The Calcium Signaling System: Aiming for a Comprehensive Explanation of Aging and the Dementia-Alzheimer's Syndrome

ARA S. KHACHATURIAN[1,3,4], ZAVEN S. KHACHATURIAN[2]

Abstract

The overarching aim of this perspective paper is to reformulate the calcium hypothesis from the standpoint of systems theory to address the complexity of the calcium signaling system to maintain homeostasis of cytosol calcium ion concentration essential for the optimal functioning of a neuron. The intent is to recast the earlier attempt of the calcium hypothesis to formulate a unifying theory linking brain aging and the dementia-Alzheimer syndrome in terms of a systems failure model[1]. This re-definition of the problem of the linkages between aging and dementia will encourage the development of novel in silico models and applications of machine learning algorithms and other quantum computing modeling approaches to tackle the complexity of neuronal calcium regulation and, eventually, describe systems failures in brain aging-dementia continuum.

Keywords: **Calcium hypothesis, aging, dementia, Alzheimer, systems failure model, complexity, systems theory, in silico model, modeling system, machine learning, artificial intelligence, calcium, signaling, neuron, multiscale.**

Introduction, Background and Context

Efforts to find treatments for chronic brain disorders such as the dementia-Alzheimer syndrome[2] are imperative, given the growing psycho-social challenges and escalating expenses associated with caring for affected individuals on a global scale. The aim of eradicating the dementia-Alzheimer syndrome from the world is a top priority for public health systems globally. However, there is still no clear path to achieving this goal. Despite significant investments in research for over 40 years, there are only a few effective interventions available to slow down the progression of the disease or delay the onset of cognitive impairment and other disabling symptoms.

One of the main challenges in developing therapies for chronic brain disorders like dementia is the complexity of the neurobiology involved. Another is the lack of a conceptual framework connecting the components and variables involved. These two factors contribute to the difficulty of discovering effective interventions.

Over the past four decades, studies on the dementia-Alzheimer syndrome have uncovered a wealth of information about the aging of the brain and the process of neurodegeneration. However, the field lacks a unifying theory[3] that can connect the dots and guide the exploration, testing, and validating of new interventions. Therefore, the calcium hypothesis, which was originally proposed as a potential unifying concept, has been restructured as a set of testable postulates based on system theory. This approach may eventually lead to a comprehensive theory that links aging and neurodegenerative disorders, such as the dementia-Alzheimer syndrome.

The task of formulating a comprehensive theory of aging and dementia-Alzheimer syndromic continuum is fraught with several conceptual, scientific, and methodological impediments. Among these the most relevant include the nature of the disease itself (i.e., an ambiguous and shifting definition), the lengthy

VM&E 2023;6:4-16
Published online October 27, 2023
http://dx.doi.org/10.14283/VME.2023.2

1. Brain Watch Coalition of the Campaign to Prevent Alzheimer's Disease, Rockville, Maryland, United States of America
2. Campaign to Prevent Alzheimer's Disease, Potomac, Maryland, United States of America
3. International Neurodegenerative Disease Research Center, International Neurodegenerative Disorders Research Center, Prague, Czech Republic
4. University of Las Vegas, Nevada, National Supercomputing Institute & Dedicated Research Network, Las Vegas, Nevada, United States of America

Corresponding to:
Ara S. Khachaturian,
9812 Falls Road Suite 114-155
Potomac, MD 20854-3963
Email: ara@pad2020.org
http://www.brainwatchcoalition.org
http://www.pad2020.org

1. The construct of a system defines a molecular signaling path, or single neuron, or neural circuit, or anatomical structure or an organism.
2. This paper discusses the concept of the dementia-Alzheimer syndrome, which is used as a broad term to encompass a range of chronic brain disorders that require extensive and personalized care. These disabilities can have a significant impact on healthcare systems and can be emotionally taxing for family caregivers. The common characteristics of these disorders include a decline in cognitive, motor, and emotional function, as well as a gradual loss of independence that necessitates labor-intensive care. With increasing life expectancy, these disabilities are lasting longer, with some individuals facing up to 30-40 years of dependency, economic strain, and reduced quality of life, especially those who live beyond their 9th or 10th decade of life.
3. A *theory* is the proven statement that represents the culmination one or more hypothesis that are tested under different conditions/experiments. Whereas a *hypothesis* is the unproven statement that suggests a possible answer for a question.

Received July 27, 2023
Accepted for publication August 28, 2023

time course for degenerative processes, the heterogeneity in the phenomenology of the syndrome, the complex interactions among genetic and other risk factors, the poorly understood nonlinear relationships between the neurobiological and the clinical phenotypes, and the paucity of appropriate modeling systems. *This paper focuses primarily on a fictional neuron system, and does not address Alzheimer's, dementia, or any other medical condition*. The primary objective is to thoroughly understand and elucidate the complex interplays—*at the conceptual level*—among different variables and factors that are essential for maintaining optimal functionality of a hypothetical neuron. Once completed, this serves as the fundamental and comprehensive framework for constructing a neural network or circuit. Once finished, this effort will *establish an essential framework for developing, testing and updating an all-encompassing blueprint* for building a neural network or circuit.

The first attempts to formulate unifying theories on brain aging and Alzheimer's disease (AD) began in the early 1980s as an integral part of planning and developing a U.S. national research program on brain aging and Alzheimer's disease at the National Institute of Aging (1). The specific aim of this initial effort, "Towards a theory of brain aging," was to consolidate existing knowledge and redirect the focus of the prospective research toward more molecular or mechanistic studies; and less emphasis on descriptive studies (2-4). Since then, the knowledge about the aging brain and the neurodegenerative process has proliferated, but unfortunately, this massive amount of information has had only a minor impact on the development of interventions and diagnostics for persons with dementia, especially AD.

Too often, the identification of a promising molecular target or the discovery of a biological marker for AD has failed to translate into an effective intervention or a robustly valid diagnostic. This problem is due partially to the lack of knowledge about the precise functional relationships between clinical features/symptoms and the neurobiological markers/mechanisms. This nonlinear relationship between molecular events and clinical symptoms remains a major challenge for neuroscience, as well as AD research.

Another major obstacle is the lack of tools for effectively managing and sharing knowledge across diverse domains. It is imperative to have a universal vocabulary and methodology to convey the intricate interconnections between molecular, biochemical, or neuronal events and the overall functioning of the brain. Currently, it is a daunting task to precisely describe clinical features, such as cognitive decline, that result from substantial changes in protein structure/function, timing, and sequence of key biological processes. Similarly, it is challenging to identify the specific role of genetic and environmental factors in symptom expression. The development of an integrated theory that links molecular data to disease behaviors and symptoms is an arduous task that is not exclusive to AD or neuroscience research.

The current state of research struggles to comprehend the behavior of complex systems through repetitive analysis of smaller, simpler constituent parts. Even with advanced research instruments, detailed information on molecular sequence-structure-function is unlikely to yield significant answers or insights on the manifestation of AD's clinical symptoms. Despite knowing the gene for Huntington's disease for several decades and ongoing clinical trials to maintain functioning early in the disease, this knowledge has yet to produce a curative therapy. In protein chemistry, even detailed information on amino acid sequences or secondary or tertiary protein structures cannot predict function, such as protein-protein interactions.

Prevailing translational theories of Alzheimer's (AD) pathogenesis presently do not consider adequately the complex relationship between the clinical and biological phenotypic expressions of the resulting dementia. The initial amyloid hypothesis, for example, is based on a conceptual model for early-onset autosomal dominant (familial) AD (FAD). Generalizing this model to sporadic AD requires a major assumption: the etiology and pathogenesis of autosomal dominant illness determined by mutations in the amyloid precursor protein (APP) or the presenilin 1 or 2 (PS1/2) genes is equivalent to the conditions that lead to the late-onset, sporadic forms of AD. This may be true. However, the latter, and the much more common form of AD, is a complex, non-Mendelian genetic entity with a multitude of protective- and risk-variant patterns. The sporadic, or late-onset, AD has a complex and variable genetic signature that may account for approximately 60 to 80 percent of disease risk (6).

Therefore, any unifying theory of AD must acknowledge that disease pathogenesis will not be reduced to a single etiological factor. Rather, a theory of AD must account for: 1) specific biological signals from multiple upstream factors/processes, 2) the intricate interactions—that include timing and sequencing—among multiple signaling paths that regulate the balance between cell repair/regeneration versus cell degeneration, 3) the complexity of convergence among a vast signaling network—upstream and downstream transmissions—from multiple sources toward a final common path (i.e., this is not equivalent to a single factor).

Where to start? Although the definition of AD continues to evolve, there are several common, known neurobiological indications at the cellular level. These include persistent waning in synaptic transmission, continuing pruning of dendritic arbors, massive loss of synapses, and decrements in repair and restoration functions1. It is the loss of neurons recognized to be the clearest and most identifiable feature of AD. Therefore, a unified theory of AD is predicated on two central questions: 1) How and why do some select sets of neurons become dysfunctional? 2) Why do some neurons die?

Neurons share many characteristics with other cell types, but in addition they have some unique features that include: 1) very high metabolic rate, 2) critical need for a constant supply of oxygen and glucose to survive, 3) loss of ability to divide by mitosis and 4) capacity to repair-regenerate-recycle essential components of the cell [e.g., membrane, organelle, receptors, channels, synapses, dendrites, etc.] in lieu of cell division by mitosis.

There are different types of neural cells in specific areas of the nervous system that serve specialized functions. The variety of cell types includes anterior horn cells, basket cells, Betz cells, granule cells, medium spiny neurons, Pukinje cells, Renshaw cells and pyramidal cells. Among these the pyramidal cells are of particular interest to AD as it may be viewed as the archetype neuron for modeling a neural system. The pyramidal neurons in the cerebral cortex, hippocampus, and amygdala, with proper connections, take part in the circuitry responsible for cognitive ability. These neurons have numerous voltage-gated Na^+, Ca^{2+} and K^+ ion-channels in the dendrites, and soma. The ability of pyramidal neurons to integrate information depends on the number and distribution of the synaptic inputs they receive. Because a single pyramidal cell receives about 30,000 excitatory inputs and approximately 1,700 inhibitory inputs, this neuron-type incorporates virtually all characteristics of self-contained "subunit" that may be used to construct rudimentary models of a more complex neural system. This opens the possibility to test the accuracy of a unified theory of AD.

There are two research avenues that have motivated the exploration of calcium ion (noted as Ca^{2+}) signaling as a theoretical basis to explain AD. First, the disruption of Ca2+ signaling could be an early upstream event where evidence from familial AD models provides important clues1. Second, there is evidence relating calcium ion dysregulation mechanistically to the neuropathological correlates of AD—namely, the presence of senile plaques and neurofibrillary tangles.

Reformulating the Calcium Hypothesis as the Calcium Systems Theory of the Dementia-Alzheimer Syndrome (CAST-DAS)

The Calcium Systems Theory of the Dementia Alzheimer Syndrome (CAST-DAS) asserts that sustained disruption of mechanisms that normally regulate intracellular Ca^{2+} signaling is pivotal for triggering adverse changes in the functioning of neurons. Ca^{2+} disruption serves as a necessary precursor and driver not only of aging-associated decrements in neuronal performance but also the molecular mechanisms underlying neuronal degeneration associated with AD pathogenesis (3). Compromised neuronal Ca^{2+} handling is thought to be an outcome of the various upstream events, including metabolic, oxidative, and proteotoxic stress (3). Changes and modulations of subcellular components [e.g., ion channels, buffers, ATP-dependent ion pumps, or other regulatory mechanisms] may also regulate the homeostasis of cytosol calcium concentration $[Ca^{2+}]i$ (1, 5). Furthermore, the deterioration of neuronal Ca^{2+} handling systems likely has an important downstream impact on virtually all of the major molecular alterations underlying the pathogenesis of AD, such as dendrite pruning, synapse loss, aggregated amyloid β-peptide (Aβ), Tau, p-Tau, mitochondrial dysfunction, oxidative stress and inflammation (1, 6, 7).

The core premise of CAST-DAS suggests conceptualizing AD as an accumulation of progressive "system failures" across an array of

interconnected networks within the brain. Using a "systems perspective" to characterize AD goes beyond identifying a single etiologic factor that leads to pathogenesis (3, 8). Instead, this theory promotes not only explaining the interactions among crucial pieces of the system [e.g., genetic, biological events, temporal changes that occur in sequence or in parallel] but also identifying approaches to optimize the overall performance of the "system" by examining its constitutive components. CAST-DAS provides a pathway to address the difficulties in conceptualizing and communicating non-linear relationships between the behavioral and clinical features of Alzheimer's disease and the underlying neurobiological mechanisms of pathology.

So how does CAST-DAS help elucidate the rules and regulatory mechanisms that operate/control molecular and network systems in the brain? The theory offers a conceptual framework to help overcome the difficulties of translating phenomenological modeling of genome-scale, protein-scale and molecular scale, and multiple other scales of information that influence the performance of neural systems. Assuming the validity of the modular network organization hypothesis (9), this assumption suggests a likelihood that genomic, proteomic, and ionic processes form subsystems of insolated functions and that emergent characteristics/behaviors should self-organize to comprise progressively higher scale systems (10). Further, these emergent characteristics will form higher-level regulatory networks with fewer interactions. From a computational and experimental perspective, this modeling schema may be calibrated so that the effective set of interactions is mathematically soluble given the data available (11).

The CAST-DAS contemplates simulations of biological systems at the molecular level to provide the most intricate mechanistic details, aid in interpreting experiments and provide predictive insight into intervention development. One of the emerging techniques is the use of multiscale computational modeling that integrates detailed knowledge from: 1) neuronal systems, 2) biomolecular/biochemical relevant parameters (12, 13), 3) brain topography that spans spatial scales from regions to a single synapse, and 4) timescales spanning single action-potentials, microsecond, hours or days. This modeling approach may also be viewed as a "knowledge management tool" that may inform strategic-level thinking of what types of key or additional data are required to validate intermediate hypotheses (14) and potentially provide an in silico platform for drug discovery (15, 16). Unlike traditional computational neuroscience—with foci on electrophysiology, synaptic signaling and corresponding network activity (17)—multiscale modeling for Ca^{2+} must include not only neuronal function but also intracellular and extracellular molecular dynamics.

Multiscale modeling computes information from a finer/smaller scale and forwards (or "couples") that information to a model at a coarser/larger scale and omits degrees of freedom along the transition from a lower to higher. These models aim to predict higher-order behaviors (so-called "upscaling or bottom-up approach") within a complex system (18). The rationale for multiscale models may be traced to Newton, Hooke, Bernoulli, Einstein, Bodenstein, and others (18), whereby omitting several degrees of freedom, and enabled them to propose continuum-based constitutive equations and simple models of relevant complex systems. In addition, multiscale models provide flexibility to simulate control conditions at a higher scale-level that then may permit simulated predictions at a lower scale-level (e.g., "downscaling" or "top-down approach"). Further, reverse engineering is another aim of top-down information flow: for AD research, this affords a rational approach to the prediction and identification of novel intervention targets (e.g., multicomponent, or multifunctional pathway combinations).

There are several applications for multiscale modeling using "well-mixed" biological systems (19). The multiscale nature of stochastic simulation for well-mixed systems arises from the separation of time scales, either disparity in rate constants or population sizes (20). The disparity of time scales—slow and fast events—is the rule rather than the exception in biochemical kinetics, irrespective of deterministic or stochastic modeling, and represents perhaps one the key innovations for CAST-DAS.

Key Assertions and Assumptions

CAST-DAS describes a unified theoretical basis of neurodegenerative processes in AD. There are several key assertions that support this framework.

Assertion #1

Sustained disruptions of intracellular calcium homeostasis are a final common pathway in brain

aging and neurodegenerative disease, which drive aging-related cellular dysfunction and diverse disease-related pathobiology in brain disorders, including dementia/Alzheimer's disease (AD). 1 Calcium is stored in several locations such as the mitochondria, endoplasmic reticulum and lysosomes, as well as protein-bound calcium that may all be relevant to the role of calcium in aging and AD. The concept of dyshomeostasis includes the possibility of impaired calcium management in organelles and other cellular compartments. Therefore, calcium regulation and homeostasis may be considered beyond cytosolic free calcium. Dyshomeostasis implies a departure from normal homeostasis that may be dynamic over time and may depend on the cell type. As a normal part of brain function and aging, changes in calcium homeostasis are dynamic (1, 5-7, 21-23).

Assertion #2

Changes in local $[Ca^{2+}]i$ levels centrally regulate the plasticity of neuroarchitecture, synaptic transmission by modulating the equilibrium between maintenance, growth/ regeneration, and regression. The direction of this equilibrium, either toward growth, synaptogenesis and synaptic plasticity, or away to synaptic and neuritic regression, is governed by intracellular calcium concentrations (1). The presumption is that neuronal dysfunction, dendritic regression, and loss of synapses observed in AD and other neurodegenerative processes reflect aberrations of inherently normal processes from optimally functioning adult brain. Shifts in the equilibrium between growth and regression are a consequence of system failures that control $[Ca^{2+}]$ i homeostasis. Small, yet chronic, elevations in $[Ca^{2+}]i$ may disrupt the equilibrium in favor of degeneration. The critical idea is that a pre-existing process is being altered or exacerbated (4, 22, 24-41).

Assertion #3

Slow progressive decrements in efficiency in one or more cellular compartments/ mechanisms for regulating $[Ca^{2+}]i$ or maintaining homeostasis could cause damage comparable to that due to a large acute insult, such as in TBI or stroke.1 The idea that prolonged small and rapid large increases of $[Ca^{2+}]i$ produce equivalent damage is yet to be validated. The postulate recognizes that both small sustained and large rapid increases of $[Ca^{2+}]i$ can each generate pathogenic conditions that contribute to aging-related deficits and pathologies associated with neurodegeneration. Each condition may recruit different compensatory mechanisms and homeostatic responses, but the net effect is that a brain is more vulnerable to AD or some other chronic brain disorder. The ability of neurons to compensate for low-level calcium dysregulation suggests that dysregulation during early and mid-life is counteracted. This concept may explain why presenilin (PS) mutations (i.e., those with early onset familial AD) have no discernable effects on brain function in youth but have progressive detrimental effects with advancing age. Presumably, "aging" produces additional changes in calcium signaling, such as reduced calcium buffering in the cells and reduced mitochondrial capacity to handle calcium. These changes, together with PS-induced calcium dyshomeostasis, lead to a neurodegenerative phenotype in aging neurons (4, 6, 24, 40, 42-51).

Assertion # 4

The concept of a final common pathway refers to the role of calcium dyshomeostasis in individual neurons. There is a presumption that the combined total effects of alterations from multiple sites regulating $[Ca^{2+}]i$ homeostasis, such as ion channels and pumps, calcium-binding proteins, etc. (1). It follows that different antecedent disease factors, including oxidative stress, impaired bioenergetics, lysosome dysfunction, and reduced neurotrophic support, may initiate individual cascade streams of pathological events. However, these aggregated streams amplify calcium dyshomeostasis to produce catastrophic effects on neural function and integrity. The assertion will be an aid therapy development in terms of developing new target leads. Different initial disruptions can result in various types of calcium dyshomeostasis but converge on common outcomes, such as dysfunctional mitochondrial calcium handling (27, 34, 51-66).

Assertion #5

The decline in optimal performance of a neuron with age or neurodegeneration is not due to a single event. Instead, these deviations are due to the convergence of multiple aberrant processes occurring in combination or in sequence over an extended period (1). The concept is related to temporal summation; taken neurophysiology where signals from two or more different neurons impinging on a third neuron must coincide in

time (rhythm) in order to receive a response. Progress in understanding the neural basis of AD requires broad implementation of the concept of nonlinearity as used in engineering. Two or more interacting processes are almost certain to lead to an outcome that is not obtained by simple additives of the two inputs; instead, they are most likely to combine in a nonlinear manner that makes combinatorial predictions difficult if not impossible (25, 28, 31, 67, 68).

Assertion #6

Replace the concepts of "Healthy vs. Disease" with Objective Measures of Performance (47, 69, 70). Although the concept of "time" is an important risk factor for neurodegeneration, the relationship between chronological (calendar) age and biological age is not perfectly correlated. This means within any chronological age-year the performance of individuals varies over a range, based on an individual's functioning. Too often chronological age [rather than biological age] is used as the anchor for comparisons of behaviors between age-matched normal versus patients with disease. Not everyone at age-80 or age-90 functions at the same level. Thus, going forward to develop a biological age construct, CAST-DAS will focus on quantitative objective measures of neuron performance on a continuous scale. This means establishing values that range from optimal performance to sub-optimal.

Moving beyond the concepts of "normal", "disease", and "aging" and by adopting objective measures of performance/functioning of a system, this affords several analytical advantages: 1) eliminates ambiguity in defining a subjective health state, 2) establishes a standard metric for ascribing performance of the system [e.g., neuron] in various settings or conditions and 3) advances neuron performance/function as a common dependent variable or outcome measure in analyses. This should allow direct comparison of various other theories of AD and should reduce current "disease" classification bias, or the problem of "AD heterogeneity."

Assertion #7

Define Neuron Performance from a Systems Perspective. Although various prevailing ideas on the pathogenesis of AD may start with very different assumptions, any viable theory must account for the deficits in functional connectivity of various neural networks associated with neurodegeneration dementia. The CAST-DAS focuses on the performance of a neuron and neuronal circuits as a system (71).

Progressive decline in the performance of normal neuron functions represents the most proximal event common to all neurodegenerative conditions. The CAST-DAS explicitly stresses this notion to account for the complex interactions among the array of molecular mechanisms for maintaining optimal functionality of individual neurons and neuronal networks as systems. The theory explains how early age-related upstream alterations at the molecular and cellular levels affect the performance of neurons and their ability to cope with genetic and/or environmental stressors (72).

The central idea of a systems model of performance enables a continuous scale to indicate varying performance levels (e.g. from optimal to sub-optimal to catastrophic failure, without imposing subjective demarcations between unaffected and affected states). In this way, the concept of AD (either as a disease or syndrome) may now be conceptualized in terms of progressive failures in an array of interconnected complex systems at the subcellular and neural network levels. Specifically, "performance degradation" is no longer considered the linear result of a single causal/etiological factor. Rather, "performance degradation" arises from multiple hits that leads to neuronal dysfunction and degeneration (73).

Further, the embedded-sets of nested subsystems describes a modeling framework that may support many other theories. The approach requires allowances for the complex interactions among several predisposing biologic events: especially focused on the timing that might occur in sequence and/or in parallel. CAST-DAS permits explanations of functional and temporal relationships among key components of a system (74).

The explicit challenge for this conceptual framework is to shift the focus of future research toward solving the complex interactions necessary to maintain or restore the optimal performance of the system, which could be defined by the functionality of: a biochemical signaling pathway, an organelle or sub-cellular compartment, an individual cell/neuron, a synapse, a neuronal network, a well-defined anatomic structure, or an emergent characteristic, a system with higher-level organization such as memory (70, 74-76).

Assertion #8

Ca^{2+} Signaling as a Final Common Path: It is the sustained decline in the performance of the neuron that sets the stage for neurodegenerative processes leading to the deterioration of performance in a neural net. There are several alternative mechanisms for dysregulation of $[Ca^{2+}]i$. Thus, there are differing paths toward the decline in a neuron's functionality – performance. Calcium is a common denominator in many signaling processes including some beneficial pathways that are crucial to normal brain function [e.g., long-term potentiation, memory mechanisms and learning] and, accordingly, its role in disease is likely to involve disruption of multiple pathways (3, 29, 68, 77-79).

Assertion #9

Emergent Behaviors and the Complex Systems: The concept of emergent behavior is often overlooked to account for the non-linear relationships between clinical features of dementia such as impairments of memory, language, and affect with the underlying neurobiological processes. CAST-DAS asserts that emergent behaviors [e.g., "cognition"] arise from complex systems [e.g., neural networks] are not the linear result of a single factors. Rather, these characteristics emerge from stochastic processes and the resulting complex interactions among signaling and metabolic pathways. Multifaceted constructs such as cognition, memory or language involve large numbers of biological phenomena temporally activated across different echelons of organization in a system. These levels are stacked within a hierarchy of increasing complexity (e.g. from least complex to most complex). Macroscopic changes of how the system functions are observed at the relatively simpler levels of complexity, yet they can also be mechanistically characterized in a bottom-up fashion to inform what might occur at higher levels. Changes in subcellular systems (i.e., signal transduction pathways) result in alterations in gene expression, protein translation and protein functionality, that mediate and propagate upwards cellular outputs. This includes structural and dynamic changes in neuronal networks at higher levels, ultimately giving rise to clinical manifestations. The concept of emergent behaviors provides a useful approach to a) examine the characteristics of the constitutive subcomponents of the neuron and neural network systems, b) explain the functional relationships among key components within a given hierarchy of a system, and c) identify approaches to optimize the functioning of the overall system, i.e., discover and test new therapeutic targets (16, 28, 70, 74, 80).

Supporting Evidence: Experimental and Observational Data

The CAST-DAS needs to be viewed in terms of systems level function—the propagation of cellular signals through multi-synaptic pathways—to address the neural correlates of cognition. It is unknown how variations in biochemical signals translate to changes in cognition. A greater appreciation for subcellular compartmentalization is very important. Dysregulated Ca^{2+} signaling can have effects in different sub-cellular compartments, including cytoplasm, nucleus, endoplasmic reticulum, and mitochondria. What are these effects? How do they change organelle function, gene expression, and cell physiology over time and with age? However, there are fascinating clues based on the CAST-DAS suggesting differences in Ca^{2+} handling systems may be pivotal determinants of selective neuronal vulnerability (57, 62, 81-84). For example, hippocampal dentate gyrus granule neurons are remarkably resistant to degeneration, whereas CA1 neurons are highly vulnerable (35, 57, 68, 85-87). This differential vulnerability is reflected in the resistance of granule neurons (and the vulnerability of CA1 neurons) to Ca^{2+}-mediated excitotoxicity and metabolic (i.e., mitochondrial) impairment. This differential neuronal vulnerability is also associated with differences in the expression of Ca^{2+}-binding proteins (e.g., calbindin) (53, 78, 88, 89). Such disturbances suggest that in neuronal Ca^{2+} handling both are "necessary for" and "sufficient to" induce degeneration of vulnerable neuronal populations in a wide array of experimental models that are relevant to AD and Parkinson's disease (1).

Interestingly, evidence now supports early pre-symptomatic roles for dysregulated cellular $[Ca^{2+}]$ i homeostasis in promoting amyloidogenesis (90), cytoskeletal pathologies, mitochondrial dysfunction, synaptic transmission and plasticity dysfunction, and oxidative stress (53, 55, 58, 59, 84, 91-99). For example, gene mutations or polymorphisms that increase AD risk promote aberrant neuronal Ca^{2+} handling early in the disease, and may thereby be early triggers for

synaptic dysfunction, neuronal degeneration and cognitive impairment. Emerging findings also suggest that exercise, intellectual challenges, social interactions, and dietary energy restriction stimulate neuroprotective-signaling pathways modulating neuronal Ca^{2+} handling and may thereby forestall AD. Evidence now may indicate that diabetes and/or obesity may adversely alter calcium channel function (100, 101).

There is a growing consensus about the upstream and downstream cellular Ca^{2+} handling mechanisms. This includes activation of innate CNS immune responses, including local inflammation and microglial activation (102). Also, associated protein aggregation likely exacerbates other age-related problems (103). In addition, there are AD pathological features that have important linkages to neuron performance including: 1) energy-generating deficits and accumulate oxidative damage (52, 104), 2) impaired lysosomal function and autophagic mechanisms leading to problems eliminating damaged molecules and organelles, repairing DNA, and maintaining protein quality (62, 95), 3) compromised ability to respond to metabolic challenges or cellular stress (54, 56). These emerging data suggest that calcium signaling alterations are related to oxidative damage (as associated with compromises in calcium and sodium regulation), adenosine triphosphate (ATP) production by mitochondria, protease activation, and other cellular processes (22, 52, 104).

Future Research Questions

The CAST-DAS asserts that early synaptic dysfunction leading to subsequent decrements in performance, or complete failure, in neural network functions are the most critical molecular mechanisms leading to cognitive decline and clinical dementia. However, several uncertainties remain that will require future experiments to validate these claims (47, 82, 105). The following is a summary of some important unanswered questions:

Question: Why are some neurons more resilient than others and what are those mechanisms that may protect against chronic [Ca2+]i dysregulation? The question is whether changes in the mechanisms for regulating $[Ca^{2+}]$ i dynamics and maintaining calcium homeostasis represent necessary and sufficient conditions for the decline in functionality of a neuron1. Future studies should include multiple cell types and brain regions. Thus, beyond CA1 hippocampal neurons, there is a need to understand $[Ca^{2+}]$i regulation in neurons of the entorhinal cortex, prefrontal cortex, parietal lobes, default mode network, and basal forebrain cholinergic neurons (47, 68, 101).

Question: Why does selective neuronal vulnerability (SNV) matter? Selective neuronal vulnerability (SNV) is often overlooked and yet a fundamental characteristic of both aging and neurodegeneration (1). There are cells, structures, systems that are affected while other vast areas are relatively unaffected or appear normal. What factors account for these differences or the underlying mechanisms of various neurodegenerative diseases that attack different but specific parts of the brain? Until recently, the molecular and cellular mechanisms to account for SNV were not well studied. New technologies now permit investigating the fundamental mechanisms regulating SNF in clinical samples of aging and dementia.

Future investigation will be needed to examine the effects of various types of stresses on SNV. Functional genomic analyses may compare the sensitivity of cells, to an oxidative insult, within specific brain regions. GeneChip-based transcriptomics may evaluate those with unaffected brain aging and AD to assess the question of how injured/vulnerable neurons are characterized by significant decreases in the expression of genes related to mitochondrial metabolism and energy production (9, 53, 56, 57, 62, 66, 84, 106). In this way, biochemical analyses may provide critical validation of lower energy levels (in the form of ATP) between different neurons (i.e., primary CbG compared with cortical neurons). In total, these future experiments would provide a foundational basis to evaluate low energy reserves and high intrinsic stress levels as two underlying factors for SNV to oxidative stress (57, 84). In a systems-based analytical framework, this future work may lead to establishing a conceptual basis to for "coupling transition" between higher and lower order sub-systems within the overall neuronal architecture.

Detecting genomic differences—between sensitive and resistant neurons—can now be used to explore suggested molecular mechanisms of cell injury in aging and AD. In the future such whole genome expression studies, may lead to further insights into the organization of SNV complexity that could be then targets

for interventions to protect vulnerable neurons (57, 83, 84). The Allen Brain Atlas may be used to explore the expression of aggregation-related genes and their localization in the Braak staging regions where AD develops early (107). The spatial transcriptomics strategy allows visualization and quantitative analysis of the transcriptome with spatial resolution in individual tissue sections. This approach enables pattern analysis proteins or messenger RNAs (mRNAs) in histological tissue sections (106).

Question: Will the Calcium System Theory be sufficiently inclusive to explain selective neuronal vulnerability (SNV)? In light of the previous question one of the lingering crucial challenges for all theories of AD is to explain the functional mechanisms for the specificity of decline in performance of the system affected; where the system could be defined either in terms of cell type, anatomical region, neural network or neurochemical system (2, 3, 8, 73). An important consideration for the theory will be whether corollaries will be required for different cell types. It is likely that in order to produce models with greater fidelity, future studies will need to identify mechanistic insights for specific cell types sufficiently so to supply cell type-specific in vivo functional data.

Question: What are the distinguishing differences between the more general decline in a neuron's performance from those changes in functionality that underlie disease-specific system failure? Is there a way to identify specific neural systems, networks, pathways, or mechanisms that are selective for various forms of neurodegeneration and/or different forms of dementia? Although there are multiple pathways for regulating $[Ca^{2+}]i$ in what way do are each of these paths involved or affected by age and/or dementia/AD. Further studies should differentially examine the nature of these nonlinear interactions and pathways. Several of the identified AD susceptibility genes will be important study targets. The objective will be to determine if and how these genes may contribute to, and if so, to what extent $[Ca^{2+}]i$ dysregulation. For example, calcium-linked susceptibility genes such as PICALM, CLUSTERIN and APOE are of particular interest in this regard (10, 21, 88, 94, 108, 109). The role of calcium in neuro-immunology as it relates to the expression or modulation of susceptibility genes is not known. Further studies should investigate how changes in $[Ca^{2+}]i$ dyshomeostasis affect or modulate the immune system in AD (70, 86, 93, 98).

Question: How does how intracellular Ca^{2+} homeostasis change during aging and what is the clinical impact? Because emerging evidence suggests that systemic abnormalities and diseases such as such as diabetes, cardiovascular disease and obesity can increase the risk of dementia/AD, it will be important to understand how such peripheral alterations due to comorbid conditions influence cellular $[Ca^{2+}]i$ homeostasis in the brain (66, 110).

Question: What are the prospects of developing Ca^{2+}-primed biomarkers to monitor neuronal performance, overall system health, and correlates of human health? The concept of Ca^{2+} biomarkers needs to be approached with an open mind. One useful biomarker could be a blood marker of Ca^{2+} dysregulation in the brain, such as might be possible by using brain-specific extracellular vesicles (exosomes) (77, 88, 90, 111). Future biomarkers should follow the performance of individual neurons as they function within neuronal networks. The important challenge will be to develop the appropriate nanotechnologies to measure and monitor the changes in Ca^{2+} in living animal models. There is a need to measure Ca^{2+} dysregulation in vivo in the clinic with human subjects. The goal will be developing ways to monitor Ca^{2+} homeostasis in living people, and to distinguish normal homeostasis from pathologic conditions, for example by using indirect measures of Ca^{2+} homeostasis or biomarkers. Links to obesity and diabetes show promise as peripheral biomarkers. There is evidence that calcium dysregulation occurs in the periphery (adipocytes, neurons) in response to diabetes (110). There is a question whether there are effects of neurodegeneration, or more specific changes associated with dementia/AD, in the peripheral nervous system that could be measured. Development of direct measures of calcium function is underway in vivo (87). One possibility is the calcium-activated K current responsible for the afterhyperpolarization. Advanced calcium imaging methods will prove valuable for studies of aging. Some other peripheral markers that are potential surrogates for neurodegeneration include retinal imaging to study retinal vasculature Ca^{2+} in retinal cells (1).

Future research should explore if the olfactory epithelium is also accessible for similar studies as biopsies of olfactory epithelium is possible. Studies of more readily accessible cells, such as fibroblasts, may also provide clues regarding changes in calcium regulation in patients with sporadic AD (1).

Question: How does CAST-DAS address the temporal and functional relationship between $[Ca^{2+}]i$ dyshomeostasis and molecular mechanisms underlying other putative etiologic factors such as amyloid, tau, inflammation, mitochondrial dysfunction etc.? This is a key consideration for the CAST-DAS: $[Ca^{2+}]i$ dyshomeostasis is considered a final common pathway leading to the decline in a damaged neuron and eventually playing a central role in the downstream neurodegeneration process (1, 2, 5, 7, 12, 70, 71, 73, 75, 96). With multiple pathogenic effects that seem unrelated, there is a need to address how $[Ca^{2+}]i$ dyshomeostasis serves as a common final path—a point of convergence—for both upstream and downstream contributors to the neurodegenerative process including amyloid beta and Tau pathologies, lysosome and mitochondrial dysfunction, and impaired adaptive cellular stress responses. Future studies should determine how well the CAST-DAS could account for: a) Large variance, measured in years, in the latency of expression in the decrement of function or deviation from optimal performance or the onset of symptoms [e.g., cognitive impairment] and, b) Heterogeneity in not only the timing of onset or the trend toward the decline in performance but also the expression of biological markers associated with aging or neurodegeneration.

Question: What are the major technical challenges for CAST-DAS? The list of immediate steps required to understand the role of calcium dysregulation in neurodegeneration includes the following (1). First, developing in vivo methods to observe sub-cellular localization of calcium dynamics and identify organelle(s) exhibiting compromised neuronal calcium behavior, without altering inherent neuronal activity. Second, improving the temporal resolution to reveal key timing features that are presently unobservable. This is important because of the extremely short duration of calcium dynamics [i.e., nanoseconds]. Third, developing bioassays to allow accurate and reliable characterization of therapeutic interventions in disease models. Fourth, developing new analytical modeling techniques that allow predictions of synaptic, neuron, and neural systems functionalities based on experimental findings of calcium dynamics.

Conclusions

The CAST-DAS posits that a decline in the functioning of a neuron due to $[Ca^{2+}]i$ dyshomeostasis is the necessary and sufficient condition that affects the performance of a neural system. This deterioration underlies many impairments of human behaviors as a consequence and the expression of clinical phenotypes related to Alzheimer's disease. Thus, CAST-DAS represents significant progress towards offering a comprehensive account for AD pathogenesis by providing explanations that are more integrative than other prevailing theories (75, 96).

Why is CAST-DAS a promising framework for developing therapies for AD and other complex chronic brain disorders? This theory has already provided the rationale for one FDA-approved medication for AD—Memantine. The conceptual framework proposed by CAST-DAS provides both an explanation for the limited or short duration of effectiveness of other current treatments and outlines the essential features for a novel drug discovery-development paradigm based on a systems approach. The primary premise of CAST-DAS is to use the complex neuronal mechanism for regulating the $[Ca^{2+}]i$ dyshomeostasis as a template to map functional relationships among multiple variables. CAST-DAS provides both a theoretical and experimental basis to develop targeted nanotherapies that can isolate specific brain regions or specific cell types for intervention. Future drug products may be designed on an individual patient-specific basis in response to their own unique physiological make-up to tailor precision drug targeting of novel neuroprotective strategies.

Conflicts of Interest

Ara S. Khachaturian, Ph.D. is an Officer and director of the Campaign to Prevent Alzheimer's Disease (PAD 20/20) and; Officer, director and employee of Khachaturian and Associates; Founding executive-editor of Alzheimer's & Dementia, The Journal of the Alzheimer's Association (retired), Founding executive-editor of Alzheimer's & Dementia: Translational Research & Clinical Intervention (retired), Founding executive-editor of Alzheimer's & Dementia: Diagnoses, Assessment & Disease Monitoring (retired); Executive Officer and Director, Brain Watch Coalition; Senior Research Fellow, University of Nevada Las Vegas, National Supercomputing Institute & Dedicated Research Network; Received payments through organizational affiliations for grants, contracts, consulting fees, honoraria, meeting support, travel support, in-kind research/professional support over the last 36 months from the Alzheimer's Association, Acadia

Pharmaceuticals, Alzheon, Biogen, Clinical Trials Alzheimer's Disease Conference, Davos Alzheimer's Consortium, Eisai, Eli Lilly & Company, and International Neurodegenerative Disorders Research Center, and Serdi Publishing.

Ethical standards

Ara S. Khachaturian is editor-in-chief of *Vitality, Medicine & Engineering*; he has recused himself from any editorial decision on this manuscript, Dr. Bruno Vellas was responsible for the editorial peer-review process.

References

1. Alzheimer's Association Calcium Hypothesis W. Calcium Hypothesis of Alzheimer's disease and brain aging: A framework for integrating new evidence into a comprehensive theory of pathogenesis. Alzheimers Dement. 2017;13(2):178-182 e117.
2. Khachaturian ZS. Towards theories of brain aging. In: Kay DSB, G.W., ed. Handbook of studies on psychiatry and old age. Amsterdam: Elsevier Science Publishers, B.V.; 1984:7-30.
3. Khachaturian ZS. Calcium hypothesis of Alzheimer's disease and brain aging. Ann N Y Acad Sci. 1994;747:1-11.
4. Landfield PW. 'Increased calcium-current' hypothesis of brain aging. Neurobiol Aging. 1987;8(4):346-347.
5. Khachaturian ZS. A hypothesis: regulation of cytosol calcium in aging and Alzheimer's disease. In: Corkin SH, Growdon, J.H., Wurtman, R.J., ed. Alzheimer's disease: advances in basic research and therapies. Zurich: The Center for Brain Sciences and Metabolism Charitable Trust; 1984.
6. Khachaturian ZS. The role of calcium regulation in brain aging: reexamination of a hypothesis. Aging (Milano). 1989;1(1):17-34.
7. Khachaturian ZS. Hypothesis on the regulation of cytosol calcium concentration and the aging brain. Neurobiology of aging. 1987;8(4):345-346.
8. Khachaturian ZS. A unifying hypothesis on the etiology of Alzheimer's disease. In: Fisher A, Nagatsu, T.,Yoshida, M., ed. Alzheimer's and Parkinson's disease II: basic and therapeutic strategies. New York: Plenum Press; 1989.
9. de Bivort B, Huang S, Bar-Yam Y. Dynamics of cellular level function and regulation derived from murine expression array data. Proc Natl Acad Sci U S A. 2004;101(51):17687-17692.
10. Stuart JM, Segal E, Koller D, Kim SK. A gene-coexpression network for global discovery of conserved genetic modules. Science. 2003;302(5643):249-255.
11. Eichler GS, Huang S, Ingber DE. Gene Expression Dynamics Inspector (GEDI): for integrative analysis of expression profiles. Bioinformatics. 2003;19(17):2321-2322.
12. Geerts H, Dacks PA, Devanarayan V, et al. Big data to smart data in Alzheimer's disease: The brain health modeling initiative to foster actionable knowledge. Alzheimers Dement. 2016;12(9):1014-1021.
13. Geerts H, Roberts P, Spiros A, Carr R. A strategy for developing new treatment paradigms for neuropsychiatric and neurocognitive symptoms in Alzheimer's disease. Front Pharmacol. 2013;4:47.
14. Xu Z, Chen N, Kamocka MM, Rosen ED, Alber M. A multiscale model of thrombus development. J R Soc Interface. 2008;5(24):705-722.
15. Neymotin SA, McDougal RA, Bulanova AS, et al. Calcium regulation of HCN channels supports persistent activity in a multiscale model of neocortex. Neuroscience. 2016;316:344-366.
16. Neymotin SA, Dura-Bernal S, Lakatos P, Sanger TD, Lytton WW. Multitarget Multiscale Simulation for Pharmacological Treatment of Dystonia in Motor Cortex. Front Pharmacol. 2016;7:157.
17. De Schutter E. Why are computational neuroscience and systems biology so separate? PLoS Comput Biol. 2008;4(5):e1000078.
18. Vlachos D. A review of multiscale analysis: examples from systems biology, materials engineering, and other fluid–surface interacting systems. Advances in Chemical Engineering. 2005;30:1-61.
19. Rao CV, Wolf DM, Arkin AP. Control, exploitation and tolerance of intracellular noise. Nature. 2002;420(6912):231-237.
20. Fredrickson AG, Stephanopoulos G. Microbial competition. Science. 1981;213(4511):972-979.
21. Dreses-Werringloer U, Lambert JC, Vingtdeux V, et al. A polymorphism in CALHM1 influences Ca2+ homeostasis, Abeta levels, and Alzheimer's disease risk. Cell. 2008;133(7):1149-1161.
22. Jiang L, Bechtel MD, Galeva NA, Williams TD, Michaelis EK, Michaelis ML. Decreases in plasma membrane Ca(2)(+)-ATPase in brain synaptic membrane rafts from aged rats. J Neurochem. 2012;123(5):689-699.
23. Pierrot N, Ghisdal P, Caumont AS, Octave JN. Intraneuronal amyloid-β1-42 production triggered by sustained increase of cytosolic calcium concentration induces neuronal death. Journal of neurochemistry. 2004;88(5):1140-1150.
24. Goussakov I, Miller MB, Stutzmann GE. NMDA-mediated Ca(2+) influx drives aberrant ryanodine receptor activation in dendrites of young Alzheimer's disease mice. J Neurosci. 2010;30(36):12128-12137.
25. Hendrickson PJ, Yu GJ, Song D, Berger TW. A million-plus neuron model of the hippocampal dentate gyrus: Dependency of spatio-temporal network dynamics on topography. Annu Int Conf IEEE Eng Med Biol Soc. 2015;2015:4713-4716.
26. Watanabe S, Hong M, Lasser-Ross N, Ross WN. Modulation of calcium wave propagation in the dendrites and to the soma of rat hippocampal pyramidal neurons. The Journal of physiology. 2006;575(2):455-468.
27. Binder DK, Scharfman HE. Brain-derived neurotrophic factor. Growth Factors. 2004;22(3):123-131.
28. Bouteiller JM, Allam SL, Hu EY, et al. Integrated multiscale modeling of the nervous system: predicting changes in hippocampal network activity by a positive AMPA receptor modulator. IEEE Trans Biomed Eng. 2011;58(10):3008-3011.
29. Chakroborty S, Stutzmann GE. Early calcium dysregulation in Alzheimer's disease: setting the stage for synaptic dysfunction. Sci China Life Sci. 2011;54(8):752-762.
30. Cirrito JR, Yamada KA, Finn MB, et al. Synaptic activity regulates interstitial fluid amyloid-β levels in vivo. Neuron. 2005;48(6):913-922.
31. Demuro A, Parker I, Stutzmann GE. Calcium signaling and amyloid toxicity in Alzheimer disease. J Biol Chem. 2010;285(17):12463-12468.
32. DeVos SL, Corjuc BT, Oakley DH, et al. Synaptic Tau Seeding Precedes Tau Pathology in Human Alzheimer's Disease Brain. Front Neurosci. 2018;12:267.
33. Enager P, Piilgaard H, Offenhauser N, et al. Pathway-specific variations in neurovascular and neurometabolic coupling in rat primary somatosensory cortex. J Cereb Blood Flow Metab. 2009;29(5):976-986.
34. Farizatto KLG, Ikonne US, Almeida MF, Ferrari MFR, Bahr BA. Abeta42-mediated proteasome inhibition and associated tau pathology in hippocampus are governed by a lysosomal response involving cathepsin B: Evidence for protective crosstalk between protein clearance pathways. PLoS One. 2017;12(8):e0182895.
35. Fujii S, Matsumoto M, Igarashi K, Kato H, Mikoshiba K. Synaptic plasticity in hippocampal CA1 neurons of mice lacking type 1 inositol-1, 4, 5-trisphosphate receptors. Learning & Memory. 2000;7(5):312-320.
36. Henneberger C, Juttner R, Rothe T, Grantyn R. Postsynaptic action of BDNF on GABAergic synaptic transmission in the superficial layers of the mouse superior colliculus. J Neurophysiol. 2002;88(2):595-603.
37. Koffie RM, Meyer-Luehmann M, Hashimoto T, et al. Oligomeric amyloid β associates with postsynaptic densities and correlates with excitatory synapse loss near senile plaques. Proceedings of the National Academy of Sciences. 2009;106(10):4012-4017.
38. LaFerla FM, Oddo S. Alzheimer's disease: Abeta, tau and synaptic dysfunction. Trends Mol Med. 2005;11(4):170-176.
39. Manczak M, Reddy PH. Abnormal interaction between the mitochondrial fission protein Drp1 and hyperphosphorylated tau in Alzheimers disease neurons: implications for mitochondrial dysfunction and neuronal damage. Human Molecular Genetics. 2012;21(11):2538-2547.
40. Parent A, Linden DJ, Sisodia SS, Borchelt DR. Synaptic transmission and hippocampal long-term potentiation in transgenic mice expressing FAD-linked presenilin 1. Neurobiology of disease. 1999;6(1):56-62.
41. Yuan A, Kumar A, Peterhoff C, Duff K, Nixon RA. Axonal transport rates in vivo are unaffected by tau deletion or overexpression in mice. J Neurosci. 2008;28(7):1682-1687.
42. Arispe N, Pollard HB, Rojas E. The ability of amyloid beta-protein [A beta P (1-40)] to form Ca2+ channels provides a mechanism for neuronal death in Alzheimer's disease. Ann N Y Acad Sci. 1994;747:256-266.
43. Davies PJ, Ireland DR, McLachlan EM. Sources of Ca2+ for different Ca (2+)-activated K+ conductances in neurones of the rat superior cervical ganglion. The Journal of Physiology.

1996;495(2):353-366.
44. Nakamura T, Barbara J-G, Nakamura K, Ross WN. Synergistic release of Ca2+ from IP3-sensitive stores evoked by synaptic activation of mGluRs paired with backpropagating action potentials. Neuron. 1999;24(3):727-737.
45. Stutzmann GE, LaFerla FM, Parker I. Ca2+ signaling in mouse cortical neurons studied by two-photon imaging and photoreleased inositol triphosphate. Journal of Neuroscience. 2003;23(3):758-765.
46. Stutzmann GE, Smith I, Caccamo A, Oddo, S., F.M. L, Parker I. Enhanced ryanodine receptor recruitment contributes to Ca2+ disruptions in young, adult, and aged Alzheimer's disease mice. J Neurosci. 2006;26(19):5180-5189.
47. Stutzmann GE. The pathogenesis of Alzheimers disease is it a lifelong "calciumopathy"? Neuroscientist. 2007;13(5):546-559.
48. Zhang C, Wu B, Beglopoulos V, et al. Presenilins are essential for regulating neurotransmitter release. Nature. 2009;460(7255):632.
49. Fitz NF, Cronican AA, Saleem M, et al. Abca1 deficiency affects Alzheimer's disease-like phenotype in human ApoE4 but not in ApoE3-targeted replacement mice. J Neurosci. 2012;32(38):13125-13136.
50. Tong BC, Lee CS, Cheng WH, Lai KO, Foskett JK, Cheung KH. Familial Alzheimer's disease-associated presenilin 1 mutants promote gamma-secretase cleavage of STIM1 to impair store-operated Ca2+ entry. Sci Signal. 2016;9(444):ra89.
51. Nixon RA. Amyloid precursor protein and endosomal-lysosomal dysfunction in Alzheimer's disease: inseparable partners in a multifactorial disease. FASEB J. 2017;31(7):2729-2743.
52. Carafoli E. Historical review: mitochondria and calcium: ups and downs of an unusual relationship. Trends Biochem Sci. 2003;28(4):175-181.
53. Zaidi A, Barron L, Sharov VS, Schoneich C, Michaelis EK, Michaelis ML. Oxidative inactivation of purified plasma membrane Ca2+-ATPase by hydrogen peroxide and protection by calmodulin. Biochemistry. 2003;42(41):12001-12010.
54. Mattson MP. Pathways towards and away from Alzheimer's disease. Nature. 2004;430(7000):631-639.
55. Abramov AY, Canevari L, Duchen MR. Beta-amyloid peptides induce mitochondrial dysfunction and oxidative stress in astrocytes and death of neurons through activation of NADPH oxidase. J Neurosci. 2004;24(2):565-575.
56. Bezprozvanny I, Mattson MP. Neuronal calcium mishandling and the pathogenesis of Alzheimer's disease. Trends Neurosci. 2008;31(9):454-463.
57. Wang X, Zaidi A, Pal R, et al. Genomic and biochemical approaches in the discovery of mechanisms for selective neuronal vulnerability to oxidative stress. BMC Neurosci. 2009;10:12.
58. Boccardi V, Pelini L, Ercolani S, Ruggiero C, Mecocci P. From cellular senescence to Alzheimer's disease: The role of telomere shortening. Ageing Res Rev. 2015;22:1-8.
59. Tarantini S, Tran CHT, Gordon GR, Ungvari Z, Csiszar A. Impaired neurovascular coupling in aging and Alzheimer's disease: Contribution of astrocyte dysfunction and endothelial impairment to cognitive decline. Exp Gerontol. 2017;94:52-58.
60. Wolfe DM, Lee JH, Kumar A, Lee S, Orenstein SJ, Nixon RA. Autophagy failure in Alzheimer's disease and the role of defective lysosomal acidification. Eur J Neurosci. 2013;37(12):1949-1961.
61. McBrayer M, Nixon RA. Lysosome and calcium dysregulation in Alzheimer's disease: partners in crime. Biochem Soc Trans. 2013;41(6):1495-1502.
62. Colacurcio DJ, Nixon RA. Disorders of lysosomal acidification-The emerging role of v-ATPase in aging and neurodegenerative disease. Ageing Res Rev. 2016;32:75-88.
63. Peng S, Wuu J, Mufson EJ, Fahnestock M. Precursor form of brain-derived neurotrophic factor and mature brain-derived neurotrophic factor are decreased in the pre-clinical stages of Alzheimer's disease. J Neurochem. 2005;93(6):1412-1421.
64. Cosker KE, Segal RA. Neuronal Signaling through Endocytosis. Csh Perspect Biol. 2014;6(2).
65. Coelho FG, Vital TM, Stein AM, et al. Acute aerobic exercise increases brain-derived neurotrophic factor levels in elderly with Alzheimer's disease. J Alzheimers Dis. 2014;39(2):401-408.
66. Mattson MP. Late-onset dementia: a mosaic of prototypical pathologies modifiable by diet and lifestyle. NPJ Aging Mech Dis. 2015;1.
67. Du F, Whetsell Jr WO, Abou-Khalil B, Blumenkopf B, Lothman EW, Schwarcz R. Preferential neuronal loss in layer III of the entorhinal cortex in patients with temporal lobe epilepsy. Epilepsy research. 1993;16(3):223-233.
68. Disterhoft JF, Wu WW, Ohno M. Biophysical alterations of hippocampal pyramidal neurons in learning, ageing and Alzheimer's disease. Ageing Res Rev. 2004;3(4):383-406.
69. Bartzokis G, Cummings JL, Sultzer D, Henderson VW, Nuechterlein KH, Mintz J. White matter structural integrity in healthy aging adults and patients with Alzheimer disease: a magnetic resonance imaging study. Arch Neurol. 2003;60(3):393-398.
70. Hampel H, Lista S, Khachaturian ZS. Development of biomarkers to chart all Alzheimer's disease stages: the royal road to cutting the therapeutic Gordian Knot. Alzheimers Dement. 2012;8(4):312-336.
71. Khachaturian ZS, Lombardo J. In silico modeling system: a national research resource for simulation of complex brain disorders. Alzheimers Dement. 2009;5(1):1-4.
72. Palop JJ, Chin J, Mucke L. A network dysfunction perspective on neurodegenerative diseases. Nature. 2006;443(7113):768-773.
73. Khachaturian ZS. Perspectives on Alzheimer's disease: past, present and future. In: Carrillo MC, Hampel, H., ed. Alzheimer's disease—modernizing concept, biological diagnosis and therapy. Vol 28. Basel: Karger; 2012:179-188.
74. Khachaturian ZS. Models and modeling systems in Alzheimer disease drug discovery. Alzheimer Dis Assoc Disord. 2002;16 Suppl 1:S9-S12.
75. Khachaturian ZS. Toward a comprehensive theory of Alzheimer's disease--challenges, caveats, and parameters. Ann N Y Acad Sci. 2000;924:184-193.
76. Brandman O, Meyer T. Feedback loops shape cellular signals in space and time. Science. 2008;322(5900):390-395.
77. Stutzmann GE, Mattson MP. Endoplasmic reticulum Ca(2+) handling in excitable cells in health and disease. Pharmacol Rev. 2011;63(3):700-727.
78. Carafoli E. Calcium-mediated cellular signals: a story of failures. Trends Biochem Sci. 2004;29(7):371-379.
79. Carafoli E. Calcium signaling: a tale for all seasons. Proc Natl Acad Sci U S A. 2002;99(3):1115-1122.
80. Hendrickson PJ, Yu GJ, Song D, Berger TW. A Million-Plus Neuron Model of the Hippocampal Dentate Gyrus: Critical Role for Topography in Determining Spatiotemporal Network Dynamics. IEEE Trans Biomed Eng. 2016;63(1):199-209.
81. Cavallari M, Dai W, Guttmann CR, et al. Neural substrates of vulnerability to postsurgical delirium as revealed by presurgical diffusion MRI. Brain. 2016;139(Pt 4):1282-1294.
82. de Wilde MC, Overk CR, Sijben JW, Masliah E. Meta-analysis of synaptic pathology in Alzheimer's disease reveals selective molecular vesicular machinery vulnerability. Alzheimers Dement. 2016;12(6):633-644.
83. Freer R, Sormanni P, Vecchi G, Ciryam P, Dobson CM, Vendruscolo M. A protein homeostasis signature in healthy brains recapitulates tissue vulnerability to Alzheimer's disease. Sci Adv. 2016;2(8):e1600947.
84. Wang X, Michaelis ML, Michaelis EK. Functional genomics of brain aging and Alzheimer's disease: focus on selective neuronal vulnerability. Curr Genomics. 2010;11(8):618-633.
85. Hamm V, Heraud C, Bott JB, et al. Differential contribution of APP metabolites to early cognitive deficits in a TgCRND8 mouse model of Alzheimer's disease. Sci Adv. 2017;3(2):e1601068.
86. Hong S, Beja-Glasser VF, Nfonoyim BM, et al. Complement and microglia mediate early synapse loss in Alzheimer mouse models. Science. 2016;352(6286):712-716.
87. Bissig D, Berkowitz BA. Testing the calcium hypothesis of aging in the rat hippocampus in vivo using manganese-enhanced MRI. Neurobiol Aging. 2014;35(6):1453-1458.
88. Ma Z, Siebert AP, Cheung KH, et al. Calcium homeostasis modulator 1 (CALHM1) is the pore-forming subunit of an ion channel that mediates extracellular Ca2+ regulation of neuronal excitability. Proc Natl Acad Sci U S A. 2012;109(28):E1963-1971.
89. Esposito D, Patel P, Stephens RM, et al. The cytoplasmic and transmembrane domains of the p75 and Trk A receptors regulate high affinity binding to nerve growth factor. J Biol Chem. 2001;276(35):32687-32695.
90. O'Day DH. Calmodulin Binding Proteins and Alzheimer's Disease: Biomarkers, Regulatory Enzymes and Receptors That Are Regulated by Calmodulin. Int J Mol Sci. 2020;21(19).
91. Plog BA, Nedergaard M. The Glymphatic System in Central Nervous System Health and Disease: Past, Present, and Future.

Annu Rev Pathol. 2018;13:379-394.
92. Clayton KA, Van Enoo AA, Ikezu T. Alzheimer's Disease: The Role of Microglia in Brain Homeostasis and Proteopathy. Front Neurosci. 2017;11:680.
93. Venegas C, Kumar S, Franklin BS, et al. Microglia-derived ASC specks cross-seed amyloid-beta in Alzheimer's disease. Nature. 2017;552(7685):355-361.
94. Jack CR, Bennett DA, Blennow K, et al. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. Alzheimers Dement. 2018;14(4):535-562.
95. Nixon RA. The calpains in aging and aging-related diseases. Ageing Res Rev. 2003;2(4):407-418.
96. Khachaturian ZS, Mesulam MM. Alzheimer's disease : a compendium of current theories. New York, N.Y.: New York Academy of Sciences; 2000.
97. Mattson MP, Engle MG, Rychlik B. Effects of elevated intracellular calcium levels on the cytoskeleton and tau in cultured human cortical neurons. Molecular and chemical neuropathology. 1991;15(2):117-142.
98. Kosik KS, Joachim CL, Selkoe DJ. Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease. Proc Natl Acad Sci U S A. 1986;83(11):4044-4048.
99. Santos RX, Correia SC, Zhu X, et al. Mitochondrial DNA oxidative damage and repair in aging and Alzheimer's disease. Antioxid Redox Signal. 2013;18(18):2444-2457.
100. Pancani T, Anderson KL, Brewer LD, et al. Effect of high-fat diet on metabolic indices, cognition, and neuronal physiology in aging F344 rats. Neurobiol Aging. 2013;34(8):1977-1987.
101. Maimaiti S, Anderson KL, DeMoll C, et al. Intranasal Insulin Improves Age-Related Cognitive Deficits and Reverses Electrophysiological Correlates of Brain Aging. J Gerontol A Biol Sci Med Sci. 2016;71(1):30-39.
102. Mrak RE, Griffin WS. Common inflammatory mechanisms in Lewy body disease and Alzheimer disease. J Neuropathol Exp Neurol. 2007;66(8):683-686.
103. Wyss-Coray T, Rogers J. Inflammation in Alzheimer disease-a brief review of the basic science and clinical literature. Cold Spring Harb Perspect Med. 2012;2(1):a006346.
104. Starkov AA, Beal FM. Portal to Alzheimer's disease. Nat Med. 2008;14(10):1020-1021.
105. Thibault O, Gant JC, Landfield PW. Expansion of the calcium hypothesis of brain aging and Alzheimer's disease: minding the store. Aging Cell. 2007;6(3):307-317.
106. Stahl PL, Salmen F, Vickovic S, et al. Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. Science. 2016;353(6294):78-82.
107. Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol. 1991;82(4):239-259.
108. Overk C, Masliah E. Perspective on the calcium dyshomeostasis hypothesis in the pathogenesis of selective neuronal degeneration in animal models of Alzheimer's disease. Alzheimers Dement. 2017;13(2):183-185.
109. Li G, Bien-Ly N, Andrews-Zwilling Y, et al. GABAergic interneuron dysfunction impairs hippocampal neurogenesis in adult apolipoprotein E4 knockin mice. Cell stem cell. 2009;5(6):634-645.
110. Thibault O, Anderson KL, DeMoll C, Brewer LD, Landfield PW, Porter NM. Hippocampal calcium dysregulation at the nexus of diabetes and brain aging. Eur J Pharmacol. 2013;719(1-3):34-43.
111. Tamura M, Gu J, Danen EH, Takino T, Miyamoto S, Yamada KM. PTEN interactions with focal adhesion kinase and suppression of the extracellular matrix-dependent phosphatidylinositol 3-kinase/Akt cell survival pathway. J Biol Chem. 1999;274(29):20693-20703.