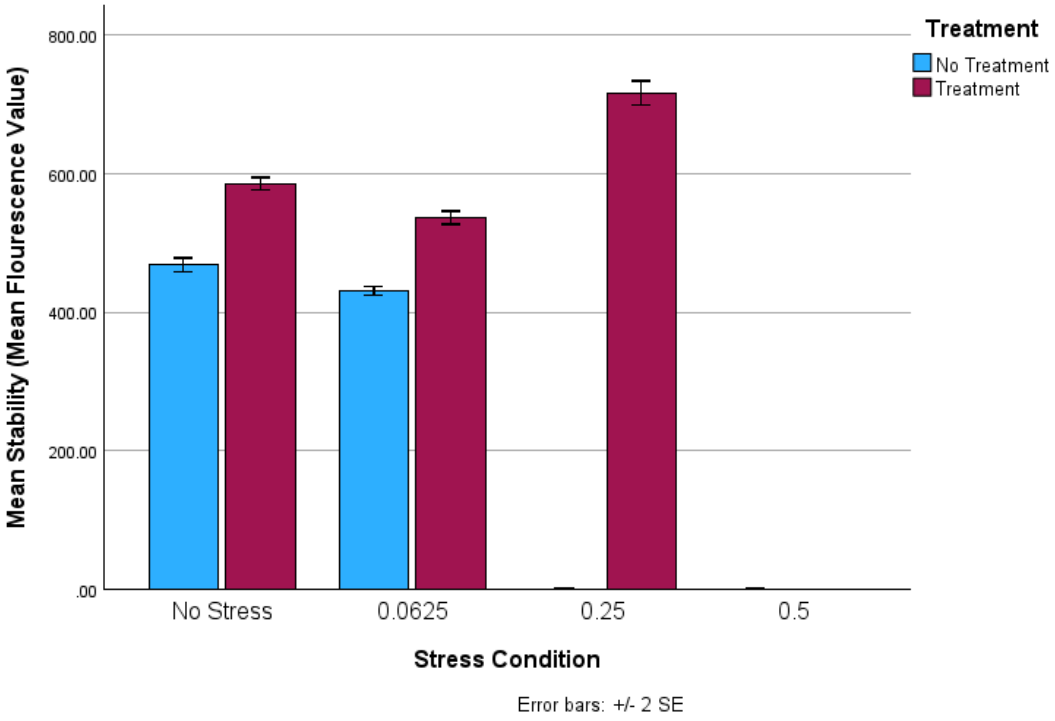


Figure 8: Sample images from the 0.25X and 0.5X non-treatment stress conditions and 0.5X stress condition with treatment



***NOTE**: No neurites were produced under the no-treatment 0.25X and 0.5X conditions. Technological challenges also did not permit for the 0.5X treatment condition data to be collected.

Figure 8: Average Intensity of Microtubule Fluorescence of Neurons Treated With and Without DCP-LA and LM11A-31 Under Oxidative Stress Conditions. Cells were stimulated with varying degrees of FAB stress (no stress, 0.0625X, 0.125X, 0.25X, and 0.5X) for five days either treated with or without the DCP-LA and LM11A-31 treatment. This graph depicts the relationship between each stress condition's no-treatment versus treatment-administered mean microtubule stability scores.

Discussion

This study sought to better understand how the combination of DCP-LA and LM11A-31 under an oxidative stress model of the aging brain may elicit neuroprotective effects promoting both cell viability and stability. LM11A-31 has been shown in mice models to increase neurite density (Knowles, J. K., et al., 2013). DCP-LA, a specific activator of PKC-ε, can help promote downstream anti-inflammatory cascades and help bind microtubule-associated proteins in a way

that is conducive to microtubule stability (Callender, J. A. & Newton, A. C., 2017). Accordingly, modulation of the p75 neurotrophin receptor, coupled with the upregulation of PKC-ε, was hypothesized to mediate these effects in this experiment.

The FABN model used in this investigation combined ferrous sulfate, amyloid-beta, buthionine, and NMDA in order to replicate key aspects of oxidative stress, excitotoxicity, and diminished antioxidant defenses that occur naturally as organisms age. Emulating the cumulative oxidative and metabolic stress that occurs in aging neurons was believed to lead to decreased cell viability and microtubule destabilization, as observed in aging and neurodegeneration. In this model, the varying concentrations of stress represent different levels of oxidative stress and cellular damage, correlating with the progression of aging at the cellular level. Lower concentrations sought to simulate mild stress conditions, akin to the early stages of aging where cellular antioxidant mechanisms are still able to manage the buildup of ROS to maintain functional integrity. As FABN concentrations gradually increased, it was hypothesized that the cells would experience a greater degree of insult, challenging their natural defense mechanisms. Likely these conditions would induce the greater structural and functional impairments mirroring aging's later stages. Higher FABN conditions reflect compounding damage that occurs in aged cells, aligning with the biological realities of aging where cellular repair mechanisms gradually weaken, leading to the inability to maintain homeostasis. This model provides insights into the particular thresholds at which age-related damage becomes irreversible by analyzing how the cells respond to different FAB conditions. Utilizing a gradient approach further emphasizes how the complications of aging exist on a spectrum from manageable oxidative challenges to severe cellular dysfunction.

FABN Stress Conditions Elicited Sufficient Neuronal Stress

The first assessment made in this experiment was whether or not the FABN stress solutions at varying degrees elicited sufficient neuronal distress on measures of cell viability and stability. In terms of cell viability, all stress conditions performed statistically significantly worse than the no stress condition without treatment. These findings are consistent with research exploring *in vivo* FAB rat models, where lower viability is quantified as the prominence of hippocampal neuron degeneration (Lecanu, L. & Papadopoulos, V., 2013). This viability decrease was not only expected, but confirms the FABN solution's ability to generate stress, modeling both the aging brain and perhaps a brain in a prolonged preclinical state. Considering measures of stability, immunocytochemical analysis indicates that across all stress conditions, neurites that were not treated DCP-LA and LM11A-31 demonstrated the lowest fluorescent values, or the most unstable microtubules. The highest stress conditions did not even generate observable neurites for data collection, meaning that these concentrations are likely too high to allow for neuronal growth and differentiation.

DCP-LA and LM11A-31 Did Not Lower Viability and Stability Scores in the Absence of Stress

The combinatorial treatment of DCP-LA and LM11A-31 did not lower viability or stability scores in the control group (without stress), suggesting that this treatment is non-toxic under normal conditions. During cell culture, 10-20% cell death takes place after around two weeks of growth. Differences in microtubule stability were noted between the non-treatment and treatment-administered conditions, where the latter yielded a higher microtubule stability average. It is possible that this occurred due to a treatment-induced protection of the cells that would otherwise undergo processes that may have destabilized their microtubules. The ability of

the combinatorial treatment to preserve cellular heath in non-stressed conditions indicates that it is selective in targeting pathological environments without negatively impacting healthy cells. These findings suggest that this approach does not impair fundamental cellular functions, such as energy production, protein transport, or synaptic communication, crucial for long-term cellular and organismal health. Some contemporary treatments, as explored through the discussion of ARIA associated with amyloid-beta targeting drugs, can interfere with baseline functions leading to significant off-target effects. At the very least, these preliminary findings from an *in vitro* point of view presents the potential of this combinatorial approach as a targeted therapeutic strategy with minimal side effects in non-pathological contexts.

DCP-LA and LM11A-31 Improve Cell Viability

An MTS assay was the first test conducted in order to gain a preliminary understanding of how this combinatorial treatment impacts neurons. As demonstrated by the data, when treated with DCP-LA and LM11A-31, cell viability was highest for all stress conditions (**Figure 4**; **Figure 5**). Following treatment, the no stress condition performed significantly worse than the two highest stress conditions (0.25X and 0.5X), and roughly the same as the two low-to-middle levels (0.0625X and 0.125X). The 0.125X condition was outperformed by the 0.25X condition, but still yielded greater cell viability than the lowest of the stressors (0.0625X). Overall, 0.25X and 0.5X outperformed all other groups when treated with DCP-LA and LM11A-31. These findings demonstrate that while drug administration significantly improves viability, its effects are more pronounced under higher levels of stress. Additionally, stress conditions alone had varied effects depending on the treatment presence (worse across the board without it and better across the board with it). Non-significant results for lower stress levels suggest that the buffering

effect of the combinatorial treatment may be more critical under moderate to high stress conditions.

This experiment raises an important notion that stress may enhance the efficacy of certain pharmacological interventions. When experiencing greater stress (under higher stress levels), neurons are likely undergoing more severe insults to their viability from worsening oxidative damage and metabolic imbalances, effects that DCP-LA and LM11A-31 actively work to counteract. It is possible that stress-induced apoptosis and microtubule protein disarray manifests frequently at higher stress levels, paving the way for these compounds to exert more potent restorative or protective effects. Furthermore, statistical analysis did reveal an interaction between stress condition and treatment, pointing to a potential threshold where the protective mechanisms of the drugs become necessary, very well being at a moderate to high stress level.

Considering lower stress levels, it may be possible that the neurons are not undergoing sufficient physiological disruption for the treatment's effects to be just as impactful. As discussed throughout this paper, there are innate cellular "resilience" mechanisms to mediate many of the deficits associated with these kinds of insults that may be adequate enough to reconcile these disturbances, maintaining cell viability. The lack of significant improvement under mild stress conditions (0.0625X and 0.125X) suggests that the cells' intrinsic resilience is adequate to maintain viability, reducing the contribution of the treatment. Additionally, the interaction between stress condition and treatment effects points to a potential threshold where the protective mechanisms of the treatment become necessary, such as at moderate to high stress levels. Viability deficits highlight a fundamental challenge for aging cells: the ability to sustain energy and repair mechanisms in the face of oxidative stress among other stressors. Reduced metabolic activity, as seen in the lower MTS values under FABN stress, suggests that neurons are less

capable of maintaining basic functions necessary for survival. In aging, it has been shown that ATP production is diminished, likely contributing to energy deficits that comprise synaptic plasticity and cellular repair (López-Lluch, G., et al., 2018). When neurons lose the ability to form or strengthen synaptic connections, the consolidation of new memories is impaired. As viability continues to decrease over time, entire networks may experience greater degradation, contributing to broader cognitive impairments.

Although MTS assays have become an assessment staple in neuronal cell culture research, they do come with particular limitations that must be considered when interpreting results. When the MTS dye is metabolized by the cells, producing a dark brown color that is later quantified through absorbance values in the assay, this metabolic activity may not precisely reflect neuronal health or survival. Neurons, especially in culture, exist in many different metabolic states that depend upon their differentiation stage, stress condition, and a whole host of other experimental variables. On top of this, MTS assays are unable to distinguish neurons that are alive but functionally impaired, or distinguish between neurons and non-neurons (like glial cells). These limitations may potentially lead to overestimations or underestimations of cell viability, making MTS assays not the most powerful stand alone assessment. It is for these reasons that dark room imaging was also performed to better ascertain if there are differences in morphology (microtubule stability) that may account for these variations as well.

DCP-LA and LM11A-31 Improve Microtubule Stability

Microtubules, as discussed, are a fundamental cytoskeletal component, known for their dynamic instability that is fostered by a wide-range of post-translational modifications or PTMs. Particularly, the acetylation of lysine 40 (K40) on alpha-tubulin (a microtubule building block)

has been widely studied (Janke, C. & Montagnac, G., 2017). Acetylation at large is linked to microtubule stability and longevity, as this PTM makes them more resistant to depolymerization. Recent studies posit that acetylation of K40 may specifically contribute to mechanical and cellular stress resistance. This heightened stabilization is believed to take place via microtubule self-repair, a process where acetylation strengthens the microtubule lattice, reducing the likelihood of stress-induced disrepair (Janke, C. & Montagnac, G., 2017). If the sentiment surrounding PTMs is beginning to adopt that these are active restorative processes, acetylation may be just one example of microtubule health that extends beyond their age and stability. Anti-acetylated tubulin was the marker utilized in this experiment to detect microtubules that have undergone this PTM. Bearing these findings in mind, perhaps it is possible that on top of assessing for stability, immunocytochemical analysis in this study may also contribute to a better understanding of how well a given microtubule is resisting stressors through engaging in stronger intracellular transport, signaling, etc., via this enhanced structural integrity.

As exemplified by **Figure 8**, when treated with DCP-LA and LM11A-31, microtubule stability was much higher than the conditions without treatment. Notably, neurites were not observable for the 0.25X and 0.5X stress conditions without treatment, indicating that the cells surviving in these conditions as exemplified by MTS assessment were either not neurons or ones without stable microtubules. In terms of microtubule stability, the 0.0625X stress condition without treatment did not yield microtubules statistically significantly less stable than the controls, suggesting that this stress condition in particular may not be sufficient enough to meaningfully affect microtubules. However, when treated with DCP-LA and LM11A-31, microtubule stability was much higher across the board for all stress levels. The control group (no stress) outperformed 0.0625X, but did worse than 0.25X, which outperformed all others.

Outlined by this preliminary research study is that also for microtubule stability, higher stress levels yield the highest stability values in the presence of the treatment. DCP-LA and LM11A-31 appear to mitigate these adverse effects, promoting stability and neuronal differentiation, even when bombarded by substantial stress. Interestingly, the no stress group fared worse than the 0.25X group when treated, which may demonstrate a potential adaptive response. Moderate stress may prime cellular pathways that enhance microtubule stabilization induced by the treatment. In a similar way to inflammatory and ROS cascades, perhaps cellular stress prompts stress-induced repair pathways that enhance each compound's accessibility to their targets. Because of this, the control group, not illustrating differences when treated, might not be benefiting as much from the treatment because these pathways are not as strongly engaged in the absence of stress. Considering one of the first indications of impaired memory is a disruption in one's ability to recall recent events, there may be issues with the stability of microtubules that might lead to memories being more at risk. Stable microtubules maintain axonal and dendritic transport of proteins, microtubules, and other essential molecules. When unstable, as indicated by reduced fluorescence intensity in FABN-treated groups, such would impair the transport of these key materials and in the context of memory, this disruption can weaken synaptic connectivity. Additionally, one may be left with an inability to effectively store and retrieve recent events. Memories, particularly short-term ones, rely heavily on active synaptic processes, and microtubule instability may increase the risk of disconnection between neurons, yielding lapses in recall (Kaganovsky, K. & Wang, C. Y., 2016).

Immunocytochemistry is a very powerful technique to visualize neuronal integrity, but it can often become susceptible to experimental error. A central challenge lies in the sensitivity and specificity of the antibodies used. Working in a lab where multiple experiments are occurring

simultaneously, manipulation of the antibodies occurs often, and prolonged exposure to room temperature can result in degradation. An antibody that is not as potent as it ordinarily would be leads to diminished staining, and consequently minimal microtubule analysis. Furthermore, working with cell culture involves the possibility of contamination, which resulted in the absence of a 0.125X stress condition for review. Additionally, immunocytochemistry requires NIS Elements, a specialized software, in order to capture images and subsequently analyze them using a variety of the program's specialized tools. During this experiment, the software was no longer accessible due to technological difficulty, leading to the inability to collect data from the 0.5X condition treated with the drugs. Moreover, it is critical to note that quantification of immunocytochemistry results is often subjective, relying heavily on visual assessment or digital image analysis. In order to avoid observer bias, a circular grid approach was adopted to standardize data collection between experiments.

Implications for an Accumulation Threshold

The concept of an accumulation threshold, while heavily discussed in the context of amyloid-beta pathology, extends to other critical aspects of AD pathology and aging at large, such as tau tangles and ROS. Both tau and ROS contribute significantly to neurodegeneration, and their roles are interconnected with broader pathological cascades. Like amyloid-beta, tau protein accumulation may also have a threshold beyond which its pathological effects become irreversible. Hyperphosphorylated tau aggregates into neurofibrillary tangles, disrupting intracellular transport and leading to neuronal dysfunction (Iqbal, K., et al., 2010). Similarly, ROS, the byproducts of cellular metabolism, accumulate during oxidative stress and can exacerbate tau hyperphosphorylation and amyloid-beta toxicity (Ristow, M. & Schmeisser, S.,

2011). Early intervention might prevent these feedback loops, but once a critical threshold is crossed, the cellular damage can become self-perpetuating, rendering therapies less effective.

In surveying present-day research, there are many different theories to explain why a once cognitively sound mind might snowball into developing dementia. The notion that there can be multiple cascades converging on one another even a decade before symptoms appear complicates current therapeutic strategies. Interventions typically begin at later stages, when these unidentified thresholds may have already been exceeded. For example, consider again drugs targeting amyloid-beta like aducanumab and lecanemab. They primarily aim to reduce amyloid-beta plaque burden, but cannot address the possible upstream effect of ROS accumulation or downstream tau pathology, which can intensify as amyloid-beta aggregates increase in prominence (Söderberg, L., et al., 2023; Maulden, A., 2022). Many contemporary treatments magnify a solution to one problem that may be secondary to another. They also tend to be introduced at more advanced stages of the disease where intervention may not be as influential.

Research Goals and Future Work

Isolating the Potential Synergistic Effect

In the present study, DCP-LA and LM11A-31 were not tested by themselves as research conducted by former students of this lab had previously devoted time and resources to investigate each molecule independently. Preliminary findings indicated that when normalized to the control group, DCP-LA on its own was able to partially recover both cell viability and microtubule stability also under an FAB stress model (around 85% recovery); however, only one concentration of FAB was explored in this project (Rinald, J., 2020). The highest DCP-LA

concentration of 100μM performed the best in comparison to the lower doses in terms of cell viability and was accordingly utilized in the cultures to be fixated and stained for microtubule stability. Overall, DCP-LA in this project did not appear to preferentially augment viability or stability scores, and effects are modest, never quite reaching or exceeding the performance of the control group.

Exploring the independent effects of LM11A-31, however, did demonstrate a similar trend to the current research study: improvement in the stress condition transcending the performance of the control group under treatment (Cevallos, J., 2022). LM11A-31 at 10nM recovered viability measures beyond that of the control group (around 30%), with the highest recovery seen for measures of microtubule stability (40%). This improvement above the control group also persisted in the current study, where the percentage of improvement was much higher, reaching over 50% for the highest stress conditions (extending as high as over 100% for the 0.25X stress condition) for cell viability and over 50% as well for microtubule stability. The degree of recovery exemplified by the current study was not observed by the pilot studies exploring the effects of DCP-LA and LM11A-31 independently of one another, suggesting there may be a synergistic relationship between the two mediating this effect. Future research must isolate whether these improvements are purely synergistic or alternatively additive. Due to the constraints in financial resources and the limited timeframe of this project, a comprehensive analysis was not feasible.

Looking Ahead

Studies have shown DCP-LA to be supportive of long-term potentiation (LTP), a process occurring in the brain whereby synapses (connections between neurons) are strengthened in

response to repeated activity. LTP is a form of synaptic plasticity that allows these connections to adapt and change over time — a cellular alteration at the root of learning and memory. The hippocampus is pivotal for memory formation and is considered a site of consistent synaptic transmission enhancement. When high-frequency stimulation (repeated activity) takes place, a significant influx of calcium ions enter the responding cell (postsynaptic neuron) via NMDA receptors, glutamate receptors (Lüscher, C. & Malenka, R. C., 2012). In doing so, the calcium ions sensitize AMPA receptors, also glutamate receptors, enabling that synapse to become even more responsive to future signals. When the structural characteristics associated with LTP take place, they can last for days at a time, enhancing both how new information is learned and how old information is retrieved in a way that is most efficacious for both. DCP-LA has been demonstrated to reverse the negative effects of a mutated form of amyloid-beta (Aβ1–42E22Δ) on the LTP process in the hippocampus (Nagata, T., et al., 2010). When the hippocampus in the presence of mutated amyloid-beta was stimulated via the Schaffer collateral pathway (a pathway designated to be critical for LTP), LTP was blocked. However, DCP-LA rectified these issues if injected prior to stimulation.

LM11A-31 has been shown to induce similar effects in the basal forebrain, a region of the brain that is functionally interconnected with the hippocampus (Simmons, D. A., et al., 2014). Damage to the basal forebrain's cholinergic neurons, such as in AD, disrupts the acetylcholine supply to the hippocampus. As a result, hippocampal function is impaired, leading to the memory and cognitive deficits characteristic of this neurodegenerative disease. In two models of AD disease (mid-stage and late-stage), LM11A-31 prompted reductions in the amount of cholinergic neuron atrophy in the basal forebrain (Simmons, D. A., et al., 2014). For mice at the mid-stage, LM11A-31 treatment for 3 months not only prevented further damage to cholinergic

neurons, but also showed improvements for the neurons that were already damaged. These findings are significant because they suggest that targeting the p75NTR may not only slow down the progression of AD but also reverse some of the damage already done.

The preliminary data set forth by the present experiment places these two drugs side by side in order to assess if there is a potential synergistic effect that would work to enhance their independent functions further and create new ones built upon their collaboration. Given the results of this experiment demonstrating significant interactions between stress conditions and drug administration for both cell viability and stability ($p < 0.001$), there is a reasonable groundwork to inquire further about what such means for the future development of therapeutic strategies utilizing these two drugs. A significant limitation of the study is that it lacks morphological data from the 0.125X condition, and because of the significant neuronal loss characteristic of the two highest stress conditions, it would be advisable to conduct more experiments utilizing a wide range of stress conditions. With a greater quantity of stress conditions to consider, the effects of these drugs would be understood on an even more specific gradual stress basis.

To further grasp how these drugs function alongside one another, *in vivo* studies offer the ability to utilize tasks involving behavioral paradigms to test different kinds of memory (spatial, associative, recognition, etc.). Applying the DCP-LA and LM11A-31 drug treatment to transgenic rat models of AD, for example, would allow for the deficits of AD to develop somewhat organically through the animal's own genetics (not from external injections). This approach would enable researchers to evaluate how these genetically-driven deficits respond to the drug treatment on a number of different tasks like the Morris Water Maze, assessing spatial memory and learning based on navigating from learned spatial cues, or the Novel Object

Recognition Test, where animals are assessed on how long they spend exploring novel stimuli opposed to familiar ones. It would also be beneficial to look at the protective effects these drugs may yield in such a setting by analyzing performance before and after age-related/AD-related deficits take place to understand if there is a significant temporal effect of the drugs on behavior. Taking this research from neuronal cell culture to an *in vivo* space would allow for the cellular improvements noted from this experiment to be linked to the context of real-world problem solving and memory.

Conclusions

Ultimately, though much of the scientific discourse surrounding treatments of this nature pertain to treatment of already existent symptoms of dementia, the argument made is one that calls to reconsider aging as an environment inviting these deficits to occur through the early accumulation of damage over time. The limited cognitive benefits observed in clinical trials for current FDA-approved treatments might reflect the timing of intervention and the multifactorial nature of AD. Future research must delve into whether targeting these processes earlier (before symptoms even develop) can yield more meaningful clinical outcomes.

Aging is often perceived as a natural and inevitable process, but in reality, it shares many characteristics with pathological conditions that should justify its classification as a treatable phenomenon. Aging is not a mere accumulation of birthday candles on top of the cake each year — it can be understood as specific cascades of biological mechanisms (like ROS, decreased glymphatic activity, altered dendritic morphology, etc.) that parallel neurodegenerative diseases. This considerable overlap challenges the idea that aging is separate from disease, begging a transformative shift in perspective: to treat aging as a medical condition worthy of intervention.

The implications of doing so are profound. Scientists wielding this perspective could target aging's underlying mechanisms before they culminate in debilitating conditions. However, societal attitudes often resist this perspective, rooted in the belief that aging is natural and therefore untouchable. Yet, many of the changes associated with aging, like cellular senescence and chronic inflammation, are far from benign. Issues like these directly contribute to a diminished quality of life, increased healthcare costs, and the loss of autonomy feared by so many as they age.

Although a cliche, it is wise never to judge a book by its cover. Just because symptoms are not apparent does not indicate the environment in which they may soon develop is not already forging from compounding stress on our fragile neural systems. For the same reasons in which one may elect to take a daily multivitamin, it may be time to consider aging as a process we can support with the right supplements. DCP-LA and LM11A-31 have not yet been analyzed in the sole context of aging, nor have they been paired together. The investigation of their potential synergy may further spark a hopeful trend in medical research: the increasing focus on how early action for the aging brain may very well revolutionize not only how we approach age-related cognitive decline and related disorders, but the emotions we feel as we get older. This is not about lifespan, but rather healthspan, or the period of our life spent in good health, free from the chronic diseases and disabilities of aging that enable older age to appear so taboo.

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