**Generation of dendritic Ca²⁺ oscillations as a consequence of altered ryanodine receptor function in AD neurons**

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**Ryanodine receptor (RyR)-mediated Ca²⁺ dysregulation is associated with Alzheimer’s disease (AD) neuropathology.** Using 2-photon Ca²⁺ imaging and patch clamp recordings in brain slice preparations from young 3xTg-AD and NonTg control mice, we recently demonstrated that RyR-mediated Ca²⁺-induced Ca²⁺ release (CICR) is substantially increased within dendrites from AD neurons, such that synaptic stimulation alone is sufficient to generate aberrant CICR. We also observed supra-additive Ca²⁺ release upon coincident RyR activation with synaptic stimulation in 3xTg-AD mice. Here, we describe an additional observed phenomenon: generation of patterned Ca²⁺ oscillations in the spines and dendrites from AD neurons upon coincident RyR and synaptic stimulation. As the temporal entrainment of Ca²⁺ signals influences many downstream cellular and synaptic functions, these abnormal oscillatory patterns may be associated with the structural and functional breakdown of synapses in AD.

The pathological mechanisms underlying Alzheimer’s disease (AD) are currently unknown. Recent studies in AD mouse models have identified an upregulation in intracellular (ER) Ca²⁺ release that precedes AD histopathology and cognitive decline, and we and others have focused on the RyR as the principal contributor. This is based on studies from AD mouse models demonstrating increased RyR-mediated Ca²⁺ responses, increased RyR expression and Ca²⁺-dysregulation reversal with RyR antagonists.¹³ We have also demonstrated that the greatest relative increases in RyR Ca²⁺ release occur in dendrites and spine heads (10- to 20-fold), which are critical sites for synaptic transmission and plasticity.²,⁴ Alterations in Ca²⁺ dynamics can play a role in spine loss and dendritic breakage, alter signal propagation, and impair plasticity mechanisms supporting learning and memory.⁵,⁶ Therefore, altered Ca²⁺ signaling within dendrites may contribute to the devastating cognitive loss that defines AD.

The RyR is a high conductance cation channel (~100–150 pS) localized to the ER membrane. It is expressed in a number of cell types and coordinates many Ca²⁺ signaling events.⁷ The tetrameric RyR is structurally similar to the IP₃R, however, at ~560 kD for each subunit, the RyR is one of the largest channel complexes identified.⁸ There are three isoforms, RyR1, RyR2 and RyR3, with the corresponding genes located on different chromosomes. The RyR1 is predominant in skeletal muscle,⁹ and in cerebellar Purkinje neurons.¹⁰,¹¹ The RyR2 is heavily expressed in cardiac muscle, and is the predominant subtype in the brain. Interestingly, RyR2 levels are upregulated in the brains of AD mouse models early in the disease.² The RyR3 has a low level, widespread expression pattern, and is also found in the brain—particularly in cortical and hippocampal regions involved in learning and memory.¹¹,¹² This isoform has also been linked to AD at later disease stages, with expression increasing with Aβ pathology.¹³ The principal activator for all three RyR subtypes is Ca²⁺ itself, although each subtype has different Ca²⁺ binding affinities.

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either synaptic stimulation or NMDAR activation generated a supra-additive Ca$^{2+}$ response in dendrites from AD neurons (Fig. 1). One aspect of this supra-additive Ca$^{2+}$ response we found particularly interesting, but outside the scope of the original study, was the observation of Ca$^{2+}$ oscillations within spines and dendrites of the AD neurons. This oscillatory behavior did not appear to be synchronized between these compartments, rather, the Ca$^{2+}$ signals fluctuated independently of each other. Rogue RyRs have also been shown to result in an increased probability of spontaneous Ca$^{2+}$ oscillations and processes and spine heads, and likely involves a reduced CICR threshold. A key finding supporting the reduced CICR threshold in the AD mice was that Ca$^{2+}$ signals evoked by synaptic stimulation alone were increased in spines and dendrites relative to NonTg controls, and this effect was blocked by RyR-antagonists. This was likely mediated through Ca$^{2+}$ influx from NMDA receptors, as direct activation of NMDA receptors also results in an enhanced postsynaptic Ca$^{2+}$ response in the AD mice, and is reversed by RyR antagonists. It was also observed that RyR activation concurrently with

Figure 1. Enhanced RyR-evoked Ca$^{2+}$ release within subcellular compartments in AD neurons. (A) Graph demonstrates relative Ca$^{2+}$ increases with caffeine wash (20 mM, 30 sec.) in the soma, nucleus, distal dendrites and spine heads of NonTg (black bars) and 3xTg (gray) neurons. (B) A comparison of relative ER Ca$^{2+}$ stores, as measured by Ca$^{2+}$—efflux upon blockage of the SERCA pump by CPA, demonstrates enhanced levels in the AD mutant mice. (C) Raw 2-photon Ca$^{2+}$ images from distal dendrites and spines in NonTg (top), and ADTg neurons (bottom) comparing Ca$^{2+}$ responses from (from left to right) (1) baseline, (2) RyR-evoked release (10 mM caffeine wash), (3) synaptic stimulation and (4) RyR + synaptic stimulation. Note that fura-2 reduces fluorescence intensity with increasing Ca$^{2+}$ levels at 780 nm 2-photon excitation, therefore a dimmer image indicates greater Ca$^{2+}$ release.

with RyR1>RyR2>RyR3. RyR1 displays a biphasic, bell-shaped response curve with maximal release at ~5 μM and complete inhibition occurring in the low millimolar range. The RyR2 and RyR3 isoforms, however, require substantially higher Ca$^{2+}$ concentrations for feedback inhibition.7 In our recent study, we demonstrated greatly increased RyR-mediated Ca$^{2+}$ release in pyramidal neurons from young AD mouse models (3xTg-AD and PS1/APP), prior to the onset of detectable histopathology or cognitive deficits.4 The increased RyR-mediated Ca$^{2+}$ release was particularly pronounced in dendritic
accelerated wave propagation in other cell systems. This is consistent with the hypothesis that the dendritic Ca²⁺ oscillations in the AD mice observed here are likely an aberrant consequence of dysregulated RyR function.

As shown in Figure 2, stimulation of RyR (with 5 mM caffeine) concurrently with synaptic stimulation (30 Hz; 1.5 seconds) in pyramidal neurons results in patterned Ca²⁺ oscillations within dendritic processes from the AD Tg mice (3xTg-AD, 4–6 weeks of age) but not the NonTg age-matched controls (Fig. 2C). This is markedly different from the sustained Ca²⁺ release response generated by either caffeine application alone, or synaptic stimulation alone (Fig. 2A). A similar effect is observed within spine heads from the AD Tg mice, with distinct Ca²⁺ peaks and rapid decay (Fig. 2D). The Ca²⁺ signaling patterns are likely distinct or heavily filtered between spines and dendrites, as the relative amplitude and frequency are higher in the spines compared to the neighboring dendritic processes. This is consistent with their differing spatial geometry and the hypothesis that the spines and dendrites can function as separate signaling compartments. The observed Ca²⁺ oscillations in the AD neurons are largely blocked when ryanodine (20 µM) is included in the patch pipette to block RyR-Ca²⁺ release (Fig. 2E and F).

The functional implications of generating aberrant Ca²⁺ oscillations are presently unclear, but it is feasible that localized Ca²⁺-dependent processes that originate within dendritic spines would be affected. The dendritic spine heads of CA1 pyramidal neurons are sites of postsynaptic excitatory neurotransmission, and where long-term plasticity, the cellular basis of learning and memory, is encoded. Long term potentiation (LTP) at these synapses requires ER Ca²⁺ through RyR activation; for example, we and others have found that blocking RyRs will block LTP induction in control and AD brains. Consistent with the rogue activation of Ca²⁺ oscillations in the AD Tg neurons, we also found that LTP expression is differentially affected by concurrent RyR stimulation (Fig. 3). Although both the NonTg and AD mouse strains show a similar increase in the baseline field potential response (via antagonism of the inhibitory presynaptic adenosine receptor), after the LTP tetanus, the NonTg control mice exhibit no further synaptic potentiation while the AD mice continue to display excessive potentiated responses, far greater than what is observed under control conditions. Although it is presently unclear what the physiological and behavioral implications are of this ‘runaway’ LTP, it presents a clear and proximal indication that critical synaptic functions are altered in these AD neurons.

The downstream consequences of aberrant Ca²⁺ oscillations within spines could also contribute to the synaptic pathophysiology described in AD. Under normal conditions, synchronized Ca²⁺ oscillations have been described in hippocampal and cortical neurons and are thought to play a constructive role in brain function. The frequency of Ca²⁺ oscillations can regulate gene expression patterns and support synaptic plasticity and memory processing. In addition, RyR-mediated Ca²⁺ release is associated with a range of physiological cascades relevant to synaptic functioning—such as NFκB and CamKII activation, ERK and CREB phosphorylation and neural stem cell proliferation linked to Hebbian synaptic function.
Disruption of these key signaling cascades can introduce subtle but dysfunctional consequences for maintenance of neuronal homeostasis. For example, we have uncovered that despite the appearance of similar synaptic physiology and plasticity expression between the NonTg and pre-symptomatic 3xTg-AD mice, the Ca$^{2+}$ dynamics underlying these fundamental mechanisms are vastly different.

In the study presented here, the 3xTg-AD mice are pre-symptomatic, meaning that detectable amyloid plaques and cognitive impairments are not yet present; however, there is an increase in Aβ$_{1-42}$ levels. Aβ$_{1-42}$ is more patogenic species of the APP cleavage products, and in addition to being a primary component of plaques found in brains of AD patients, it has been shown to alter RyR gating properties. Exposure to Aβ$_{1-42}$ increases the RyR channel open probability 10-fold, and the mean open time two-fold, with the net effect of increased Ca$^{2+}$ release resulting from RyR alterations at the biophysical level. Increased ER store levels, as suggested by enhanced Ca$^{2+}$ release upon CPA application (Fig. 2) and evidence presented in Zhang et al., can also alter RyR sensitivity and upregulating ER Ca$^{2+}$ release properties. Additionally, IP$_3$-R-evoked Ca$^{2+}$ release can feed into the Ca$^{2+}$ dysregulation through more than one mechanism; IP$_3$-R-evoked release can be a Ca$^{2+}$ source for the RyR-mediated CICR, and IP$_3$ channel gating properties are altered with AD-linked PS mutations resulting in an increased open channel probability and sensitivity to low IP$_3$ levels.

The disruption of functional RyR-mediated Ca$^{2+}$ signals, or the induction of aberrant oscillations as observed here, could likely interfere with synaptic transmission, synaptic plasticity, and the related learning and memory processes—each devastating components of AD whose underlying pathogenic mechanisms are poorly understood.

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