ly discovered adhesin of *C. albicans* was reported earlier this year in *Science* by Sundstrom and colleagues (*8*). They demonstrated that Hwp1, a glycoprotein, is a substrate for mammalian transglutaminase and that this microbial adhesin covalently cross-links the pathogen to its host cell. *HWP1* was not discovered in a search for adhesin genes, but the function of its protein product in stable adherence became apparent through careful experiments and correlative observations. This has been the pattern for virulence gene discovery in fungal pathogens—candidate genes are identified on the basis of characteristics such as predicted biochemical properties, sequence similarities to known virulence determinants, transcriptional upregulation in models of infection, or functional complementation in heterologous systems (for example, genes that confer adhesive properties on *Saccharomyces cerevisiae*). Signature-tagged mutagenesis complements these approaches, allowing the identification of interesting genes according to the fate of randomly generated mutants in mixed infection experiments.

Mycologists should now be able to apply signature-tagged mutagenesis and related mutant screening strategies to the study of other fungal pathogens. Powerful molecular genetic systems are now in place for some of these fungi, and the most comprehensive sets of tools have been developed for *Candida* species, *Cryptococcus neoformans*, *Aspergillus fumigatus*, and *Histoplasma capsulatum*. Within the past year, new developments have included novel strategies for fungal gene disruption (*9–11*), transformation with freely replicating plasmid vectors (*12, 13*), the use of reporter genes for studying transcriptional regulation (*14, 15*), and insertion mutagenesis (*2, 16*). The medically important fungi, although not as well studied as many bacterial pathogens, offer a fascinating smorgasbord of model systems for research into adherence, mechanisms of intracellular survival, tissue invasiveness, evasion of host defenses, phenotypic switching and variation, chronic and latent infections, modulation between saprophytic and parasitic lifestyles, and transmission. Even though signature-tagged mutagenesis has previously been used only in bacterial systems, it was originally conceived by the Holden lab as an approach for their ongoing studies of virulence genes in *A. fumigatus*. The paper by Cormack and colleagues returns signature-tagged mutagenesis to its Kingdom of destiny, and not a moment too soon for the rapidly emerging field of molecular mycology.

References

1. B. P. Cormack, N. Ghori, S. Falkow, *Science* **285**, 578 (1999).
2. B. P. Cormack and S. Falkow, *Genetics* **151**, 979 (1999).
3. M. Hensel *et al.*, *Science* **269**, 400 (1995).
4. J.-M. Mei, F. Nourbakhsh, C. W. Ford, D. W. Holden, *Mol. Microbiol.* **26**, 399 (1997).
5. A. J. Darwin and V. L. Miller, *ibid.* **32**, 51 (1999).
6. S. Chiang and J. Mekalanos, *ibid.* **27**, 797 (1998).
7. A. Polissi *et al.*, *Infect. Immun.* **66**, 5620 (1998).
8. J. F. Staab, S. D. Bradway, P. L. Fidel, P. Sundstrom, *Science* **283**, 1535 (1999).
9. J. Morschhäuser, S. Michel, P. Staib, *Mol. Microbiol.* **32**, 547 (1999).
10. R. B. Wilson, D. Davis, A. P. Mitchell, *J. Bacteriol.* **181**, 1868 (1999).
11. T. T. Brandhorst, M. Wüthrich, T. Warner, B. Klein, *J. Exp. Med.* **189**, 1207 (1999).
12. J. P. Woods, E. L. Heinecke, W. E. Goldman, *Infect. Immun.* **66**, 1697 (1998).
13. A. Varma and K. J. Kwon-Chung, *Curr. Genet.* **34**, 60 (1998).
14. J. B. Patel, J. W. Batanghari, W. E. Goldman, *J. Bacteriol.* **180**, 1786 (1998).
15. M. Del Poeta *et al.*, *Infect. Immun.* **67**, 1812 (1999).
16. J. S. Brown, A. Aufauvre-Brown, D. W. Holden, *Mol. Gen. Genet.* **259**, 327 (1998).

PERSPECTIVES: DEVELOPMENTAL NEUROSCIENCE

Spontaneous Activity: Signal or Noise?

David A. McCormick

The brain is constantly active. From well before birth, till death's final hour, neurons in the central nervous system generate barrages of electrical discharges. Electrical activity that does not bear any obvious relationship to, for example, the task of processing sensory information or the generation of movement is commonly referred to as spontaneous. Although the appropriateness of this term is questionable, as it presupposes detailed understanding of the action potential discharges in a neuronal network, spontaneous electrical activity is clearly something that the brain does generate. For example, the first investigators to record from the cerebral cortex and thalamus of sleeping animals were surprised to find strong, rhythmic barrages of action potential activity. Indeed, the average rate of action potential generation during sleep could be significantly higher than that in waking animals. We now know that much of this activity is truly spontaneous in that it can be detected in brain slice preparations in vitro, despite the prior cessation of all activity and the lack of a clearly defined stimulus from the environment (*1*).

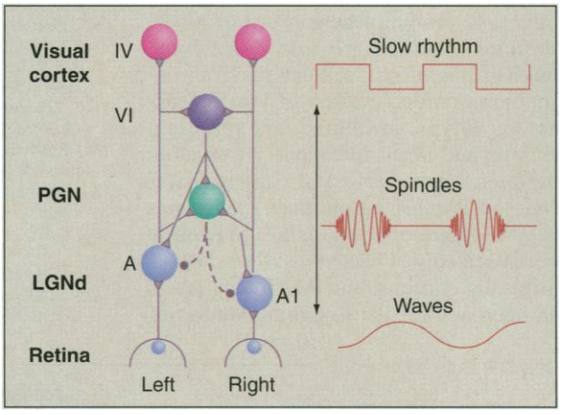
What is the function of this spontaneous activity? Is it an epiphenomenon of the neuronal circuitry that is irrelevant to the true task of the neuronal pathway, or does it have some other significance? In a report on page 599 of this issue, Weliky and Katz set out to answer this question by investigating functional neuronal connections in the visual system of awake baby ferrets before their eyes are open, when external sensory stimuli cannot be perceived (*2*).

Many processes in neurons, and even some aspects of the formation and refinement of neuronal circuits during development, are dependent on, or influenced by, action potential activity (*3*). During development, the basic connections that define the complex of neuronal circuits making up the nervous system are determined largely through genetic preprogramming that depends on a wide variety of molecular guidance cues (*4*). Refinement of these neuronal connections during development is highly sensitive to experience during a window of time referred to as the critical period (*5*). However, in many animals, especially primates, significant development of neuronal connections occurs before precise sensory experience (*6*). This is especially true of the visual system because no patterned information can reach retinal photoreceptors in utero. Nevertheless, many of the basic connections, for example, between the ocular dominance layers in the lateral geniculate nucleus (LGN) of the thalamus, and receptive field properties of neurons, such as orientation tuning in the primary visual cortex, are determined during fetal development. Although it is well known that competitive interactions between inputs before birth are involved in sharpening the terminal fields of axons (*7*), the underlying mechanisms are unclear. They may in part depend on the presence of action potentials, even in the absence of visual experience (*3*). For example, blocking retinal activity in one eye of the cat with an intraocular injection of the Na^+ channel poison tetrodotoxin in utero results in a competitive disadvantage in the formation of connections by that eye in the LGN; the competing eye then innervates more than its fair share of the LGN (*3*). If these connections form before patterned sensory

The author is in the Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06510, USA. E-mail: david.mccormick@yale.edu

experience, then where does the activity come from that drives this competition? It has been proposed that spontaneous activity may replace sensory-evoked activity in this process during the early stages of development (*3*). One could expand this hypothesis even further and ask: Does spontaneous activity in the nervous system during sleep or development modify network properties, such as synaptic connectivity, in preparation for the operation of the awake and mature brain?

Spontaneous activity in the nervous system often takes the form of rhythms, especially during periods of sleep (see the figure). Some of the cellular mechanisms underlying the generation of these rhythms are understood, and it is now clear that each region of the nervous system can generate its own cyclical patterns that interact with those of the other regions to which it is interconnected (*8*). For example, during slow wave sleep, the thalamus generates a waxing and waning 7 to 14 Hz synchronized rhythm (spindle waves), whereas the cerebral cortex generates periodic barrages of activity and inactivity that alternate about once every 1 to 5 seconds (the slow rhythm) (*8*). In the intact animal, these two rhythms interact to form widely synchronized barrages of activity called "K-complexes" (*8*). Lesion studies reveal that although connections within the thalamus can synchronize this activity locally (*1*), larger scale synchronization is achieved through connections between the thalamus and cortex and within the cerebral cortex itself (*9*). The spontaneous activity of the retina, present even in the dark, interacts with these rhythms in a complex manner. Importantly, this slow rhythmic activity is abolished upon arousal and attentiveness, and therefore plays little if any immediate role in the processing of information during the waking state.



Spontaneous activity in the visual system. During development of the visual system, the retina, dorsal lateral geniculate nucleus (LGNd), and visual cortex all generate spontaneous rhythms (waves, spindles, and slow oscillations, respectively). These interact and are synchronized through neuronal connections between the LGNd, the associated collection of inhibitory neurons in the perigeniculate nucleus (PGN), and the cerebral cortex. Spontaneous activities such as these are proposed to influence the precise pattern of connections between neurons that is determined during development.

Could this spontaneous activity supply a signal that contains sufficient information to guide or refine the formation of connections during development? The report by Weliky and Katz (*2*) suggests that it might. In their study, the authors demonstrate that the pattern of activity generated in the LGN of ferrets before opening of the animal's eyelids is generated in response to barrages of activity from the retina that interact with the intrinsic properties of the LGN, and are synchronized by the massive connections between the LGN and the visual cortex. The properties of this activity possess at least some of the prerequisites necessary for guiding activity-dependent development of retinal-thalamic, thalamocortical, and intracortical connections. For example, the higher correlations between the activities of cells with similar receptive field properties could facilitate the formation of ON and OFF subregions (important for many features of visual processing including the detection of edges) of neuronal receptive fields in the cerebral cortex. The interaction of neuronal inputs from the two eyes is crucial to the formation of binocular receptive fields that are aligned in space, and this is essential for accurate depth perception (*2*, *10*).

How could spontaneous activity regulate formation of detailed point-to-point neuronal connectivity and define the characteristics of neurons and synapses? Numerous cellular processes, including the strength and location of synapses, the localization of receptors for neurotransmitters, the expression of genes, and the electrophysiological properties of the neuron itself, may be influenced by neuronal activity, particularly when this activity results in an increase in the intracellular concentration of Ca^{2+} (*11*, *12*). Rhythmic patterns of activity, such as those during sleep or development, may be particularly pertinent because they are associated with rhythmic increases in intracellular Ca^{2+}. The amplitude and frequency of intracellular Ca^{2+} oscillations may regulate which genes are expressed, the state of activation of biochemical pathways, as well as other neuronal functions during development such as neurite extension at growth cones and neuronal migration (*12*). Given these diverse responses to Ca^{2+} fluxes, it seems likely that rhythmic spontaneous activity may control the activation of genes that are required for the cellular processes associated with either sleep or development. Indeed, the induction of the calcium/cyclic AMP response element (CRE), whose activation regulates gene transcription, is associated with plasticity in visual cortical pathways (*13*).

The leading hypothesis of how spontaneous activity may be useful to the nervous system holds that the correlational structure of synchronized oscillations provides a signal that adjusts the strength of existing synapses, especially during development, and perhaps in the adult as well (*14*). For example, prolonged periods of spontaneous activity could be used either to "read out" memories formed during the day from one structure to another (*15*) or, through some type of sliding threshold mechanism (*14*), to determine which synaptic modifications formed during the day are to be kept, and which are to be reversed, returning the synaptic strength to some basal level.

Before becoming overenthusiastic about hypotheses such as these, it must be remembered that only certain aspects of network development and function are influenced by activity-dependent plasticity. There are susceptible periods for various aspects of vision in all stages of development, from prenatal to puberty (*16*). Some functional subsystems, such as the magnocellular and parvocellular (M and P) synaptic streams (which carry different types of visual information), may develop independently of action potential activity, whereas others, such as ocular dominance, may be susceptible to changes in the level and pattern of activity (*17*). Furthermore, in the adult the plasticity of neuronal networks is highly state dependent. Dreams, for example, are notoriously difficult to recall unless the person having them is awakened during or shortly after their occurrence. It is possible, and perhaps even likely, that synaptic plasticity during sleep is relatively low, perhaps owing to a low level of release of modulatory neurotransmitters or the dynamic state of the neuronal network. Whether there are cycles of susceptibility to synaptic plasticity in the developing human brain is unclear. Interestingly, newborns spend an inordinate amount of time in a rapid eye movement-like sleep state that is typified by activity closer to that of the awake brain than of the brain in slow wave sleep.

Considerable work remains to be done before we will truly understand the role of spontaneous activity in the nervous system and whether it is instructive or permissive in the development of neuronal connections and the maintenance or modulation of these connections in the adult. The analysis of knockout mice that lack particular patterns of activity owing to the loss or disruption of a particular ion channel, receptor, or protein involved in synaptic transmission seems to be a particularly promising avenue for future research (*13*, *18*).

References

1. M. von Krosigk, T. Bal, D. A. McCormick, *Science* **261**, 361 (1993); D. A. McCormick and T. Bal, *Annu. Rev. Neurosci.* **20**, 185 (1997); D. A. McCormick, F. Trent, A. S. Ramoa, *J. Neurosci.* **15**, 5739 (1995).
2. M. Weliky and L. C. Katz, *Science* **285**, 599 (1999).
3. L. C. Katz and C. J. Shatz, *ibid.* **274**, 1133 (1996); C. J. Shatz and M. P. Stryker, *ibid.* **242**, 87 (1988); D. W. Stretavan, M. P. Stryker, C. J. Shatz, *Nature* **336**, 468 (1988); A. A. Penn, P. A. Riquelme, M. B. Feller, C. J. Shatz, *Science* **279**, 2108 (1998); S. M. Catalano and C. J. Shatz, *ibid.* **281**, 559 (1998).
4. M. Tessier-Lavigne and G. S. Goodman, *Science* **274**, 1123 (1996).
5. T. N. Wiesel and D. H. Hubel, *J. Neurophysiol.* **26**, 978 (1963); *ibid.*, p.1003; D. H. Hubel and T. N. Wiesel, *J. Physiol.* **206**, 419 (1970).
6. P. Rakic, *Nature* **261**, 467 (1976); *Philos. Trans. R. Soc. London Ser. B* **278**, 245 (1977); M. C. Crair, D. C. Gillespie, M. P. Stryker *Science* **279**, 566 (1998).
7. P. Rakic, *Science* **214**, 928 (1981).
8. M. Steriade, D. A. McCormick, T. J. Sejnowski, *ibid.* **262**, 679 (1993); M. Steriade and F. Amzica, *J. Sleep Res.* **7** (suppl. 1), 30 (1998).
9. D. Contreras, A. Destexhe, T. J. Sejnowski, M. Steriade, *Science* **274**, 771 (1996); A. M. Sillito, H. E. Jones, G. L. Gerstein, D. C. West, *Nature* **369**, 479 (1994).
10. E. Erwin and K. D. Miller, *J. Neurosci.* **18**, 9870 (1998).
11. N. S. Desai, L. C. Rutherford, G. S. Turrigiano, *Nature Neurosci.* **2**, 515 (1999); A. M. Craig, *Neuron* **21**, 459 (1998); M. J. Berridge, *ibid.*, p. 13; R. D. Fields, F. Eshete, B. Stevens, K. Itoh, *J. Neurosci.* **17**, 7252 (1997); D. V. Lissin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 7097 (1998).
12. R. E. Dolmetsch, R. S. Lewis, C. C. Goodnow, J. I. Healy, *Nature* **386**, 855 (1997); R. E. Dolmetsch, K. Xu, R. S. Lewis, *ibid.* **392**, 933 (1998); H. Bading, D. D. Ginty, M. E. Greenberg, *Science* **260**, 181 (1993); W-H. Li, J. Llopis, M. Whitney, G. Zlorkarnic, R. Y. Tsien, *Nature* **392**, 936 (1998); X. Gu and N. C. Spitzer, *ibid.* **375**, 784 (1995); T. M. Gomez, and N. C. Spitzer, *ibid.* **397**, 350 (1999); H. Komoro and P. Rakic, *J. Neurobiol.* **37**, 110 (1998); P. De Koninck and H. Schulman, *Science* **279**, 227 (1998).
13. T. A. Pham, S. Impey, D. R. Storm, M. P. Stryker, *Neuron* **22**, 63 (1999).
14. M. F. Bear, *ibid.* **15**, 1 (1995); C. D. Rittenhouse, H. Z. Shouval, M. A. Paradiso, M. F. Bear, *Nature* **397**, 347 (1999).
15. W. E. Skaggs and B. L. McNaughton, *Science* **271**, 1870 (1996); H. S. Kudrmont, C. A. Barnes, B. L. McNaughton, *J. Neurosci.* **19**, 4090 (1999).
16. N. Daw, *Visual Development* (Plenum, New York, 1995).
17. C. Meissirel, K. C. Wikler, L. M. Chalupa, P. Rakic, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 5900 (1997); C. J. Snider, C. Dehay, M. Berland, H. Kennedy, L. M. Chalupa, *J. Neurosci.* **19**, 220 (1999).
18. M. M. Huntsman, D. M. Porcello, G. E. Homanics, T. M. DeLorey, J. R. Huguenard, *Science* **283**, 541 (1999); T. K. Hensch *et al.*, *ibid.* **282**, 1504 (1998).

PERSPECTIVES: NATURAL GAS DEPOSITS

Methane in the Deep Blue Sea

Bilal U. Haq

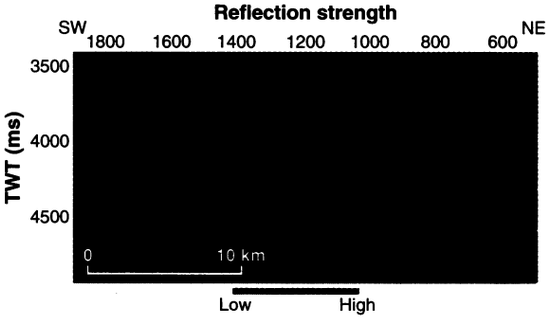
Natural gas hydrates have been known to exist in marine sediments since the 1970s, but the pace of research into their nature has only recently picked up. Research efforts have been focused on investigating their efficacy as an alternative energy resource and on their potentially important roles in global climate change and the stability of the continental slope. Gas hydrates are considered to represent an immense, although as yet largely uncharted, source of fuel for future consumption (*1*). Indeed, hearings have been held in the U.S. House and Senate on a revived bill to inject as much as $42.5 million into hydrate research over the next 5 years.

Gas hydrates consist largely of a mixture of methane and water frozen into a solid crystalline state. At moderately high pressure and low temperature, the methane molecule is captured inside a cage of water molecules and chilled into a solid hydrate, while expelling salt. Methane hydrates exist at water depths greater than ~500 meters in the pore spaces of marine sediments on the continental slope and rise and on the sea floor where gas escapes from fault conduits (especially in the Gulf of Mexico). Because of the very low temperatures in the Arctic, hydrates also occur on land in association with permafrost. Hydrate methane is largely of biogenic origin, derived from the decay of organic material trapped in the sediments. Initial results of a field investigation (*2*) show that methanogenic microorganisms can produce methane at substantial depths in the sediment, at pressures of about 400 atmospheres.

In marine sediments, hydrates are commonly characterized by the presence of acoustic reflectors known as bottom simulating reflectors (BSRs) that are caused by the acoustic velocity contrast between a solid hydrate layer and the free gas below. BSRs mimic the sea floor (see the figure). They have been observed on many continental margins of the world (*3*), but hydrates have only rarely been sampled through drilling or brought to the laboratory in their natural state. Because of this paucity of direct sampling and analysis, estimates of the volumes of methane trapped in hydrates, or the associated free gas beneath the hydrate stability zone, remain speculative (*1*). However, even relatively conservative estimates indicate that on the order of 10,000 gigatons of carbon, or double the amount of all known fossil fuel sources, may be stored in gas hydrates (*3*).

Thus far, the petroleum industry has not been interested in methane hydrates as a resource, because they may not be easy to recover or cost-effective to exploit, particularly if most marine hydrate is thinly dispersed in the sediment. Drilling on the Blake Ridge off the East Coast of the United States (*4*) indicates that they are very rarely locally concentrated in the otherwise widespread field of thinly dispersed hydrate.

Gas hydrates have been implicated in massive slumps and slides on the continental slopes, when hydrates break down as a result of structural changes, increased bottom temperature, or reduced hydrostatic pressure (see the figure). Ongoing experiments show that methane hydrate has a markedly higher mechanical strength than water ice (it is 10 times stronger than ice at 260 K) (*5*). The sediment thus gains considerable strength when the hydrate forms but then loses it near the base of the hydrate stability zone during dissociation. When a hydrate dissociates, it changes from a solid to a mixture of sediment, water, and gas.



When a gas hydrate collapses. Seismic profile of a collapse structure on the crest of the Blake Ridge, off the coast of the Carolinas. The vertical scale shows two-way travel time (TWT) in milliseconds, or depth. The prominent BSR (just below 4000 milliseconds) is intact on the left but is disrupted below the collapse structure. The collapse is confined to the hydrate depths and was most likely caused by overpressure below the hydrate stability zone. It may have been accompanied by substantial injection of methane into the seawater and atmosphere.

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The author is in the Division of Ocean Sciences, National Science Foundation, Arlington, VA 22230, USA, E-mail: bhaq@nst.gov