

albeit in a different way, by modulating ryanodine receptors in blood vessels. If so, the absence of FKBP12.6 would lead to an increase in blood pressure, requiring the heart to pump more forcefully. And the predicted outcome of more forceful cardiac contraction in the face of lasting high blood pressure is enlargement of the heart. Indeed, Xin *et al.* found that male, but not female, FKBP12.6-null mice had high blood pressure¹. This is consistent with the idea that cardiac hypertrophy in the males is a secondary consequence of elevated blood pressure, and that oestrogen opposes the effects of a lack of FKBP12.6 on blood pressure.

There are other possibilities, however. The increases in intracellular calcium levels seen in FKBP12.6-deficient animals might lead directly to an increase in the activity of the calcium-dependent transcription factor NFAT. Alternatively, because FKBP12 and FKBP12.6 not only regulate calcium release through ryanodine receptors but are also important components of the calcineurin protein complex, which regulates NFAT activity, it is possible that disruption of FKBP12 or FKBP12.6 in the heart or blood vessels leads to the activation of NFAT

through effects on calcineurin. The activation of NFAT by either of these two mechanisms might induce a gene programme that causes an increase in blood pressure or cardiac hypertrophy. It is conceivable that oestrogen decreases the expression or activity of NFAT or its target genes in the blood vessels or hearts of females. Whatever the answer, an understanding of the molecular mechanisms at work will no doubt be crucial to comprehending gender-based differences in cardiovascular function. ■

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Materials science

Breaking the neural code

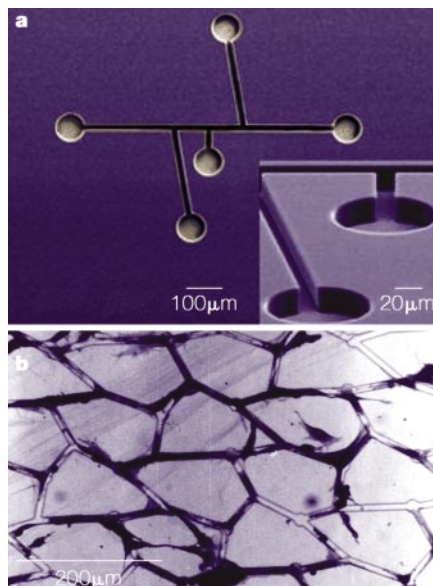
Adam Curtis

The precise information that is conveyed between nerve cells remains unknown. Networks of nerve cells grown on silicon chips, using a polyester as a guide, may bring us closer to translating the elusive neural language.

Within a nerve cell, or neuron, differences in electrical potential encode information, and messages can be passed on to other neurons through connections that may be chemical or electrical. But, like nineteenth-century linguists contemplating the hieroglyphs of the Rosetta Stone, we have only vague clues as to the symbolism and language, let alone the grammar or syntax, of neural messages. We will never dig up a neurobiological Rosetta Stone — but we might be able to synthesize our own. In a notable paper in *Advanced Materials*, Merz and Fromherz¹ have taken us at least one stage towards this goal, and their work brings with it the promise of further discoveries.

To decipher the neural code, the aim is to grow networks of neurons in culture, with each neuron connected to others through synapses that form at the ends of the neuron's tendril-like extensions, or neurites. We need to reproduce the connectivity that is seen anatomically in nervous systems and which, when destroyed, leads to loss of correct function. We suspect that the message passing across a synapse carries instructions for how the information should be processed and

possibly also routed, and in many cases we know what the correct (or at least normal) input and output are. What we would like to test are the effects of different connectivities and input messages on the neuronal output.



Work on growing neuronal networks has been in progress for several years, although previous experiments paid insufficient attention to preventing the neurites from moving or becoming rearranged in the cultures; this can happen through the mechanical forces generated by the cells themselves. But secure, long-term positioning of neurites (on a scale of days to weeks) has been achieved², with lines of neurons immobilized using the protein laminin. Maher *et al.*³ have also grown cells in deep pits, and recorded data from them. An alternative to network growing has also been tried. In an earlier, seminal experiment, Syed and colleagues⁴ reconstructed neuronal circuits by delicate hand surgery and managed to rebuild a working circuit. But they did not explore modified circuits or the effects of changes in the message.

Now Merz and Fromherz¹ have built on the results of such experiments, and have obtained well-defined, if very small, stable networks of cultured neurons from the pond snail *Lymnaea stagnalis*. Using micro-fabrication techniques, they etched a series of pits and grooves in a polyester surface on a silicon substrate (Fig. 1a). The design traps the cell bodies but allows neurites to grow along the grooves. Merz and Fromherz managed to identify pairs of nerve cells that had formed connections and to record electrical signals from them. They did this by injecting current into one neuron and showing that a voltage change occurred in the neighbouring cell, indicating that electrical synapses had formed. Moreover, the electrical events observed in the networks were as expected, proving that the behaviour of the neurons was not being appreciably affected by the chemistry or structure of the polyester-silicon growth material.

To record and stimulate electrical signals in their neuronal network, Merz and Fromherz used conventional intracellular

Figure 1 Artificially grown networks of neurons could be the key to deciphering the intricate code of neural messages. a, Merz and Fromherz¹ etched pits and grooves in a polyester-silicon material to control the growth of neurites into a functioning network; pits 70–80 μm in diameter match the size of the pond-snail neurons used in the experiment, and grooves up to 15 μm wide and 30 μm deep confine and guide the neurites into forming synaptic connections between neurons. b, Following a pattern suggested by Wilkinson⁶, Sandison *et al.*⁵ grew this neuronal network in grooves in silica — although the difficulty of confining the growth within the structure is apparent.

a, M. MERZ & P. FROMHERZ / b, A. CURTIS

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 micro-electrode, can kill them. In fact, my own group has recently invented a manipulable extracellular electrode array⁵ 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 Fromherz¹ 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.

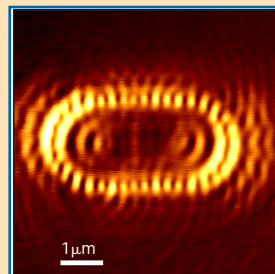
The picture of the killing process that has dominated thinking over the past 40 years is that the lethal agents are the highly reactive

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 through 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