Symposium 1: Amyloids in Human Disease

14-Symp
Dysregulation of Intracellular Calcium in Alzheimer's Disease
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Alzheimer's disease is characterized by the deposition of senile plaques in the brain resulting in focal neurotoxicity that ultimately leads to neural network disruption. Intracellular calcium is a tightly regulated second messenger whose activation leads to numerous downstream events, including cell death. It has been suggested that dysregulation of calcium homeostasis plays a role in Alzheimer's disease, however this has not been demonstrated directly. We combined in vivo multiphoton cell-resolved calcium imaging to quantitatively image resting and dynamic calcium signaling in both neurons and astrocytes in the brains of mouse models of AD. We found that resting calcium was elevated in a subset of neurons and throughout the astrocytic network in mice with cortical plaques. This increase in calcium levels in neurons but not astrocytes, depended on the proximity to individual senile plaques. The neuronal calcium overload was not the result of presenilin mutations, and led to the loss of spinodendritic compartmentalization, important for synaptic coordination. In astrocytes, we observed increased spontaneous calcium transients that were not dependent on neuronal activity. Astrocytes were functionally coupled across long distances in APP transgenic but not wildtype mice with evidence of in vivo intercellular calcium waves originating near plaques and spreading to astrocytes nearly 200 microns away. These data reveal disruptions in calcium homeostasis in both neurons and astrocytes in mouse models of AD with differing spatial ramifications. Together, the results demonstrate that the aberrant intracellular calcium levels in the brain provide insight into the pathophysiology of AD and that specific manipulation of calcium levels may lead to new drug targets.

15-Symp
Alzheimer's Presenilin Regulation of InsP3R Ca2+ Release Channel Gating
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Familial Alzheimer's disease (FAD) is caused by mutations in amyloid precursor protein and presenilins (PS1, PS2). Many FAD-linked PS mutations affect intracellular calcium (Ca2+) homeostasis by proximal mechanisms independent of amyloid production, although the molecular details are controversial. Here, we demonstrate that several FAD-causing PS dramatically enhance gating of the inositol trisphosphate receptor (InsP3R) intracellular Ca2+ release channel measured in native endoplasmic reticulum membranes by nuclear patch clamp electrophysiology. In contrast, wild type or mutant PS that cause frontotemporal dementia have no such effect. FAD PS alter InsP3R channel gating by modal switching to a high open probability burst mode. Single channel recordings of endogenous InsP3R in FAD patient B cells as well as cortical neurons of asymptomatic PS1-AD mice revealed they have higher occupancy in the burst mode than controls, resulting in enhanced intracellular Ca2+ signals. These results indicate that exaggerated Ca2+ signaling through InsP3R-PS interaction is a disease specific and robust proximal mechanism in AD.

16-Symp
Structural Diversity of Amyloid Oligomers
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Recent evidence suggests that soluble amyloid oligomers may represent the primary pathological species of protein aggregates in neurodegenerative diseases. Conformation-dependent, aggregation specific antisera indicate that there are three widely distributed and distinct classes of oligomer structures: prefibrillar oligomers, fibrillar oligomers and pore like annular protofibrils. Fibrillar oligomers are soluble at 100,000 x g, rich in β-sheet structures but yet bind weakly to thioflavin T. EPR spectroscopy indicates that fibrillar oligomers display significantly more spin-spin interaction at multiple labeled sites than prefibrillar oligomers and are more structurally similar to fibrils. Fibrillar oligomers are approximately one half to one third the height of mature fibrils, suggesting that they may represent small pieces of a single fibril protofilament. Fibrillar oligomers seed the formation of fibrillar oligomers from Aß monomers, but do not seed the formation of fibrils. The fibrillar oligomers resulting from seeded reactions have the same dimensions and morphology as the initial seeds, suggesting that the seeds replicate by growing to a limiting size and then splitting. We have also isolated a number of monoclonal antibodies that recognize generic, sequence-independent epitopes associated with prefibrillar oligomers. Analysis of synthetic Aß oligomers by dot bots using prefibrillar oligomer specific monoclonal antibodies indicates that structural polymorphisms exist in Aß prefibrillar oligomers that vary in their reactivity with monoclonal antibodies. These results suggest that distinct structural variants of soluble Aß oligomers exist, analogous to different strains of prions. These structural polymorphisms may contribute to disease heterogeneity. This work was supported by NIH NS 38298, AG00538, the Cure Alzheimer Fund and a grant from the Larry L. Hillblom Foundation.

17-Symp
Mechanisms Underlying Neuronal "hyperactivity" in a Mouse Model of Alzheimer's Disease
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Alzheimer's disease (AD) is a progressive, irreversible neurodegenerative disorder characterized by deposition of amyloid plaques and accumulation of intraneuronal neurofibrillary tangles. Mouse models of the disease, expressing human amyloid precursor protein and/or presenilins with mutations typically causing early onset AD in humans, recapitulate many hallmarks of the human disease. They develop senile plaques and neurofibrillary tangles; exhibit dysregulation of the intracellular Ca^{2+} homeostasis, brain inflammatory response and memory impairment. Because the dysregulation of the intracellular Ca^{2+} homeostasis was postulated to act as an important progenitor of AD, we studied intraneuronal Ca^{2+} dynamics in APP23xPS45 mutant mice. In vivo two-photon Ca^{2+} imaging in these mice revealed 3 different classes of layer 2/3 cortical neurons: ''silent cells'' showing no spontaneous Ca^{2+} transients during a prolonged recording period, neurons with normal frequency of Ca^{2+} transients (< 4/min) and ''hyperactive'' cells with unusually high frequency of Ca^{2+} transients (Busche et al., 2008). Here we studied the mechanisms underlying Ca^{2+} transients in normal and hyperactive cells. The Ca^{2+} transients in both cell types had similar amplitudes and kinetics. They were tetrodotoxin-sensitive and thus caused by action potential firing. Moreover, these transients were of synaptic origin because they were completely and reversibly blocked by a mixture of glutamate receptor blockers CNQX and APV. Surprisingly, APV alone was sufficient to block the transients in both cell types, suggesting a key contribution of NMDA receptor-channels. According to our data, the increased frequency of Ca^{2+} transients in hyperactive cells was not due to an increase in intrinsic excitability but rather to a relative loss of synaptic inhibition. Thus, neuronal hyperactivity in APP23xPS45 mice is caused by a local synaptic rewiring with a relative increase in the excitation vs. inhibition.

Symposium 2: The Cytoskeleton: Variations on a Theme

18-Symp
Function and Regulation of the Bacterial Cytoskeleton
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During the fast paced multiplication of bacteria, cell growth, DNA replication, DNA segregation and cell division must occur and these essential processes must be coordinated temporally and spatially to achieve homeostasis. How this is achieved is not well understood. In the Gram-negative bacterium *Caulobacter crescentus*, the assembly and cellular positioning of the cytokinetic ring made of the tubulin homolog FtsZ is coordinated with chromosome origin segregation through the *parS*/ParB/MipZ kinetochore complex. We found that the polarity factor TipN plays a major role in the segregation of the *parS*/ParB/MipZ complex by affecting the dynamics of the ParA cytoskeletal element. This in turn governs the timing and placement of FtsZ ring formation, which ultimately affects cell growth, division and the size of the progeny.

19-Symp
Actin and Bacterial Actin-Like Proteins: Insights Into Evolution
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Protein polymers are ubiquitous in biology, from cytoskeletal filaments to bacterial pili, and in many cases contain most of the protein in the cell. While it has been assumed that each polymer has a defined structure, we can show that many polymers exist in a multiplicity of states. Conserved subunits, such as bacterial flagellin or Type IV pilin, can be assembled in different ways, giving rise to abrupt changes in quaternary structure. As new quaternary structures emerge, these can have very new functions. For example, the bacterial ParM protein, a homolog of eukaryotic actin, forms filaments that are very different in structure than F-actin, and have a very different function, being involved in DNA segregation. New insights have emerged about the structural plasticity