

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

- Balice-Gordon, R.J., and Lichtman, J.W. (1993). *J. Neurosci.* 13, 834–855.
- Brown, M.C., Jansen, J.K.S., and Van Essen, D. (1976). *J. Physiol.* 261, 387–422.
- Campanot, R.B. (1982). *Dev. Biol.* 93, 13–21.
- Feng, G., Mellor, R.H., Bernstein, M., Keller-Peck, C., Nguyen, Q.T., Wallace, M., Gross, J., Nerbonne, J.M., Lichtman, J.W., et al. (2000). *Neuron* 28, 41–51.
- Gan, W.-B., and Lichtman, J.W. (1998). *Science* 282, 1508–1511.
- Keller-Peck, C.R., Walsh, M.K., Gan, W., Sanes, J.R., and Lichtman, J.W. (2001). *Neuron* 31, this issue, 381–394.
- Lichtman, J.W., and Colman, H. (2000). *Neuron* 25, 269–278.
- Purves, D., and Lichtman, J.W. (1980). *Science* 210, 153–157.
- Redfern, P.A. (1970). *J. Physiol.* 209, 701–709.
- Riley, D.A. (1977). *Brain Res.* 134, 279–285.

## Electrical Wiring of the Oscillating Brain

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

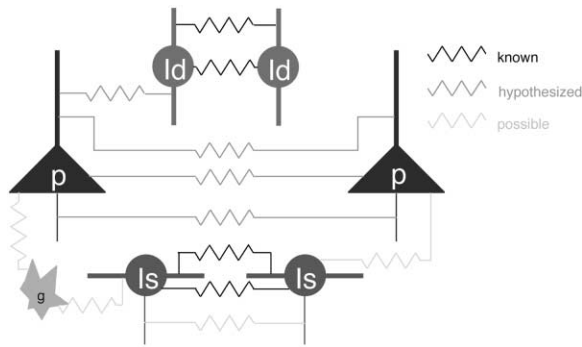
In comparison to a digital machine, the brain is a very slow device. The sluggish nature of chemical synapses is usually blamed for this tardiness. Most of our motor actions are slow, our perceptions build up gradually, and retrieval of memories may take hundreds of milliseconds. Chemical synapses have 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  $\theta$  to  $\gamma$  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



#### Are Electrical Junctions Everywhere?

P, pyramidal cell; Is, GABAergic neuron with somatic targets; Id, interneuron with dendritic targets; g, glia.

et al., 1985). The other kind of error seen with this technique is that cells actually connected by gap junctions may not be dye coupled. For example, biocytin labeling of basket interneurons in vitro or in vivo hardly ever results in dye coupling, even though neighboring basket cells can communicate with each other electrically through as many as 17 anatomically confirmed gap junctions (Katsumaru et al., 1988).

In vertebrates, gap junctions are formed by proteins encoded by the connexin family of genes (Bennett, 2000). There are many connexins to choose from in the brain, including Cx32, Cx36, Cx43, and Cx47, all of which have been claimed to be neuronal using in situ hybridization and immunocytochemical methods. Unfortunately, these techniques have also developed a dubious reputation in the identification of neuronal electrical junctions. There is a reliable and accepted (but laborious) method for the unequivocal identification of gap junctions: electron microscopy. Recent evidence using freeze fracture and immunogold labeling indicates that only one known connexin is expressed in neurons: Cx36 (Rash et al., 2000). Again, Cx36 initially was reported to be everywhere and in all types of neurons. The “hard” evidence now indicates that, in the adult isocortex and hippocampus, Cx36 is expressed primarily in nonpyramidal cells and only members of the same interneuron family are connected (Venance et al., 2000; Deans et al., 2001). Electrical coupling has not thus far been reliably demonstrated between cortical pyramidal or stellate neurons (but see Draguhn et al., 1998). Thus, after much hard work and many blind alleys, the complex circuit of possible electrical junctions (Figure) may perhaps be reduced to this: hippocampal and isocortical inhibitory interneurons of the same class are connected by Cx36 channels, and there are no other known neuronal connexins to choose from.

Against this background, the papers by Deans et al. (2001) and Hormuzdi et al. (2001) are welcome examples of how the tools of molecular biology can be exploited to address functional questions. It is almost a rule in science that ideas mature virtually simultaneously in different laboratories. This is certainly the case for the experiments reported by Deans et al. and Hormuzdi et al. in this issue. The two groups independently generated a Cx36 knockout mouse. The mice walk without ataxia

(inferior olive, what happened to you?) and are not different behaviorally from their wild-type littermates in any obvious ways. Even the physiology of brain slices does not look very different. Population bursts of hippocampal neurons in the absence of chemical neurotransmission were present in both knockout and wild-type. Network oscillations from 3 to 50 Hz could be induced by either kainate, carbachol, or ACPD in both knockout and wild-type. As expected, electrical coupling between interneuron pairs was not observed in knockout mice. However, closer examination of the oscillatory patterns revealed that business was not as usual. Coupling of inhibitory postsynaptic potentials (IPSPs) in interneuron pairs was more variable and spatially more restricted in the isocortex (Deans et al., 2001), and the power of  $\gamma$  frequency oscillation is weaker in the hippocampus (Hormuzdi et al., 2001) of the knockout compared to the wild-type. The periodicity of spontaneous IPSPs is about twice as low in hippocampal pyramidal cells and interneurons in knockout as in wild-type. In light of this latter observation, it is surprising that the peak frequency of the induced rhythms was not different, given that IPSPs are thought to play a critical role in determining network frequency. The experiments of Hormuzdi and collaborators also suggest that the absence of the only known neuronal gap junction (Cx36) does not affect ultrafast (200–600 Hz) oscillations. This leaves us two possibilities: either ultrafast oscillations can be generated by conventional chemical synapses or we have yet to discover additional neuronal gap junctional molecules that may be responsible for these oscillations.

Of course, the real fun is just about to begin. Various complex functions have been attributed to network oscillations in the  $\gamma$  range from binding to memory to consciousness. Now we have a mouse to play with. The first critical step in the game is to examine whether  $\theta$ ,  $\gamma$ , and ultrafast oscillations are altered in the intact brain. After all, the slice models of the in vivo oscillations are only models. In addition, Cx36 is abundant soon after birth in the wild-type newborn and decreases substantially with age. Does this imply more abundant  $\theta$  and  $\gamma$  oscillations in the newborn than in the adult as well as higher consciousness? The experimental means are now available to approach these spooky issues more objectively. I can hardly wait for answers.

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#### Selected Reading

- Bennett, M.V. (2000). *Brain Res. Rev.* 32, 16–28.
- Deans, M.R., Gibson, J.R., Sellitto, C., Connors, B.W., and Paul, D.L. (2001). *Neuron* 31, this issue, 477–485.
- Draguhn, A., Traub, R.D., Schmitz, D., and Jefferys, J.G. (1998). *Nature* 394, 189–192.
- Galarreta, M., and Hestrin, S. (2001). *Science* 292, 2295–2299.
- Gutnick, M.J., Lobel-Yaakov, R., and Rimon, G. (1985). *Neuroscience* 15, 659–666.
- Heiligenberg, W. (1991). *Neural Nets in Electric Fish* (Cambridge, MA: MIT Press).

Hormuzdi, S.G., Pais, I., LeBeau, F.E.N., Towers, S.K., Rozov, A., Buhl, E., Whittington, M.A., and Monyer, H. (2001). *Neuron* 31, this issue, 487–495.

Kandel, A., and Buzsaki, G. (1997). *J. Neurosci.* 17, 6783–6797.

Katsumaru, H., Kosaka, T., Heizmann, C.W., and Hama, K. (1988). *Exp. Brain Res.* 72, 363–370.

Rash, J.E., Staines, W.A., Yasumura, T., Patel, D., Furman, C.S., Stelmack, G.L., and Nagy, J.I. (2000). *Proc. Natl. Acad. Sci. USA* 97, 7573–7578.

Venance, L., Rozov, A., Blatow, M., Burnashev, N., Feldmeyer, D., and Monyer, H. (2000). *Proc. Natl. Acad. Sci. USA* 97, 10260–10265.

Ylinen, A., Bragin, A., Nadasdy, Z., Jando, G., Szabo, I., Sik, A., and Buzsaki, G. (1995). *J. Neurosci.* 15, 30–46.