

GEIGER/AAAS

Subthreshold stimuli (top) propagate to distant sites in neurons (bottom).

Analogue brains

Digital computers and mammalian brains share not only a talent for computation but also a method that favors absolutes. The ones and zeros of a computer are analogous to the all-or-none character of action potentials in the brain. Neurons sum all incoming depolarizing signals until, at a threshold level of depolarization, an action potential fires along the axon. Now, however, Henrik Alle and Jörg Geiger (Max Planck, Frankfurt, Germany) show that the rat brain also uses graded signals for communication.

This analogue or subthreshold processing has been seen in invertebrates, but “in the central mammalian brain it was never shown,” says Geiger. The presumption was that the all-or-none action potentials were the whole story. “To be honest,” says Geiger, “I don’t know how this kind of thinking developed.”

Alle and Geiger looked in the hippocampus, the center of memory and learning, using tissue slices. They introduced subthreshold signals in the cell body and saw that these reached synapses up to 1 mm away. If the subthreshold signals reached the synapse just before an action potential arrived, the response in the postsynaptic neuron was increased.

Although the information in the subthreshold signal can only be communicated in combination with an action potential, it may have great power. Most synapses in the brain are close to their cell bodies—within range of the subthreshold signals. And so-called theta oscillations are a potentially crucial form of subthreshold signaling. These oscillations are thought to act as a kind of clock in the brain, helping to “bind” different sensory inputs into a single experience.

Geiger now plans to look for subthreshold signals generated by receptors in the brain, and to test whether individual axons are carrying out analogue computations. The molecular mechanisms that transduce the signal are also a mystery. “Classically one would expect it to be calcium, but we have evidence against that,” says Geiger. “This is completely new territory.” **JCB**

Reference: Alle, H., and J.R.P. Geiger. 2006. *Science*. 311:1290–1293.

Curing TB with sunlight

Ultraviolet (UV) light makes vitamin D, and vitamin D turns on innate immunity to tuberculosis (TB), say Steffen Stenger (Universität Erlangen, Germany), Philip Liu, Robert Modlin (University of California, Los Angeles, CA), and colleagues. The lower absorption of UV light by African Americans, leading to lower vitamin D levels, may be one reason why this group is more susceptible to TB.

Chemicals from bugs turn on the innate immune response via Toll-like receptors (TLRs). Modlin had already found that activating TLRs killed off intracellular *Mycobacterium tuberculosis*. Nitric oxide (NO) was the downstream mediator for this in mouse cells, but “we’ve been grasping for a decade to find a mechanism in humans,” says coauthor Barry Bloom (Harvard School of Public Health, Boston, MA).

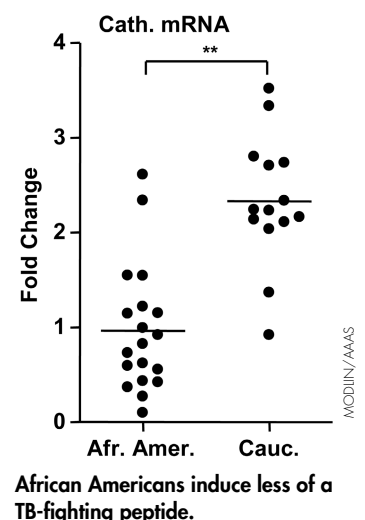
The answer came from gene arrays. Active TLR turned on production of both an enzyme (which converts 25D3 into active vitamin D) and the vitamin D receptor. The activated pathway produced an antimicrobial peptide called cathelicidin, which attached itself to intracellular *M. tuberculosis*, and is a prime suspect for causing its death.

Serum from white-skinned donors had enough of the precursor (25D3) to keep this pathway active, but serum from African Americans was short on 25D3 and supported a much lower output of cathelicidin. The shortfall was corrected by adding 25D3.

The finding may explain why Hermann Brehmer’s 19th century trip to the Himalayas cured him of his TB, and why the fresh air at his sanatoria helped cure others. “Our forefathers knew a lot more about this than we give them credit for,” says Modlin. Eventually, however, sanatoria coddled their patients behind glass, which would have blocked the beneficial UV light.

Although pigmented skin blocks out a lot of UV light, Africans and Asians probably got their fair share before the modern age introduced clothes. Now, however, supplementation may be needed. Bloom plans to test whether vitamin D supplementation can reduce TB transmission within families, speed cures by anti-TB drugs, or slow TB reactivation. “These are not trivial studies to undertake,” he says, “but it could make a big difference.” **JCB**

Reference: Liu, P.T., et al. 2006. *Science*. doi:10.1126/science.1123933.



MODLIN/AAAS

Stepwise receptor activation

The FGF receptor gets its multiple phosphorylations not randomly but in a strictly ordered sequence, say Cristina Furdul, Erin Lew, Joseph Schlessinger, and Karen Anderson (Yale University, New Haven, CT). The intermediate phosphorylation states—neither fully off nor fully on—may act as members of a carefully controlled activation and recruitment pathway.

The Yale group relied on rapid reaction techniques, including time-resolved mass spectrometry, to catch events that others have missed. They saw sequential phosphorylations at five sites, with an invariant order of phosphorylation. For example, every triphosphorylated receptor had the same 3 sites phosphorylated.

"The fact that it's so precise, without any redundancy, suggests it's significant," says Schlessinger. The mechanism for ordering is not yet known, but one possibility is that phosphorylation at each site simply has different kinetics.

One consequence is a stepwise activation of catalysis rate. The first phosphorylation increased the receptor's catalysis rate 50–100-fold; the last increased it a further 10-fold.

In addition, each state "will have a lower or higher propensity to activate different pathways," says Anderson. She speculates that other tyrosine kinase receptors that look similar may turn out to have very different activation sequences. "That order could control the time when each downstream signaling module is activated," says Anderson. The different activation times may in turn change the way that the signaling pathways interact, thus producing a different final outcome. **JCB**

Reference: Furdul, C.M., et al. 2006. *Mol. Cell.* 21:711–717.



Five phosphorylations of the FGF receptor occur in a strict order.

Nuclear actin filaments

Frog oocyte nuclei are huge: 100,000 somatic nuclei could fit inside them. With size comes the threat of rupture. Markus Bohnsack, Dirk Görlich, and colleagues (Universität Heidelberg, Germany) find that these nuclei are protected from breakage by a network of intranuclear actin filaments.

The German team had previously defined exportin 6 (Exp6) as a nuclear export protein with only one known cargo: β -actin. "I asked my post-doc to do the most boring experiment—to test if exportin also exported α -actin and γ -actin," says Görlich. "The experiment was not just boring but very frustrating—he couldn't see any export of any substrate."

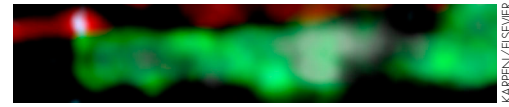
Export failed because Bohnsack was using frog oocytes, and this cell type turns off Exp6 production. Adding back Exp6 restored actin export but made the nuclei so fragile that they could rarely be isolated intact. "It was this series of frustrations that got us into something very interesting," says Görlich.

The fragility arose because oocytes with Exp6 became like all other known cell types: they no longer accumulated enough actin in their nuclei to make actin filaments. In untreated oocytes, however, painstaking fixation revealed heavily branched nuclear actin filaments.

One oocyte nucleus is packed with a huge store of proteins, including enough histones for the DNA of $\sim 15,000$ cells. The resulting gigantic size and fragility are counteracted by a filamentous network that restores sturdiness. Görlich now hopes to reconstitute the network formation in vitro, and to identify the proteins that help actin to form this structure. **JCB**

Reference: Bohnsack, M.T., et al. 2006. *Nat. Cell Biol.* 8:257–263.

Kinetochores to order



Overproduced CENP-A (green) induces an ectopic kinetochore (blue) with attached microtubule (red).

Extra functional kinetochores can be created by overexpressing just a single protein, according to Patrick Heun, Sylvia Erhardt, Gary Karpen (University of California, Berkeley, CA), and colleagues.

The protein, called CENP-A or, in flies, CID, makes sense as an initial foundation stone for kinetochores. It substitutes for histone H3 and thus, of all kinetochore proteins, gets closest to the DNA. Human tissue culture cells overexpressing CENP-A did not make extra kinetochores, possibly because of a shortage of other factors. But in fly cells the extra CENP-A incorporated into ectopic chromosomal sites and, at some of those sites, recruited inner and outer kinetochore proteins, and motor proteins. Microtubule connections at these sites appeared to be exerting force on the chromosomes and caused chromosome breaks and aneuploidy.

Additional factors may be needed to help define kinetochore identity, but "this tells us we are on the right track," says Karpen. He says evolution may have tolerated the occasional creation of new kinetochores because acentric products of chromosome rearrangements need a way to be rescued.

Karpen hopes to find sequences or histone modification patterns that favor either ectopic CENP-A incorporation or recruitment of other kinetochore proteins to these sites. The second challenge will be to find a loading factor specific for CENP-A, in the hope of localizing this factor and thus inducing kinetochore formation at a specific site. **JCB**

Reference: Heun, P., et al. 2006. *Dev. Cell.* 10:303–315.