could vary with the time of day — is fundamentally important. Most organisms, including humans, show daily rhythms in their physiology and behaviour. Although most of these rhythms are seen at the level of the output of a physiological system, the new study shows that even the most basic property of cell–cell communication in the nervous system (that is, whether the release of transmitter excites or inhibits the postsynaptic cell) can also vary with the circadian cycle. Add to this observations that the properties of a cellular membrane, its second-messenger systems and its transcriptional/translational machinery can all be rhythmically regulated[4,5], and the emerging view is that even the most basic cellular and molecular properties of a cell can be subject to circadian regulation.

What does the work by Wagner *et al.*[1] mean for our understanding of the circadian rhythms that are generated by cells in the SCN? Most of these neurons send out processes containing GABA, which form synapses with other cells in the SCN. We now have to consider the possibility that these cells switch from being excitatory to inhibitory interneurons, depending on the time of day. So is this switch responsible for generating the daily rhythm in spontaneous neural activity that is characteristic of SCN cells? Probably not, as there is evidence to suggest that this rhythm is generated by intracellular processes that do not depend on synaptic communication[6]. But the switch in sign may boost the amplitude of the neural rhythm, and be involved in driving the outputs from the SCN.

Agonists towards GABA can have an impact on the circadian system[7], so there is every reason to think that understanding the physiological role of GABA-mediated synaptic transmission in the SCN will help to explain circadian phenomena in mammals. The findings of Wagner *et al.* also force us to re-evaluate our thinking about GABA as a signalling molecule — they raise the possibility that under certain physiological conditions, or at certain times, GABA can act as an excitatory transmitter. Certainly, for SCN cells, there are times to be excited by GABA. □

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Biosensors

Switching channels makes sense

Anthony P. F. Turner

The Atlas moth can follow a thinly laid trail of single molecules. Can we mimic it? Building a device to do that, and so pass the sternest test of sensitivity, is one of the ultimate goals of the biosensor technologist. Pursuit of this prize is likely to lead to considerable commercial benefit from the production of highly sensitive diagnostics, and exciting possibilities for computers that use biochemicals in place of conventional solid-state electronics. On page 580 of this issue[1] Cornell *et al.* describe an important step forward — a stable lipid membrane that can act as an ion gate, opened or closed by the binding of single molecules.

Over the past decade, biosensors (sensors incorporating a biological or biologically derived sensing element[2]) have achieved considerable commercial success, with current worldwide sales of over half a billion US dollars a year. The field has been dominated by enzyme-based biosensors, and in particular by disposable enzyme electrodes for home blood-glucose measurement[3]. Immunodiagnostics and other affinity systems have so far been relatively unaffected by this emerging technology, with consumer products focusing on immunochromatographic approaches such as the new test strips for pregnancy and fertility.

Biosensor technology has had a smaller but significant impact on affinity assays performed in the laboratory, especially in the pharmaceutical industry. Several companies sell real-time bioaffinity assays based on measuring the change in refractive index resulting from antibody–antigen interactions at a sensing surface. The best known of these exploit surface plasmon resonance (oscillations of electrons in a thin metal layer) at an optical interface to measure affinity reactions occurring in a thin polymer matrix coating[4]. Many companies are working to miniaturize this and related technologies, in an attempt to produce a hand-held immunosensor that might be similar in both cost and size to the pocket-sized glucose biosensors already on the market. Such instruments could be used for home testing or for non-medical applications such as environmental or food monitoring. Target analytes include toxins, allergens, disease markers, hormones, bacteria, viruses, antibodies, DNA, drugs and pesticides.

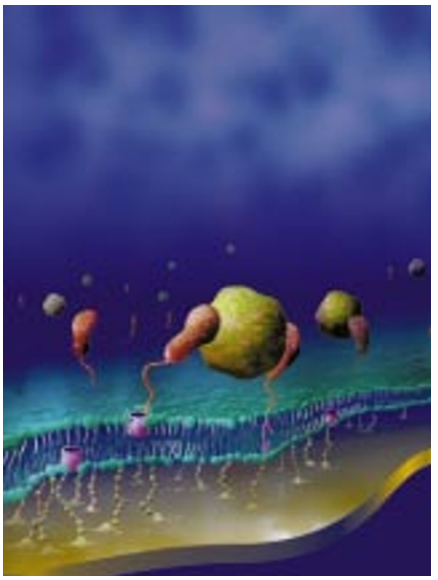
The idea of mimicking nature by harnessing an affinity reaction to open a gate or switch in a membrane is not new. Various ion-channel sensors were described in the late 1980s, and by 1989 there remained three

100 YEARS AGO

In connection with the recent correspondence in these columns on luminous phenomena observed on mountains, it is interesting to direct attention to a very remarkable series of observations of electrical storms on Pike's Peak, Colorado, contained in vol. xxii. of the Annals of the Astronomical Observatory of Harvard College, and described in NATURE... . Luminous jets appeared very often along the telegraph wires for the length of an eighth of a mile, and the anemometer cups looked like revolving balls of fire. Upon touching the anemometer under these conditions, an observer found "his hands instantly become aflame. On raising them and spreading his fingers, each of them became tipped with one or more cones of light nearly three inches in length."
From *Nature* 3 June 1897.

50 YEARS AGO

Prof. J. Kaplan proposes the name 'active oxygen' for certain luminous phenomena observed by him "just as the name active nitrogen was given to similar phenomena in nitrogen". I write to point out that this is not historically correct. ... The chemical activity referred to was the combination with metals such as sodium and mercury, to form nitrides, and with organic materials to form cyanogen compounds. Striking luminous phenomena often accompany these chemical actions, but it is wrong to regard these effects as the essence of the matter. As a matter of fact, the number of nitrogen molecules which emit a photon of afterglow light is very small compared with the number which become chemically active. For this reason, I do not think that the afterglow should be regarded as the essential phenomenon of active nitrogen, and I do not think that in the present state of knowledge the term 'active oxygen' should be applied by analogy, when only luminous phenomena are so far known to be involved. This would only cause confusion.
From *Nature* 7 June 1947.



R. PACE/CRC/P. CAMPBELL/INFOGRAPHIC DESIGN

Figure 1 **An artist's impression of the new ion-channel biosensor in operation. A molecule of the desired analyte (yellow) is bound to two tethered antibodies. One of them is fixed, the other tethered to a mobile gramicidin molecule (pink cylinder), which has thus been pulled away from its fixed lower partner, severing the ion channel and interrupting current to the gold electrode below. A single (and highly specific) molecular interaction results in a detectable change in electrical conductance.**

technical problems[5] in achieving a practical device: the fabrication of stable lipid membranes; the incorporation of a receptor into these structures; and the marriage of the modified membrane to a transducer, to convert the chemical signal into an electrical one. Despite excellent work since then[6,7], these problems have remained largely unresolved.

But Cornell *et al.*[1] have gone some way towards making a practical device (Fig. 1). To improve stability, they include membrane-spanning lipids (as in thermophilic bacteria), and the membrane is tethered to an underlying gold electrode. Each ion channel is formed of two linked gramicidin molecules; a fixed one, tethered to the electrode below, and a mobile one in the upper layer of the membrane, linked to an antibody fragment specific to the chosen analyte. The availability of mobile gramicidin to form channels is modulated by a second tethered antibody fragment (see Fig. 1). Polar molecules between the membrane and the electrode leave a space that acts as a reservoir into which cations can flow when the channel is opened, and the electrode acts as the transducer.

An advantage of such gated devices is the very high flux (up to a million ions per second) that occurs as the result of a single molecular interaction. This type of amplification lies at the heart of biological systems, and has yielded artificial sensors capable of detecting sub-picomolar concentrations within ten minutes.

It will be possible to incorporate ion-channel sensors in miniature sensor arrays, designed to respond to a wide range of analytes. Indeed, as the membrane area decreases, the membrane leakage conductance decreases, whereas the conductance per channel remains constant. So with small electrodes (<30 μm across) it should be possible to resolve individual channels. In principle, a wide range of receptor molecules could be incorporated into such a system. Antibody libraries or combinations of synthetic receptors could provide a diverse range of potential binding partners, which could be selected empirically by exposure to the target analyte. A certain amount of redundancy is usual in living systems, and high-density arrays could exploit duplication and accommodate crossreactivity by using neural-network approaches to produce the bioelectronic nose or tongue. Such arrays should be able to cope with the most stringent of demands, including chiral discrimination — the subject of a paper by Bodenhöfer *et al.* on page 577 of this issue[8], in which right- and left-handed enantiomers of a chiral receptor are coated onto sensor arrays that respond to the mass or optical thickness of the surface layer.

On a more speculative note, the creation of a stable bioamplifier has implications beyond the field of analytical chemistry. Single-molecule modulation of a gated event is a cornerstone of biomolecular electronics, and so this new device brings us a small step closer to a new generation of organic machines. The immediate commercial impact of the work by Cornell *et al.*[1], however, is likely to be restricted to a new range of simple immunodiagnostics. If their results translate to superior practical devices, that alone could have an enormous effect on users and providers of affinity assays for medicine, the environment, food, process monitoring, security and defence. And one can envisage numerous applications as a research tool, ranging from screening of pharmaceuticals to *in vitro* studies of cellular activation. □

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Microbial genetics

Hypermutation under stress

Bryn A. Bridges

When a population of microorganisms is unable to grow because nutrients are exhausted or cannot be used, it could make good sense for individuals in that population to experiment with their genomes to try to overcome the deficiency. One approach would be for a subpopulation to start mutating vigorously at random (hypermutation), in the hope that a mutation might arise that would enable growth to begin again. An alternative strategy would be to mutate only those genes that might possibly be effective if mutated (directed mutation), an idea that was raised by John Cairns and his colleagues in much-cited work published in 1988[1]. Two new papers, one from Pat Foster[2] and the other from Susan Rosenberg and her group[3], turn the spotlight firmly on the first of these strategies.

Whereas some evolutionary biologists found the idea of directed mutation disturbing because of its echoes of lamarckism, molecular biologists displayed their customary ingenuity in proposing ways in which it might be achieved without threatening conventional dogma (for review see ref. 4). It is now clear that mutations may arise at an unexpectedly high rate during nutritional stress, and the mechanisms concerned are equally interesting whether they involve hypermutation or directed mutation (or even both). Although there have been many attempts to prove or disprove the hypothesis of directed mutation, none has been totally convincing.

The most studied system is the FC40 strain of the bacterium *Escherichia coli,* in which the chromosomal *lac* operon is deleted and in which there is an F′ episome carrying a *lacI* gene with a +1 frameshift mutation[5]. Being *lac*$^-$, the bacteria cannot use lactose. But on agar plates containing lactose as the sole carbon source, mutant *lac*$^+$ colonies arise at a high rate provided the bacteria are recombination-proficient. The F′ episome is a sexual (conjugational) plasmid with some homology to the bacterial chromosome, and conjugational potency is necessary for the high mutation rate.

A few years ago, Foster[6] found that although *lac*$^+$ mutations accumulated during selection for lactose utilization, unselected mutations at the chromosomal *rpoB* locus did not accumulate in the *lac*$^-$ population. Now, however, the same author[2] has shown that unselected tetracycline-resistance mutations arise at roughly the same