

Seeing Is Believing: GFP Transgenics Illuminate Synapse Elimination

Synapses are lost during developmental synapse elimination. Until now, it has been impossible to follow elimination in the entirety of any neuron's branches and synapses. Using transgenic mice in which one or two of a muscle's motoneurons express variants of GFP, Keller-Peck et al. show in this issue of *Neuron* that elimination occurs asynchronously by local competitive interactions at each synaptic site, apparently independent of events elsewhere in the neuron's terminal field.

Neurogenesis, axon pathfinding, and synaptogenesis are principal features of neural development. However, regressive events like synapse elimination also play a critical role in shaping neural circuits during development and perhaps beyond (Lichtman and Colman, 2000). Although ubiquitous, synapse elimination is best understood from studies of the neuromuscular junction of vertebrates. In the 1970s, physiologists examining the innervation of developing muscle rediscovered what anatomists had described some 60 years earlier: the single synaptic site on each muscle fiber in the adult is formed by the terminus of a branch of one of the several axons innervating the muscle; however, multiple axon branches make synaptic contact at these same synaptic sites in the immature muscle (Purves and Lichtman, 1980). Physiologists revealed this polyneuronal innervation by showing that the synaptic potential in muscle fibers could be fractionated into a number of amplitude components, each associated with an axon in the muscle nerve having a distinct stimulus threshold (Redfern, 1970). Jansen and his colleagues (Brown et al., 1976) showed that the number of motoneurons innervating rat muscles during the period of synapse elimination was unchanging and that individual, immature motoneurons evoked a larger fraction of the muscle's tension than in the adult. These observations strongly suggested that elimination occurred by the regression of axonal branches from a constant set of motoneurons. Indeed, anatomical studies during synapse elimination provided evidence of such a retraction in the form of processes with bulbous endings ("retraction bulbs") located at and near synaptic sites (Riley, 1977). Competition was believed to occur at each synaptic site, since one victor always emerged without evidence of transient denervation. Lichtman and colleagues then provided direct evidence for a competitive process by repeated, vital imaging of the pre- and postsynaptic components of individual synapses during synapse elimination (cf. Balice-Gordon and Lichtman, 1993). They could follow the loss of individual axonal inputs and show that this loss was accompanied by loss of some of the postsynaptic apparatus. They also encountered retraction bulbs that formed as eliminated axons withdrew. However, as their vital dyes stained all the axonal processes at each synaptic site, the details of individual inputs and how they changed during elimi-

nation could not be discerned. Labeling of individual axons with lipophilic dyes circumvented this problem (Gan and Lichtman, 1998), but such preparations could not be studied repeatedly over time. Also disappointing was the inability to discern the entire intramuscular arbor of one motoneuron and fathom how the different portions of this arbor were altered during elimination. The ability to do so should allow one to distinguish among a variety of possible scenarios for events during synapse elimination (as discussed below).

In this issue of *Neuron*, Keller-Peck et al. (2001) report a novel approach to analysis of neuromuscular synapse elimination. Previously, Feng et al. (2000) had generated transgenic mice expressing fluorescent proteins (FPs) in neurons. Amazingly, a few of the transgenic lines express FPs in only one or two motoneurons innervating individual muscles. These mice thus allow visualization of the entirety of the branches and synapses of single motoneurons during synapse elimination. Analysis of these mice has not only confirmed previous hypotheses but also provided some startling new insights into the dynamics and mechanism of synapse elimination. Keller-Peck et al. show the number of synaptic contacts by individual motoneurons declines precipitously during the period of heaviest synapse elimination, providing direct evidence that the arbors of individual motoneurons are pruned during the elimination process. From previous work (Gan and Lichtman, 1988) and this study, different stages in the elimination process can be distinguished morphologically: the terminal processes of the several motoneurons are initially extensively intermixed but later become segregated from each other into different regions of the synaptic site. Finally, due to expansion of the victorious axon, loss of receptor areas under the losing axon, or a combination of both, the single remaining axon occupies all of the synaptic site. The synaptic sites of individual FP-labeled motoneurons are shown to be at different stages of synapse elimination. Thus, elimination occurs asynchronously among these sites. Such an asynchrony was assumed by previous investigations, but this study conclusively makes the point. This asynchrony shows that elimination is determined by local competitive events at each synaptic site. In further support of this conclusion, Keller-Peck et al. find no evidence that the elimination at one site is influenced by position within the entire terminal field of the motoneuron, the branch order within the intramuscular arbor, or the proximity to other sites where the same axon is successful/failing in synapse elimination. Another striking finding by Keller-Peck et al. is that, as elimination proceeds, an entire preterminal branch progressively decreases in diameter and ultimately withdraws in a retraction bulb; this withering occurs all the way back to the branch point of origin. This is reminiscent of selective withering of axonal branches deprived of trophic support in the experiments of Campenot (1982). Whether the eliminated terminals are out-competed for trophic support remains to be determined.

Keller-Peck et al. have advanced our understanding of a long-studied phenomenon by simple, elegant observations in FP-transgenic mice. However, issues remain. At the end of synapse elimination, motoneurons can innervate very different numbers of muscle fibers. How are these differences in "motor unit size" achieved? As

reported here, motoneurons undergo elimination at different times. This suggests an influence on local competitive events that is neuron wide, the nature of which is unknown. Motor innervation is topographically mapped across many muscles. Does synapse elimination contribute to the generation of this topography? At the time elimination is occurring, muscle fibers have begun differentiation into fiber types, and the role of elimination in insuring that an individual motoneuron innervates fibers of only one type is controversial. The type of approach taken by Keller-Peck et al. is likely to shed light on these additional issues. Finally, there is the promise that these mice can be used to examine similar issues in neuron-neuron synapse elimination.

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Selected Reading

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Electrical Wiring of the Oscillating Brain

In this issue of *Neuron*, two laboratories (Deans et al. and Hormuzdi et al.) find that cortical γ oscillation in vitro is impaired in the Cx36 knockout mouse. What are the implications?

In comparison to a digital machine, the brain is a very slow device. The sluggish nature of chemical synapses is usually blamed for this tardiness. Most of our motor actions are slow, our perceptions build up gradually, and retrieval of memories may take hundreds of milliseconds. Chemical synapses have been thought to do a pretty good job on this time scale (below 100 Hz). However, occasionally, neurons must be coordinated at a super speed and with super precision. For such jobs, electrical synapses (and their structural correlates: gap junctions) are called in for help (Galarreta and Hestrin, 2001). The electric organ of the weakly electric fish is

the fastest synchronously firing neuronal network known, and its extraordinary speed and precision is attributed to extensive gap junctional connections (Heiligenberg, 1991). Networks of the mammalian isocortex and hippocampus also produce transient ultrafast oscillatory patterns (200–600 Hz) which appear too fast to be synchronized by ordinary chemical synapses (Ylinen et al., 1995; Kandel and Buzsaki, 1997). Since halothane, a gap junction blocker, abolishes these in vivo oscillations, it was logical to assume that the ultrafast oscillations are brought about by the known gap junctions between fast spiking interneurons (Katsumaru et al., 1988). The papers by Deans et al. (2001) and Hormuzdi et al. (2001) in this issue of *Neuron* do not support a role for them in generating the ultrafast rhythms but instead suggest that gap junctions may play a role in coordinating interneuronal networks in the production of slower oscillations in the θ to γ range (3–50 Hz).

Electrical junctions in the adult brain were considered a rarity not too long ago. However, over the past decade, they have been suggested to be at work in virtually every central nervous structure from olfactory bulb and retina to spinal cord and cortex, with connections among somata, dendrites, and axons of principal cells and interneurons and even between glia and neurons (Figure). Numerous functions previously explained by old-fashioned chemical synapses have been attributed to these more esoteric channels of neuronal communication. In short, gap junctions became fashionable.

The presence of electrical and mixed synapses in the mammalian brain was classically inferred using primarily two techniques: the intercellular spread of small molecules after intracellular injection (dye coupling) and the near synchronous spread of electrical activity seen using dual intracellular recordings from cell pairs. The functional significance of this work has been difficult to assess because of two problems. First, no reliable practical methods are available to verify and document the existence and the exact anatomical location of the hypothesized gap junctions. Second, the available pharmacological means, i.e., gap junction blockers, have so many side effects that the specificity of action is hard to determine.

Additional methods are especially necessary to verify the existence of gap junctions in all cases where they are inferred from the presence of dye coupling. Because gap junctions are permeable to small molecules, dye coupling of biocytin- or Lucifer yellow-filled neurons has been taken as evidence for electrical coupling. Unfortunately, the strength of the link between electrical junctions and dye coupling is questionable. Cells not actually coupled by gap junctions may be dye coupled. Thus, cortical pyramidal cells are often shown to be dye coupled to other pyramidal neurons, GABAergic interneurons, or even glia. However, the dye can diffuse from one cell to another by artificial holes freshly created by membrane ruptures, as well as through gap junctions. The artifactual nature of this coupling can be seen from the fact that dyes like Fura-2 and Calcium Green, too large to cross gap junction pores, also often spread between cells. Slicing of the tissue, perfusing it with hyper- or hypoosmotic solution, and changing the pH of the extracellular fluid are especially efficacious ways of creating membrane fusion among brain cells (Gutnick