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Perspective on the calcium dyshomeostasis hypothesis in the pathogenesis of selective neuronal degeneration in animal models of Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative disease associated with the abnormal accumulation of amyloid β ($A\beta$) and microtubule-associated protein tau (τ). Although the mechanisms through which abnormal accumulation of proteins leads to neuronal vulnerability are still under investigation, intracellular calcium homeostasis is dysregulated and has been implicated in the neurobiology of AD [1–6].

Mouse models of neurodegenerative diseases have been used to investigate the proteins associated with neurodegenerative diseases, including $A\beta$. These animal models have led to invaluable insight into the mechanisms and progression of the diseases. A number of animal models of AD have been created [7].

Calcium dysregulation has been proposed as a mechanism through which aggregated $A\beta$ may lead to neurodegeneration [1,8]. Two-photon microscopy is a powerful imaging tool used to acquire neural data in vivo and has been used to capture and analyze changes in calcium signaling in various models of AD. Previous reports have used in vivo two-photon imaging in awake behaving mice to show that altered calcium homeostasis in amyloid precursor protein (APP) tg mice was associated with $A\beta$ plaques [9,10], but not in anesthetized PS1 tg animal models [11], or neurofibrillary tangle-bearing neurons in a tau (P301L) mutant model [12]. Moreover, a recent study using patch clamping and two-photon imaging confirms alterations in APP/PS1 but found that these alterations are independent of plaques [13]. A recent study characterizing multiple animal models of neurodegeneration found abnormal calcium dynamics in a mouse model of AD [14]. Specifically, the normalized 1-Hz stimulus-driven neural calcium transients exhibited anomalous features in their geometric shapes (Fig. 1). Temporal signal variations were analyzed using statistical measures of the calcium transients using such features like the area under the curve (AUC) or peak width of calcium transients [14]. The AUC measures, for example, were significantly increased, and therefore distinctive, for APP mice across three cortical depths compared to non-tg mice, and spontaneous calcium transient AUC was significantly lower for APP [14].

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Although the cause(s) of the aberrations in the calcium transients for these in the APP mice are unclear, there are a number of possible mechanisms including increase in spiking, increase in the amount of calcium that enters the cell (Fig. 2), and failure of organelles or cytosolic calcium buffer to sequester calcium [15] (Fig. 2). There are several organelles that sequester calcium in neurons including the endoplasmic reticulum and mitochondria. APP has been implicated in mitochondrial dysfunction which could also lead to abnormal calcium function [16] (Fig. 2). APP and A β have the potential to destabilize endoplasmic reticulum (ER) calcium homeostasis by increasing levels of stored calcium, attenuating calcium entry, and altering interaction with proteins such as calsenilin, ryanodine receptor, or calmyrin [17].

The connection between calcium and synapse loss is currently under investigation. One possible link that has been suggested is destabilizing ER calcium [18], specifically aberrant processing of calcium in the ER (Fig. 2), may weaken the mushroom-type postsynaptic spines associated with long-term memory [19]. Another possible link between calcium and synapse loss is alterations in mitochondria (Fig. 2), particularly synaptic mitochondria (reviewed [20]), which are responsible for maintaining calcium in the synapse as well as fulfilling the large energy demands in the synapse. As part of maintaining homeostasis, synaptic mitochondria have been shown to uptake the transiently increased calcium in the presynaptic terminal quicker than ER [21,22] and thus prevent cellular damage due to prolonged calcium stimulation [23]. Synaptic mitochondria are more vulnerable to cumulative damage than mitochondria in the soma [24], and A β has been shown to accumulate in mitochondria leading to abnormal synaptic mitochondria behavior in AD (reviewed [20]). Therefore, failure of synaptic mitochondria to maintain synaptic activity is a potential mechanism that may lead to synaptic loss.

Future studies investigating the interaction between APP/ A β and neural calcium dynamics will be critical in elucidating the mechanisms involved in neurodegeneration. In particular, we believe the following scientific steps will be key in our understanding of calcium dysregulation in AD. (1) Developing methods for subcellular localization of calcium dynamics in vivo without altering inherent neuronal activity (normal or abnormal) could lead to the identification of organelle(s) exhibiting compromised neuronal calcium behavior. (2) Since calcium dynamics occur over a very short time scale, improving the temporal resolution of the data could further reveal key temporal features that are presently unobservable. This is similar to switching from a 4 $\times$ objective to a 100 $\times$ objective on a microscope, which can reveal previously unseen structures. (3) Bioassays allow accurate and reliable characterization of therapeutic interventions in disease models. They can also help confirm the role of a specific mechanism/pathway in nondiseased models. The rapidly emerging statistical pattern recognition (SPR) methods and proliferating software tools have great potential in helping formalize and solve the increasingly data-driven puzzles in neuroscience. In our case, SPR methods can unmask subtle characteristics in neuroimaging data similar to how antibodies reveal pathological features. A recent publication provides an example application of SPR to create a neuroimaging data-driven bioassay for highly efficient testing and characterization of the effects of a drug in a neurodegenerative mouse model [14]. Expanding on this application and drawing on SPR methods' inherent design for optimal extraction of information embedded in the data can provide access to knowledge

beyond the reach of historically used simplistic measures such as mean, standard deviation, and post hoc statistical tests.

In conclusion, we need to expand beyond the current limits of neuroimaging for seeing and capturing neural events in greater spatiotemporal resolution and develop the analytical tools for analyzing the data to help hypothesize, simulate, and validate models of calcium dynamics to uncover and target mechanisms involved in AD and potentially other neurodegenerative diseases.

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Fig. 1.

Representative examples of calcium activity from barrel cortex neurons in non-tg, APP, 3RTau, and WT α-syn tg mice. (A) Representative two-photon images of the calcium indicator dye, OGB (green), and astrocyte marker, SR101 (yellow–orange) in the different mouse models. (B) Respective two-photon images indicating the SR101-positive astrocyte structures. (C) Selected regions of interest for neurons and astrocytes for each image. Neurons indicated in green; astrocytes are in red. (D) The average 1-Hz stimulus time course for the neuronal calcium signal compared to NTG mice. The arrows indicate the difference in calcium transients between the neurodegenerative mouse model and the NTG mouse model. Scale bar for photomicrographs is 10 μm.

Fig. 2.

Schematic illustration of calcium alterations triggered by Aβ, tau, and α-syn in the synapse. During stimulus, calcium rushes into the synaptic terminal through calcium channels, which leads to neurotransmitter release. Under normal conditions, calcium is returned to baseline by sequestration in the endoplasmic reticulum (ER) and the mitochondria, as well as to the extracellular space via sodium/calcium exchangers. However, abnormal accumulation of Ab, tau, and α-syn may lead to calcium dyshomeostasis, most likely via alterations in calcium buffering by ER and/or mitochondria.