glass microelectrodes, although their earlier work had used extracellular electrodes, based on field-effect transistors to amplify the weak electrical signals. Extracellular electrodes have an advantage over the intracellular kind because trapping neurons with fixed electrodes, or piercing them with a microelectrode, can kill them. In fact, my own group has recently invented a manipulable extracellular electrode array[5] that can be rapidly moved from site to site. The electrode patterns can be matched to that of the network under test, although the array's extracellular position does reduce the magnitude of the signal that can be extracted.

The growth technique of Merz and Fromherz, when combined with advances in electrode design, should open the way to investigating how connectivity and patterns of connection modify neural processing. In other words, it should be possible to test the consequences of specific changes in connectivity or in the electrically injected message on the output. Furthermore, as Merz and Fromherz[1] point out, their system should allow the role of subthreshold signals to be assessed — these are events that do not lead directly to transmission of a neural message, but which may influence the electrical potentials of nerve cells.

With the tools in place, the outstanding question is how a neuronal network could be designed. There are two sensible approaches: first, to try to mimic what is already found in nature; and second, to formally develop logically designed networks. Judith Wilkinson (quoted in ref. 6) has applied graph and tiling theory to design networks that should be able to propagate and compare messages (Fig. 1b). These are probably the only *in vitro* systems that feature branching networks that are just like those in real nervous systems. Such logically designed networks are just now being realized.

Despite the progress reported by workers such as Merz and Fromherz, we should still proceed cautiously — for instance, we do not even know whether there are (or aren't) several types of neural coding, or language, in a single animal. But, as in other code-breaking efforts, there will probably be a long period of data collection — revealing more and more of the neurobiological Rosetta Stone — until we have enough information to start interpreting the neural code. ■

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Immunology

The Wright stuff

Walter Gratzer

White cells in the bloodstream seek out and destroy invading bacteria. The explanation for the actual killing mechanism turns out to be wonderfully more subtle than previously thought.

On page 291 of this issue, Segal and colleagues (Reeves *et al.*)[1] cast new and unexpected light on one of the cornerstones of the immune system. Their observations overturn the established wisdom that has guided the study of phagocytosis and pose questions about the role of reactive oxygen species in destroying invading pathogens.

It is 120 years since Elie Metchnikoff discovered phagocytes — cells that engulf and devour bacteria and other invaders of the bloodstream. This 'innate immunological response' is the body's first line of defence against such violations, and it depends predominantly on the most abundant class of phagocytic cells, the most numerous white cells in the blood, the neutrophils. Sufferers from the rare, hereditary chronic granulomatous disease (CGD), whose neutrophils are defective, die from bacterial and fungal infections if they are not treated. (Metchnikoff later came to believe that, with the passage of the years, phagocytic cells become incontinent, rampage through our tissues, gnaw at our vitals and cause the catastrophe that we recognize as ageing.)

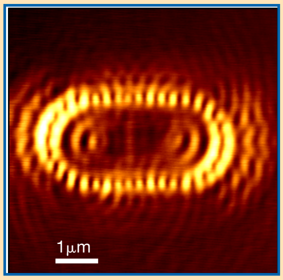
Neutrophils surge towards the site of an infection and engulf bacteria by a well-characterized process (opsonization) that requires blood serum proteins. They then destroy the involuntary guest trapped within a phagocytic vacuole. The cytoplasm of the neutrophil is rich in granules (whence its original description as a granulocyte), which, on activation, discharge their contents inside the phagocytic vacuole.

The picture of the killing process that has dominated thinking over the past 40 years is that the lethal agents are the highly reactive

Quantum optics

Light corralled

Traditional optical experiments, such as splitting rays of light into various colours with a prism, have had the attraction of being visible to the naked eye. Modern methods of confining light within microscopic structures, and tailoring its interaction with matter on atomic scales, are taking optics into the quantum realm. Making the results visible is not straightforward. But a beautiful example comes from C. Chicanne *et al.* (*Physical Review Letters* **88,** 097402; 2002), who have designed a way of taking snapshots of intricate light interference patterns in tiny photonic structures. One of their snapshots is shown here.



The images are reminiscent of the 'quantum corrals' for electrons, first created by Donald Eigler and colleagues in 1993, and produced with the scanning tunnelling microscope (STM). This instrument probes surfaces with a sharp needle, which can also be used to move loose atoms around on a metallic surface and position them into a closed loop. The electrons confined within these corrals interfere with one another and produce beautiful patterns, which the STM images make manifest. These experiments have been highly instructive for illustrating the quantum-mechanical principle that electrons can behave as waves.

Chicanne *et al.* now present an optical analogue of the quantum corral. Light has wave properties, of course, but interpreting the interference effects is not straightforward within a radius around a light source that is comparable to the wavelength of the light itself. To study this zone, Chicanne *et al.* made use of a relative of the STM, the scanning near-field optical microscope. Here, the surface probe is an optical fibre tapered to a sharp end that also illuminates the sample.

Much as in the experiments by Eigler and colleagues, Chicanne *et al.* first created corrals by carefully positioning particles of gold in a loop, in this example in the shape of a stadium. The optical corral is then imaged by scanning the fibre over the surface while collecting light transmitted though the transparent sample. The result is images of light interference patterns in a microscopic structure. The principles involved will help direct future observations, and tailored distribution, of light at atomic dimensions.

Liesbeth Venema

superoxide ions and hydrogen peroxide, generated by enzymes in the vacuole[2]. Not only were these held to act directly on the microbe, but the hydrogen peroxide also served as substrate for destructive halogenation reactions, catalysed by another enzyme, myeloperoxidase. This scheme received what appeared to be conclusive support from the discovery of CGD patients, whose neutrophils engulfed bacteria but failed to unloose the toxic agents. Much later, moreover, it was reported that genetically modified mice lacking myeloperoxidase are highly sensitive to infection by the common fungal pathogen *Candida albicans*. But it has also long been known that the neutrophil granules contain proteases — protein-degrading enzymes — and two studies revealed that mice deficient in two of these enzymes, elastase and cathepsin G, fared poorly when challenged with a fungal[3] or bacterial[4] infection.

Here, then, was the starting point for the re-examination of the problem by Reeves *et al.*[1]. First they established that mice deficient in both enzymes are indeed unable to combat infections by two of the most prevalent pathogens, *C. albicans* and *Staphylococcus aureus* (one of which, they further found, is attacked only by elastase, the other only by cathepsin G). Yet the neutrophils in these animals are otherwise fully functional and display the swift appearance of the reactive oxygen species on ingesting the bacteria. The effect was simulated *in vitro* when protease inhibitors added to normal neutrophils were seen to prevent killing of trapped bacteria.

To find explanations for these effects, Reeves *et al.* undertook a minute examination of the events that unfold in the phagocytic vacuole. In the first place, the release of reactive oxygen species — the 'respiratory burst' — is accompanied by a large disturbance in the internal pH, which rises from 6 to 8. This transcends the release of the predominantly acidic granule contents, for protons are consumed in neutralizing the huge concentration of basic superoxide ions and radicals. In addition, much of the anionic charge is offset by a massive influx of potassium ions through the vacuolar membrane. This, and especially the accumulation of osmotically potent degradation products from the disintegrating microbe, renders the vesicle grossly hypertonic, so that the bacterium within shrinks to half its original volume. If degradation is impeded by the addition of protease inhibitors, swelling is suppressed. After a while, this process supervenes as water slowly enters to counter the osmotic gradient.

What, then, regulates the rate and extent of the water uptake? It seems that the water permeability of the vacuolar membrane is much like that of other membranes, and Reeves *et al.* therefore inferred that expansion of the vacuole might be restricted by a dense network of cytoskeletal proteins under the membrane, which disperses as the pathogen is digested. Indeed, the recovery of the vacuolar volume could be inhibited by the addition of jasplakinolide, a toxin that stabilizes the actin filaments on which the cohesion of the network depends.

But how do the respiratory burst and the ionic surges in the vacuole induce the process of killing, which superoxide and hydrogen peroxide are by themselves incapable of accomplishing? The key lies in the highly charged matrix within the granules to which, it turns out, the proteases are normally adsorbed. In this state they are inactive and so do not create mayhem in the resting cell. But when the ionic strength inside the vacuole rises, the enzymes are liberated: unleashed, the enzymes are active and attack the microbe, and at the elevated pH their activity is maximal. The membrane-associated network must, of course, prevent the vacuole from swelling by an attendant reversal of the increase in ionic strength, and it is striking that certain microbes (mycobacteria), which evade the innate immune response, can disrupt actin filaments in another cell type[5]. Reeves *et al.* concede that the neutrophil myeloperoxidase does play a significant part in killing. They conjecture that it may protect the proteases themselves from oxidative damage, to which cathepsin G appears to be especially liable. The apparently unwonted complexity of the entire system may have evolved to protect the cell from its own toxins.

The results of this study[1] have implications beyond immunology, for the supposed action of reactive oxygen species on microbes has been taken as a model for their destructive effect on animal tissues. But in particular, it clarifies the action of the innate immune system, the primacy of which was recognized well before the discovery of antibodies and acquired immunity.

That may please the turbulent shade of the despotic Professor Colonel Sir Almroth Wright, founder of the Inoculation Department at St Mary's Hospital Medical School in London. For Wright believed that immunology was the key to the treatment of nearly all important diseases. George Bernard Shaw, in his play *The Doctor's Dilemma*, put into the mouth of Sir Colenso Ridgeon, alias Wright, the insistent injunction to "stimulate the phagocytes". ■

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Daedalus

Fast forgetting

Botulinum toxin, the deadly nerve poison that inhibits the neurotransmitter acetylcholine, has many cosmetic uses these days. In extremely small quantities, it cancels facial tics, erases forehead worry-lines, and so on. The effect lasts for weeks or months, until the toxin decays; repeated treatments can be permanent. Daedalus sees psychiatric implications. The brain is the repository of many inappropriate or downright harmful reflexes, memories and sensory illusions. They could well be eliminated, if only we knew where they were stored.

Acupuncture and reflexology both claim that the whole body is mapped onto the skin surface. They work, if they do, by 'gating' nerves from the skin, launching signals that interfere with or override messages from the organs being treated. Nerves are tubes. Once inside a nerve, a virus or small particle is safe from immunological surveillance. Rabies, inflicted by the bite of a rabid animal, injects a virus at the bite site that slowly ascends the local nerve and then multiplies in the brain. So Daedalus will inject botulinum toxin into an acupunctural or reflexological skin location, chosen under psychiatric examination, and hope that it travels to the site of the psychiatric trouble. Unlike a virus, the toxin will not multiply and spread. Thus, to eliminate a troublesome memory the patient will recall it in detail, and the therapist will find which area of skin has been sensitized. Indeed, even if the patient cannot recall the memory (it may have been repressed), a sensitized area of skin should act as an indicator.

The toxin will be injected into the nerve as microencapsulated particles. By the time the encapsulation decays, the toxin should be on site. With luck the particles will slowly ascend that nerve alone, and travel exactly to that part of the brain concerned with the chosen memory. The process may take some time; but many psychiatric drugs also take a long time to act.

Psychiatry should be made much more precise. Bad memories, complexes, illusions such as tinnitus, each could be addressed exactly and removed without interfering with anything else. Even better, the effect should decay in a month or two with the toxin. The therapist will thus learn what other mental effects depend on the eliminated symptom. Repeated treatments could then be targeted more exactly.

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