Effects of age and PS1 mutations on ER Ca^{2+} dysregulation in normal and Alzheimer's disease brains

Grace E. Stutzmann*[1], Salvotore Oddo[2], Antonella Caccomo[2], Ian Parker[2] and Frank LaFerla[2]

[1]*Dept. of Neuroscience, Rosalind Franklin University of Medicine and Science / The Chicago Medical School*

[2]*Dept. of Neurobiology and Behavior, UC Irvine*

grace.stutzmann@rosalindfranklin.edu

207.13

Neuronal ER Ca^{2+} release is regulated by inositol trisphosphate and ryanodine receptors (IP_3R and RyR), and disruptions in neuronal ER Ca^{2+} signaling are implicated in neurodegenerative diseases such as Alzheimer's (AD). Previously, we demonstrated that an AD-linked presenilin 1 mutation ($PS1_{M146V}$) leads to exaggerated ER Ca^{2+} responses in cortical neurons (Stutzmann et al., 2004). However, transgenic $PS1_{M146V}$ mice do not develop the characteristic AD markers of amyloid plaques and hyperphosphorylated *tau*. Here we characterize Ca^{2+} signals in a triple transgenic (3xTg-AD) mouse expressing mutant *PS1*, *amyloid precursor protein (APP)* and *tau* that does develop AD histopathology at older ages (Oddo et al., 2003). Cortical brain slices were studied by whole-cell patch clamp, flash photorelease of IP_3 and 2-photon imaging. IP_3-evoked Ca^{2+} signals were > 2-fold larger in pyramidal neurons of 3xTg-AD mice than in age-matched (4-8 week) controls; but were comparable to $PS1_{M146V}$ mice. This enhancement arose largely through recruitment of RyR, and we found that IP_3R and RyR colocalized in Layer V neurons. Application of caffeine and CPA demonstrated that the ER contains larger Ca^{2+} stores in the AD-Tg mice. We conclude that $PS1_{M146V}$ causes exaggerated ER-Ca^{2+}responses, but that *APP* and *tau* mutations do not add appreciably to this effect. The exaggerated Ca^{2+} signals are evident in young mice well before development of plaques and tangles, and result from over-filling of ER-Ca^{2+} stores and recruitment of RyR. It remains to be determined whether these Ca^{2+} signaling disruptions subsequently promote AD histopathology.

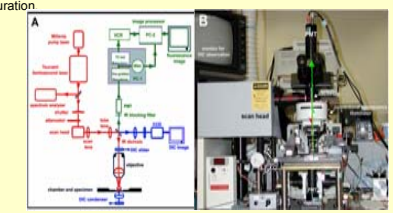
Techniques:

Whole-cell recordings and acquisition

Visualized whole-cell patch clamp recordings were performed in 300 µM thick sections of the medial prefrontal cortex using an infra red/differential interference contrast (IR/DIC) setup. Patch pipettes(4MΩhms) were filled with a solution containing (in mM): K-methlysulfonate, 120; HEPES, 10;, 5; sucrose, 20; $MgCl_2$ 1; K_2ATP, 1 and GTP, 0.1. (pH adjusted to 7.32-7.35 with KOH) as well as 50 µM fura-2 (Molecular Probes; Eugene, OR) and 50 µM caged IP_3 (Molecular Probes).

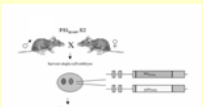
Ca^{2+} imaging and flash photolysis

Imaging was performed using a 'home-made' video-rate two-photon microscope, as described (see below, Nguyen *et al*., 2001). Excitation was provided by trains (80 MHz) of ultra-short (ca. 100 fs) pulses at 780 nm from a Ti:sapphire laser (Tsunami, Spectra-Physics, Mountain View, CA) pumped by a solid-state laser (Millenia 5X, Spectra-Physics). The laser beam was scanned by a resonant galvanometer (General Scanning Lumonics, Waterton, Mass.) allowing rapid (7.9 kHz) bidirectional scanning in the *x*-axis and by a conventional linear galvanometer in the *y*-axis, to provide a frame-scan rate of 30 fps. For clarity, images and traces of fura-2 fluorescence are expressed as a pseudo-ratio $F_0/\Delta F$ (where F_0 is resting fluorescence and ΔF is the decrease of fluorescence on stimulation), so that increases in $[Ca^{2+}]$ correspond to increasing ratios. Photolysis of caged IP_3 was accomplished by flashes of UV light (340-400 nm). The irradiance at the specimen was about 400 mW cm^{-2}, and stimulus strength was controlled by varying the flash duration.



Generation of Triple Transgenic (3xTg-AD) mice (Oddo et al., 2003)

Using the pronuclear microinjection technique, two independent transgene constructs encoding human APP_{Swe} and tau_{P301L} (4R/0N) were coinjected, both under the control of the mouse Thy1.2 regulatory elements, into single-cellembryos harvested from mutant homozygous $PS1_{M146V}$ knockin mice ($PS1_{KI}$; Guo et al., 1999). The injected embryos were reimplanted into foster mothers and the resulting offspring genotyped to identify 3xTg-AD mice.

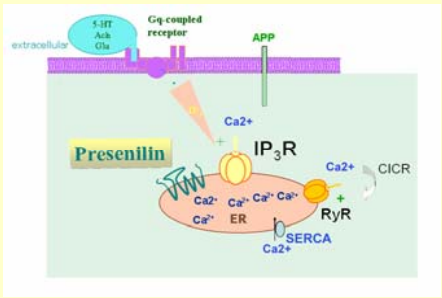


References:

Guo et al., (1999) Nat. Med. 5:101-06; Nguyen et al., (2001) Cell Calcium 30:383-93; Oddo et al., (2003) *Neuron* 39:409-421 ; Stutzmann et al., (2003). *J Neurosci.* **23**(3): 758-65.; Stutzmann et al., (2004) *J. Neurosci.* 24:508-513

Supported by NIH GM48071 and NIA AG16573

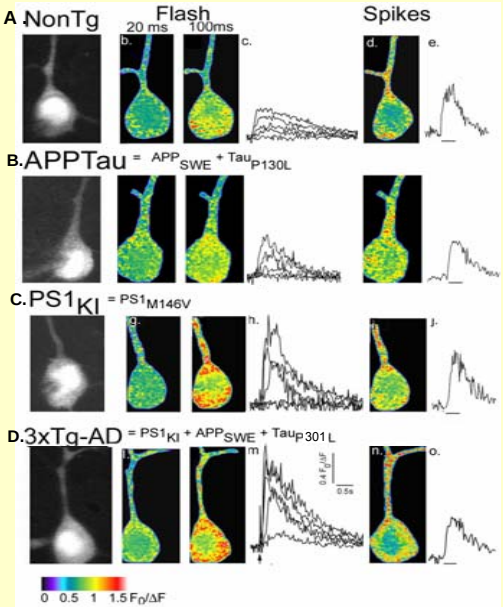
1). ER Ca^{2+} signaling dynamics in cortical neurons



2). Mutations in PS1 lead to enhanced IP_3-mediated Ca^{2+} release, but have little effect on spike-evoked Ca^{2+} signals

Imaging IP3- and spike-evoked Ca2+ signals in 6 week old Non-Tg and Tg neurons.

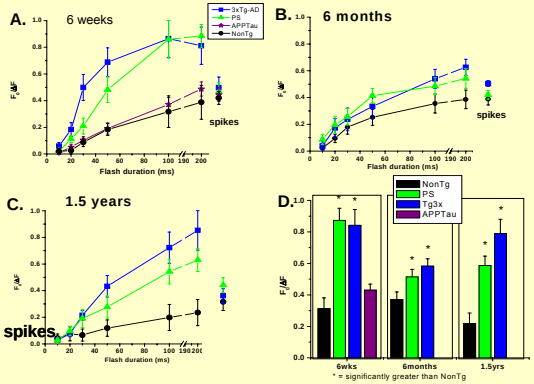
A, Resting fura-2 fluorescence in a 6-week old Non-Tg neuron, left panel. Center panels show pseudocolored images of Ca2+ signals evoked following photolysis of caged IP3 (20 ms flash duration, left; 100ms, right). Superimposed traces show increasing somatic fluorescence ratio signals evoked by flashes of 10, 20, 30, 50, and 100ms. Far right panels show the Ca2+ image and the time course of somatic Ca2+ signal during a train of spikes. B, Ca2+ images and traces in a APPTau neuron. C, Ca2+ images and traces in a PS1KI neuron. D, Ca2+ images and traces in a 3xTg-AD neuron.



3) . ER Ca2+ signaling remains elevated over the lifetime of PS1 mutant- expressing mice

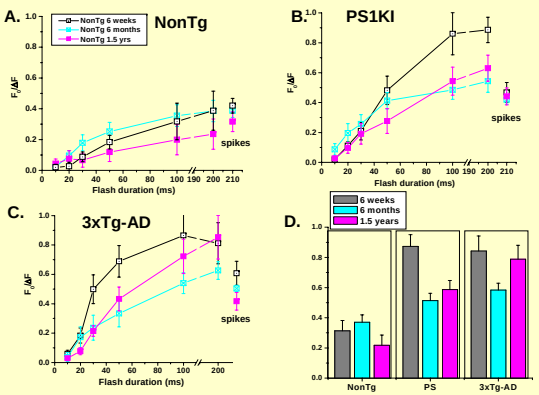
Effect of different AD transgene expression on IP3- and spike-evoked Ca2+ signals in young, adult and old neurons

A, IP_3-evoked Ca^{2+} signals in cortical neurons from Non-Tg (●), $PS1_{KI}$ (▲), 3xTg-AD (■) and APPTau (★) mice at 6 weeks of age. Data points show mean fluorescence ratio changes evoked by photolysis flashes of increasing durations, and (at right) signals evoked by trains of action potentials. B, Corresponding data from 6 month old mice. C, Corresponding data from 1.5 year old mice. D, Summary histograms, showing maximal IP_3-evoked Ca^{2+} responses (pooled 100 and 200 ms flash data) for each of the transgenic groups at 6 weeks, 6 months and 1.5 years. Asterisks indicate significant differences (p<0.05) from the NonTg controls within each age group.

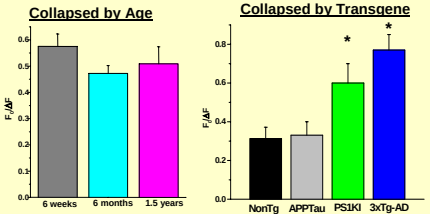


Effect of age on neuronal Ca^{2+} signals within AD transgenic models

A-C, IP_3- and spike-evoked Ca^{2+} signals measured, respectively, in neurons from Non-Tg, $PS1_{KI}$, 3xTg-AD mice. In each panel, data points show mean responses from 6 week (□) 6 month (⊠) and 1.5 year old (■) mice. Data are the same as in previous figure, but presented grouped by transgene, rather than by age. D. Summary histograms, showing maximal IP3-evoked Ca2+ responses (pooled 100 and 200 ms flash data) for each age within the different transgenic groups.

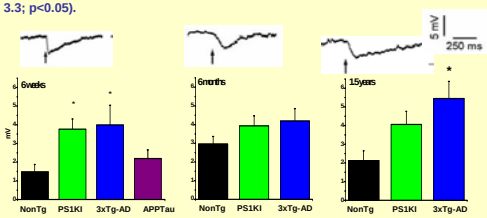


Peak IP_3- Ca^{2+} signal amplitude by age, and by transgenic model demonstrate that PS1KI transgene expression, not age, is critical factor in ER-Ca2+ dysregulation



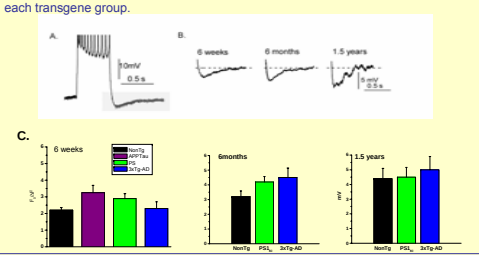
4). Amplitude of the IP3-evoked hyperpolarization increases with PS1 mutation expression, not age.

A. Representative IP3-evoked hyperpolarizations evoked by 100 ms photolysis flashes (arrows) in neurons from * mice at 6 weeks (left), 6 months (center) and 1.5 years (right) of age. Resting potential was -62 mV in all cases. B. Bar graphs show mean (n = **) IP3-evoked hyperpolarizations for all transgenic groups at 6 weeks (left), 6 months (center) and 1.5 years (right). Asterisks indicate significant increases in IP3-evoked hyperpolarizations with respect to NonTg controls at the equivalent age (6 weeks, F(3, 44) =3.0; p<0.05; 1.5 year, F(2,50) = 3.3; p<0.05).**

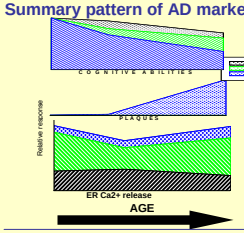


Amplitude of the spike-evoked AHP increases with age, regardless of AD transgene expression

A. Representative spike train and subsequent afterhyperpolarization used to measure the maximal amplitude of the AHP. Resting potential was -62 mV. Area in gray indicates regions of traces shown in B. **B.** Representative AHPs recorded in 3xTg-AD neurons at 6 weeks, 6 months and 1.5 years of age. **C.** Bar graphs indicate mean AHP amplitudes in NonTg and AD-Tg mice. Asterisks indicate significant increases in AHP amplitude relative to the 6-week measurement within each transgene group.



Summary pattern of AD markers over a lifetime



As AD-transgenic mice age, ER Ca2+ signals remain elevated,and plaques develop coincident with cognitive decline. However, Ca2+ levels and plaque deposition appear to be separate events.

Conclusions:

1). Cortical neurons from $PS1_{KI}$ and 3xTg-AD mice demonstrate enhanced IP_3-evoked Ca^{2+} responses. The 3xTg-AD and $PS1_{KI}$ neurons are not different from each other, while the APPTau and NonTg mice are similar.

2). Although age has some effects on ER Ca2+ signaling, expression of the PS1 mutation is the critical element in the ER-Ca2+ dysregulation.

3). Dowstream Ca2+ events linked to ER stores are enhanced in mutant PS1 expressing neurons and not affected by age; Ca2+ events linked to VGCC are affected by age, not by AD transgene expression.

4). Plaque deposition does not appear to affect ER Ca2+ dysregulation.