and expensive, currently costing more than US$10 million. In one minute, a stepper may be required to expose 60 images, each 2 × 2 cm and containing more than 10 billion features. The smallest possible feature is a square with sides 130 nm long, and the accuracies (or tolerances) of the size and position of features are around 25 nm and 65 nm, respectively; moreover, there must be no detectable defects within the image. To follow Moore's law, the minimum feature size and the respective tolerances will need to be halved over the next three years, while still maintaining the same image size and speed of the process. Over the same period, the stepper cost is projected to rise to more than $20 million. But with four times as many features created in a single image, the required reduction in cost per exposed feature could still be met.

Physicists will notice that a projected feature size of 65 nm is beyond Rayleigh's resolution limit for a microscope — the minimum distance that can be resolved owing to the effects of diffraction. (This limit is about 110 nm for ultraviolet light with a wavelength of 193 nm.) To achieve this resolution is difficult and expensive, though not impossible, and it is not clear that we can keep reducing feature size and the cost per exposed feature by continuing to push the technique of optical projection. If progress falters, Moore's law will fail and this could be disastrous for the semiconductor industry.

Thus industry is investing in 'next generation lithography', in particular in two favoured technologies, electron-projection and extreme-ultraviolet lithography. Both rely on high-resolution focusing of a projected image. However, progress has been slow and expensive, and there are doubts that either technique can maintain Moore's law.

But a new technology based on conventional, mechanical printing could eliminate the focusing problem altogether. Derived from the imprinting process used to manufacture compact disks, this process can generate sub-micrometre features over an area of 100 cm^2 in less than a second at a cost of about 50 cents — that's between two and three orders of magnitude less than the cost of optical projection. In 1996, Chou and colleagues demonstrated[4] that a modification of this technique could generate 10-nm features over an image field of about 3 cm^2, an extraordinary combination of minimum feature size and image size.

There have been other significant advances, such as the development by Whitesides and colleagues[5] of another mechanical printing process, 'soft lithography', with which features smaller than 100 nm can be printed on non-flat surfaces. In 1998, the US Defense Advanced Research Projects Agency launched a research programme on 'molecular-level, large-area printing' (MLP). And the semiconductor industry is beginning to take notice; results are emerging from a joint development programme in this area launched by Motorola and the University of Texas[6].

The latest development reported here by Chou *et al.*[3] is one of the most exciting. They have demonstrated that, instead of imprinting in a thin plastic resist film, they can pattern silicon directly by a combination of imprinting and flood illumination (Fig. 1b). They call the process 'LADI', for laser-assisted direct imprint. The printing mask is made of quartz, with a relief image on the surface that presses against the silicon. Flashing laser light through the mask causes the top, sub-micrometre layer of silicon to melt momentarily and take on the shape of the relief image of the mask. The mask is then separated from the silicon, apparently without any unwanted adhesion between the silicon and the quartz.

Chou *et al*. created features of 140-nm dimension (the limit of their mask). But detail at the level of 10 nm on these features was also replicated, indicating the extraordinary resolution of the process. This technique can also be used for patterning polycrystalline films of silicon, one of the most critical steps in patterning silicon chips. Because there is no need for expensive focusing optics, the printing equipment is much simpler than a stepper. And the absence of a resist eliminates that cost too.

But there are technical challenges to be met. Defects caused and propagated by contact led to the abandoning of contact photoprinting in the early history (pre-1970) of silicon chips, so we might expect similar problems here. But in the decades since, there have been tremendous advances in managing defects. Making a suitable mask with features as small as those required on the wafer is a challenge, but there is at least one tool, Nippon Telephone and Telegraph's experimental electron-beam system 'EBX-3', that seems to be up to the task.

Thus, on grounds of cost, speed and resolution, LADI, or some other form of mechanical printing, may displace optical projection as the preferred manufacturing technology for fashioning silicon chips. So we might expect Moore's law to hold for, maybe, another two decades. ■

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Neurobiology

Understanding the consequences

Thomas J. Carew

We learn in several ways, one of which involves forming an association between an action and its consequence. Studies of a marine mollusc shed light on how this creature forms a connection between biting and food.

The ability to assess the consequences of one's actions is fundamental to survival: an animal must learn an effective hunting strategy if it is to eat, and to elude predators if it is to live to see another day. Writing in *Science*, Brembs, Lorenzetti and colleagues[1] describe their studies of the neural basis of this type of learning in the marine mollusc *Aplysia*, which could serve as a useful model for understanding more complicated organisms.

This general class of learning is known in the trade as 'operant' or 'instrumental' conditioning[2]. It was first brought into the laboratory over a century ago by Thorndike[3], who studied the ability of cats to learn to escape from a 'puzzle box'. At about the same time, another form of learning was discovered by Pavlov[4], who described how animals form an association between a neutral (also termed a 'conditioned') stimulus, such as a ringing tone, and an 'unconditioned' stimulus that has inherent meaning, such as food. This is known as classical or pavlovian conditioning. Together these two types of learning provide most of the tools that all animals need to negotiate their environment successfully, enabling them to associate their behaviour with its consequences and to learn predictive relationships about the world.

J. H. BYRNE

Figure 1 **Learning model — the marine mollusc *Aplysia*. The new paper by Brembs *et al.*[1] gets to grips with how *Aplysia* learns to associate biting with a food reward, a type of 'operant' conditioning.**

In modern neuroscience, the analysis of learning mechanisms is a thriving enterprise. But although classical and operant conditioning are both important, the mechanisms of classical conditioning have received far more attention. Why should this be? The reason lies, at least in part, in how researchers approach the problem. The basic goal of a neurobiological analysis of associative learning is twofold: first, to identify the site of the association in the brain; and second, to characterize the neural mechanisms involved in forming the association. For classical conditioning, this strategy is relatively straightforward. One would first identify the pathways of neurons that respond to the conditioned and unconditioned stimuli. The points at which the two pathways converge would be good candidates for the sites at which an association between the stimuli is formed.

But matters are potentially more complicated in the case of operant conditioning. Here, an association is made not between two stimuli but between an action and its consequence, such as a benefit (or 'reward', in learning parlance) or punishment. So the site of association is not intuitively obvious. For example, it could occur where information about the reward converges with the brain region that initiates the behaviour, which could be more difficult to locate than the regions that respond to conditioned and unconditioned stimuli.

Brembs *et al.*[1] have overcome these difficulties by studying the operant conditioning of feeding in *Aplysia* (Fig. 1). The authors attacked the problem at several levels, from the behaviour of the whole animal down to the electrophysiological properties of single neurons. They focused on feeding in *Aplysia* because this is known to be capable of operant conditioning[5] and, more importantly, because the neural circuitry underlying feeding has been well characterized.

First, Brembs *et al.* looked at the electrical activity of the oesophageal nerve in whole animals, and found that it increased when the animals ingested food. Presumably, this activity signals the presence of a reward — food. Next, Brembs *et al.* 'trained' the animals to associate spontaneous biting (whether or not food was ingested) with a reward by stimulating the oesophageal nerve, mimicking the usual reward signal, during biting. The result was that the molluscs made significantly more spontaneous bites than controls, both immediately and 24 hours after training. In other words, the operant response (biting) can be reinforced by a food-related reward signal (stimulation of the oesophageal nerve); moreover, the memory of the association between biting and reward can persist for at least 24 hours.

The authors then turned their attention to where this memory might be stored. Here, the previous detailed characterization of the neural circuitry underlying feeding behaviour was a big help. In the central nervous system, a particular group of neurons — the buccal ganglia — controls biting, and the authors studied one of these, called B51, because it is essential in generating the correct programme of neuronal activity. By recording the electrical activity of B51 neurons in buccal ganglia that had been surgically isolated from *Aplysia*, Brembs *et al.* showed that the burst threshold was lower and the input resistance higher in neurons from trained animals than in controls. The changes in these properties together increase the likelihood that B51 will become active, and hence improve its ability to generate ingestion-related neural programmes.

So it seems that operant conditioning can alter certain properties of the B51 neuron. But it was not clear whether B51 is a genuine site of convergence between bite behaviour and reward — that is, if it is where the association is formed and stored — or whether it is simply affected by that site. To find out, Brembs and colleagues isolated and cultured B51 neurons and examined whether similar changes in properties could be induced by mimicking the operant conditioning procedure at the single-neuron level. They paired the activation of B51 (that would generate a bite) with brief pulses of dopamine, a neuromodulator that probably serves as the reward signal in this system[6] and many others[7]. They found a significant reduction in burst threshold and increase in input resistance in B51. This did not occur when dopamine application and B51 activation were unpaired.

How do these observations relate to the learned association between biting and reward? The idea is this. B51 activity is required for an animal to attempt to take a bite of food. If that attempt is successful — if food ends up in the mouth — the oesophageal nerve is stimulated. This causes the release of dopamine in the buccal ganglia, increasing the excitability of B51. Given that direct activation of B51 can elicit ingestion-related activity in isolated ganglia[8], one can envisage that a direct consequence of enhancing B51 excitability would be a greater likelihood of further biting.

So, collectively the data show that B51 is directly affected by operant conditioning, implying that it is at least one important site of memory storage. Moreover, an exciting aspect of the study is that it provides a neuronal mechanism for operant conditioning that can explain not only its associative features, but also how that associative component might control future biting behaviour.

But the significance of the paper extends further. Now that an associative site for operant conditioning has been found, it will be possible to examine the underlying cellular and molecular mechanisms in detail — though this is no easy chore, to be sure. More-

100 YEARS AGO

Mr. Marconi brought forward two interesting pieces of information in his lecture at the Royal Institution last Friday. The first relates to the new form of magnetic detector which he has been employing in place of the coherer. The instrument is found to be more sensitive and trustworthy than the coherer, and gives promise of a great increase in the speed of working. Already a speed of thirty words a minute has been attained, and this may possibly be increased to several hundred. The second point relates to the recent Transatlantic signalling. It seems that on the occasion of Mr. Marconi's journey across the Atlantic in the *Philadelphia*, the signals transmitted during the day failed entirely at a distance of 700 miles, although a message was successfully sent at night more than 1550 miles, and a signal more than 2000 miles. This effect Mr. Marconi suggests may be due to the diselectrification of the aërial waves by the daylight. The difficulty can, however, be got over by the use of greater transmitting power — as is evidenced partly by the fact that the signal received at Newfoundland was transmitted during the daytime.
From *Nature* 19 June 1902.

50 YEARS AGO

It is known that the isotopic composition of carbon in living matter and related materials is different from that in carbonates. About a hundred plants, representing most of the major plant groups, have been investigated... Various hypotheses have been postulated in order to explain the results, and they can easily be described in terms of the 'local carbon dioxide cycle', well known to botanists and geochemists. It is assumed that there is a difference in the rate of assimilation of the light and the heavy carbon dioxide molecules. This difference is accentuated by the cycle: 'local air'–plant–soil–'local air', which works as an isotope enrichment process, assuming that there is an exchange between the 'local' air and the 'main' atmospheric air. At places where this cycle is intense the isotope effect is large; where it is almost absent, for example, in deserts or very windy places, the isotope effect will be small... These results are also of some interest in connexion with the carbon-14 method for age determinations. The observed effects will be accentuated because the difference in rates of assimilation will be much larger.
From *Nature* 21 June 1952.

over, *Aplysia* shows classical conditioning[9], and much is known about the molecular and cellular mechanisms underlying this form of learning[10–12]. So it might be possible to compare classical and operant conditioning in *Aplysia* in mechanistic terms. If they have features in common, an exciting principle might emerge: evolution may have come up with a neural 'associative cassette' that can be used in either type of conditioning, depending on the neural circuit in which it is embedded. Of course, this is pure speculation, but the work by Brembs and colleagues will be instrumental in exploring this intriguing possibility. ■

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Light microscopy

Beyond the diffraction limit?

Ernst H. K. Stelzer

The wave nature of light manifests itself in diffraction, which hampers attempts to determine the location of molecules. Clever use of microscopic techniques might now be circumventing the 'diffraction limit'.

The two best-known physical limitations are Abbe's resolution limit in optical physics and Heisenberg's uncertainty principle in quantum physics. Each defines a natural limit to the resolution or accuracy with which certain parameters can be measured. But, writing in *Physical Review Letters*, Marcus Dyba and Stefan Hell[1] claim to have taken a step beyond one of these limits.

In 1873, Ernst Abbe[2] realized that the smallest distance that can be resolved between two lines by optical instruments has a physical and not just a technical limit. The distance — the diffraction limit — is proportional to the wavelength and inversely proportional to the angular distribution of the light observed. No matter how perfectly an optical instrument is manufactured, its resolving power will always have this natural limit.

About 50 years later, Werner Heisenberg[3] realized that the parameters describing a quantum particle, such as its location and momentum, are not independent. The accuracy with which one parameter can be determined is coupled to the accuracy with which the other can be determined: one can have an accurate idea either of the location or of the momentum, but not of both at the same time. Improvement in accuracy in one parameter will always occur at the expense of decreasing the accuracy in the other. Heisenberg's 'uncertainty principle' is probably one of the most thoroughly tested relationships in physics, a firm foundation of modern quantum theory, and there is no reasonable account that suggests it is incorrect. Although not a great surprise, it has only recently been shown that Heisenberg's and Abbe's formulae are related[4].

Over the past few years, several groups have claimed to have achieved image contrasts that have taken the resolution of light microscopes beyond that of classical instruments, and beyond Abbe's limit. For example, confocal fluorescence microscopy has had an enormous impact in biology. Using lasers to induce emission from fluorescent chromophores (the molecules responsible for the colour of the material), it combines point-by-point illumination with synchronous point-by-point detection. This technique has proved especially useful for imaging biological objects because of its optical sectioning capability: deep inside optically dense objects (such as embryos), it is possible to record fluorescent images that show the chromophore distribution in just a single focal plane. The advantage with this type of instrument comes from its nonlinear behaviour — it is essentially sensitive to the square of the light intensity, not to the intensity itself, and this discriminates against light that is out of focus.

On the basis of the work of Lukosz[5], Gustafsson and colleagues[6] have achieved excellent resolution with their images of actin protein networks, collected by illuminating the object with light patterns whose intensity varied in a sinusoidal fashion. These and several other contributions to the field have pushed the image resolution down to about 100 nm — which does not contradict Abbe's resolution limit.

Imaging the common soil bacterium *Bacillus megaterium* with focused light of wavelength 760 nm, Dyba and Hell[1] claim to have observed excited molecules with a resolution of 33 nm — that is, down to a distance considerably smaller than Abbe's limit. The authors' technique relies on two essentially independent technologies. The first is '4Pi confocal fluorescence microscopy', which uses two opposing lenses of high numerical aperture to illuminate an object coherently from two sides[7]. This creates an interference pattern that is modulated along the optical axis and reduces the observed volume by a half. The main maximum in the pattern of diffracted light — one 'volume element' in the image sought — is surrounded by other peaks of light intensity, which can generally be removed by further computations and by using knowledge gained about the object with other means.

The second technique is more complicated. First, the fluorophores are excited with a regular, well-focused beam of light. Picoseconds later, a second beam operating at the emission frequency of the fluorophores but with a light-intensity distribution that has a gap in its centre, stimulates the emission of the fluorophores outside the gap region. A few more picoseconds later, the regular fluorescence emission, stemming from the volume defined by the gap, can be observed. Dyba and Hell used the 4Pi technique to create the gap pattern for the stimulated emission.

The question is what the observed spot width of 33 nm tells us. Have Dyba and Hell achieved a spatial resolution of 33 nm? Although the experiments shown in their paper seem convincing at first, I question whether this is actually a demonstration of resolution improvement. Their images of membranes of *Bacillus megaterium* show a two-dimensional view of well-spaced membranes that are already well resolved in the straightforward confocal image. The images recorded with the new technique seem to localize the two membranes more exactly. This is at best an improvement in the precision of their locations, but not actually in the resolution of the image, which is defined as the minimum distance between two features that can be resolved with a certain contrast[8].

As already seen in the work of Toraldo di Francia and others[9,10], the central diffractive peak can be made much narrower, but this always generates huge side 'lobes' of light, which appear outside the area of interest. This effect is equally visible in the images shown by these authors[1]. Extensive computations are required to discriminate the information in the central lobe from that in the side lobes. So the improvement is not entirely due to the instrument but in fact stems from combining the imaging techniques and from using *a priori* knowledge. Another problem is that the signal-to-noise ratio becomes worse.