

havioral effects of DHA/PFA manipulations at younger ages, when nontransgenic controls show steeper learning curves and better memory retention, and transgenic mice are already relatively impaired even on regular chow (Westerman et al., 2002). Additional behavioral tests will be required to assess the significance of the diet- and genotype-dependent changes in thigmotaxis.

Since dietary and pharmacological manipulations of cholesterol have profound effects on plaque load and neuritic dystrophy in the Tg2576 model (Wolozin, 2004), it is surprising that these hallmarks of AD were not assessed by Calon and colleagues. If alterations of DHA in neural membranes affected the production, deposition, or clearance of A β , the neuronal alterations observed on the low DHA/PFA diet might represent, at least in part, an A β dose effect. Naturally, confirmation of such an effect would not diminish the potential therapeutic significance of the results Calon and colleagues obtained.

In conclusion, Calon and colleagues' study raises the intriguing possibility that reduction in brain DHA levels, induced by dietary depletion and A β -dependent oxidative stress, impairs cognitive functions through a causal chain involving decreased p85 α expression \rightarrow decreased PI3-kinase activity \rightarrow decreased activity of Akt and BAD \rightarrow disinhibition of caspases \rightarrow actin degradation \rightarrow destabilization of postsynaptic proteins and dendrites. Although the investigators have not yet proved that each step in this cascade is necessary and sufficient for the next, the proposed cascade provides a thought-provoking framework of testable hypotheses for future studies. In a similar vein, DHA, PI3-kinase, and caspases affect many other factors besides those examined by Calon and colleagues. It will therefore be interesting to determine which of the many biochemical alterations DHA depletion might elicit has the most important impact on AD-related cognitive decline. Whether further enrichment of the typical human diet with DHA would decrease AD risk and benefit patients with the disease is a challenging question. For those who wish to hedge their bets while waiting for the answer: eat more fish.

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Calcium Waves Rule and Divide Radial Glia

Radial glial proliferation is a critical step in the construction of cerebral cortex. In this issue of *Neuron*, Weissman and colleagues use time-lapse calcium imaging techniques to demonstrate that spontaneous calcium waves sweeping through cohorts of radial glia in the ventricular zone can modulate their proliferation during cerebral cortical development.

Radial glial cells provide a template for the generation and migration of neurons that eventually form different cortical layers. Radial glial cell morphology is characterized by a soma situated in the ventricular zone and an elongated fiber that extends the width of the developing cerebral cortical wall. During very early stages of corticogenesis, symmetric divisions of radial glia give rise to other radial glial cells and form the glial scaffolding upon which cortex is constructed. The essential role of radial glia in guiding neuronal migration to the cortical plate is well established (reviewed in Rakic, 2003; Marin and Rubenstein, 2003). Recent findings, however, indicate that asymmetric divisions of radial glia can generate neurons and radial glia that act as migratory guides for their own neuronal progeny (Noctor et al., 2001; Gotz et al., 2002; Anthony et al., 2004). Alternately, asymmetrically dividing radial glial cells may also give rise to somally translocating neurons, which retain and use the apical radial fibers to ascend to the cortical plate (Miyata et al., 2001). During cortical development, neurons regulate radial glial cell function, and radial glial cells, in turn, support neuronal cell migration and differentiation. As neuronal migration and placement dwindles, radial glia differentiate into astrocytes (Rakic, 2003). This sequential and coordinated unfolding of distinct radial glial phenotypes (i.e., neural precursor, migratory guide, and astroglial precursor) is essential for the emergence of laminar architecture and the mature functional neural circuitry of the cerebral cortex. How do radial glial cells

in cerebral cortex then coordinate their function as neuronal precursors, as neuronal migratory guides, and as astroglial precursors? This complex orchestration of diverse radial glial functions may require intrinsic plasticity and interradian glial interactions during corticogenesis. However, the dynamics of interradian glia interactions during cerebral cortical development and its functional implications remained unexplored. The work of Weissman, Kriegstein, and colleagues presented in this issue of *Neuron* (Weissman et al., 2004) provides an exciting first glimpse into the mechanisms underlying interradian glial interactions and the functional significance of such interactions for corticogenesis.

Using time-lapse calcium imaging techniques, Weissman et al. convincingly demonstrate that spontaneous calcium waves propagate through clusters of radial glia in the cortical ventricular zone. A trigger cell releasing a diffusible signal appears to initiate a spontaneous calcium wave in a cohort of radial glia cells surrounding it. This diffusible signal turns out to be ATP, not a neurotransmitter as would have been predicted by previous studies. The initiation of calcium waves involves the opening of connexin hemichannels expressed in ventricular zone cells and the release of ATP. ATP then selectively affects the calcium dynamics of cells only in the ventricular zone, not elsewhere in the cerebral wall. Pharmacological and immunohistochemical profiling of ATP receptors on the ventricular zone indicates that the P2Y₁ ATP receptor in radial glia is a critical mediator of ATP-triggered calcium wave propagation. Activation of this receptor leads to the activation of the phospholipase C (PLC) pathway, increased IP₃ production, and the release of intracellular calcium from IP₃-sensitive stores. In contrast, extracellular calcium is not required either for the initiation or propagation of radial glial calcium waves. To determine if these calcium waves regulate radial glial proliferation, Weissman et al. used an elegant strategy to label the ventricular zone cells initiating the calcium waves with hemichannel permeable Lucifer yellow and tested whether these cells were undergoing DNA synthesis with a BrdU pulse. These experiments showed that the ventricular zone radial glial cells initiating calcium waves are S phase cells undergoing DNA synthesis. Furthermore, double labeled cells (Lucifer yellow and BrdU positive) were often found in the upper VZ, known to contain mostly S phase radial glial cells. More importantly, developmental increases in both the sensitivity of the VZ cells to ATP and the calcium wave dynamics (i.e., number of participating cells and distance of wave spread) parallel the developmental increase in neuronal output from the ventricular zone. Disruption of the ATP-mediated calcium waves significantly decreases cell proliferation in the ventricular zone. Together, these data provide tantalizing new evidence for a hitherto undefined mechanism, i.e., spontaneous calcium waves sweeping through clusters of radial glia, in modulating neurogenesis during cerebral cortical development (Figure 1A).

A fundamental challenge in the study of cortical development is the elucidation of mechanisms that determine how radial glia cells are generated and how they differentiate to function as neuronal precursors, neuronal migratory guides, or as astrocyte precursors. In this regard, the results of Weissman et al. raise several important

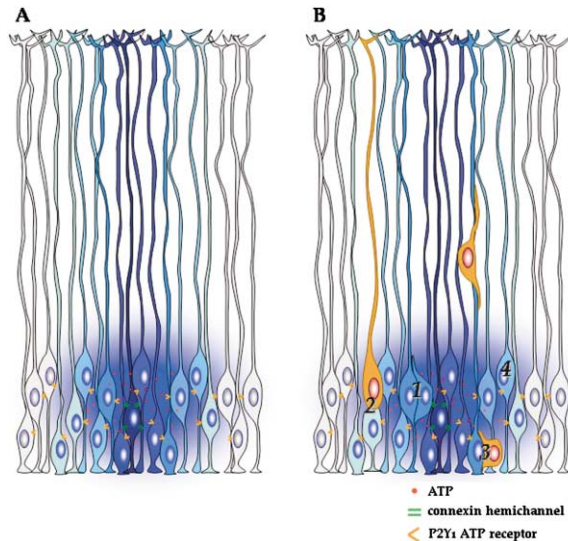


Figure 1. Calcium Waves and Radial Glial Proliferation

(A) Calcium waves (blue) propagating through cohorts of radial glia can modulate their proliferation. Initiation and propagation of these waves involve hemichannels (green), diffusible ATP (red), and P2Y₁ ATP receptors (yellow). Dark blue cell at the center is a trigger cell initiating the wave.

(B) How these spontaneous calcium waves regulate symmetrically dividing (1), asymmetrically dividing (2, 3), and stable (4) radial glial cells in the cluster remains to be characterized. Orange cells are neurons.

questions. First, how is the information encoded in calcium waves translated into different patterns of radial glial proliferation? Symmetric divisions of radial glia give rise to more radial glia (1, Figure 1B), whereas asymmetric divisions can give rise to somally translocating daughter neurons and radial glia (2, Figure 1B), or to glial guided migrating neurons and radial glia (3, Figure 1B). During late stages of cortical development, asymmetric divisions can also give rise to intermediate precursors and transforming radial glia (Noctor et al., 2004). At any given stage, there are also stable, nonmitotic radial glia (4, Figure 1B; Rakic, 2003) in the ventricular zone. Radial glia undergoing these diverse patterns of differentiation do coexist in the ventricular zone (Miyata et al., 2001). Thus, can radial glia cells within a calcium wave cluster respond differently to the increase in intracellular calcium? Weissman, Kriegstein, and their colleagues suggest that calcium increases in a cohort of radial glia may synchronize their proliferation, thus leading to the production of isochronic neurons destined to the same cortical layer or column. Whether this remains the case during different stages of cortical development is unclear. Simultaneous observation of calcium dynamics and proliferative behavior of radial glial cells from different developmental stages can be instructive in this regard. Previous studies have shown that distinct patterns and frequency of spontaneous calcium elevations can induce distinct phenotypic outcomes in neural cells (Gu and Spitzer, 1995; Kumada and Komuro, 2004). Furthermore, earlier studies by Kriegstein and colleagues have shown that calcium increases induced by neurotransmitters (GABA and glutamate) in the ventricular

zone cells can decrease progenitor proliferation? Determining whether changes in the pattern and mode (e.g., neurotransmitter versus ATP induced) of calcium dynamics underly the developmental changes in the proliferative behavior of radial glia is likely to reveal novel insights into the significance of calcium waves or fluxes in the ventricular zone.

A second related question raised by these studies is how do calcium signals actually lead to radial glial proliferation? Do they play a permissive role, enabling the activities of other proliferation-inducing molecular signals, or do the patterns of calcium dynamics play an instructive role in directing proliferation? Calcium can directly or indirectly regulate the activation of transcription factors essential for neural cell differentiation. Furthermore, recent studies by Chenn and Walsh (2002) indicate that neural progenitor proliferation/differentiation decisions in the ventricular zone are critically modulated by β -catenin. How do calcium waves influence the function of such genes? Thus, the identification of calcium wave-induced transcriptional programs and the relevant target genes in the developing cerebral cortex will be essential to understand the role of calcium waves in radial glial differentiation during the emergence of cerebral cortex. Another intriguing possibility raised by this study is that ATP-induced calcium increases in radial glia may have additional effects such as the release of neurotransmitters (e.g., glutamate or GABA). These neurotransmitters in turn can modulate or trigger glial-guided neuronal migration (Marin and Rubenstein, 2003). Thus, spontaneous calcium waves in the VZ may provide a mechanism for the coordination of neural precursor proliferation in the VZ and the appropriate targeting of neuronal progeny to the cortical plate.

The initial demonstration of the significance of spontaneous calcium waves in the developing retina suggest that they play a role in synchronizing the activities of neighboring clusters of retinal cells and refine the patterns of connectivity of retinal ganglion cells (Wong et al., 1995; Feller et al., 1996). Recent studies in the embryonic retina indicate that a distinct type of spontaneous calcium wave is also present in the retinal ventricular zone and it may be correlated spatially and temporally with the previously described inner retinal waves (Feller, 2004; Syed et al., 2004). This correlation of calcium waves in distinct domains of developing retina may coordinate the generation and differentiation of different classes of retinal neurons. Similar mechanisms involving spontaneous calcium waves in distinct domains of developing cerebral wall may also sculpt both the generation of neurons and their patterns of connectivity in cerebral cortex (Weissman et al., 2004).

Considering the significance of radial glial-like cells as neuronal stem cells in the adult brain, the competence to respond to calcium waves appropriately could be a determining feature in the ability of astroglial cells to give rise to new neurons in the mature cortex. Further characterization of the significance of this mode of calcium wave signaling in coordinating the diverse functions of ensembles of radial glia in the developing cortex or analogous astroglial cells in the mature brain is likely to open up new vistas in the study of cerebral cortical development and function.

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How Early Is Firing Required for Wiring?

Activity is known to be important for the refinement of neural connections in the developing brain. In this issue of *Neuron*, Hanson and Landmesser provide evidence that GABA-dependent spontaneous bursting of motor neurons in the embryonic spinal cord is required for the correct execution of an early axon pathfinding decision.

It was the pioneering studies of Hubel and Wiesel in the 1970s that first alerted us to the prominent role that activity plays in the formation of neural maps in the brain (Hubel and Wiesel, 1970). These and subsequent studies in the visual system have outlined roles for activity in sculpting and refining connections in the visual cortex, lateral geniculate, and superior colliculus (Shatz and Stryker, 1988; McLaughlin et al., 2003; Grubb et al., 2003). In the developing somatosensory system, NMDA-dependent neural activity is also required for refining the sensory map in the primary trigeminal sensory nucleus and in “whisker barrels” in layer IV of the somato-