Neuroscience

Optical mapping of activity in primate visual cortex

from D. Van Essen and H. S. Orbach

MUCH of our current understanding of cortical function has been gained by analysing how different types of information are represented in a systematic fashion within the three-dimensional architecture of the cortex. This is most evident in the case of primary visual cortex (striate cortex) of primates, where cortical architecture is specialized to a notably high degree and where many of these specializations have interesting neurophysiological correlates. In a report on page 579 of this issue, Blasdel and Salama[1] use a relatively new technique involving *in vivo* application of voltage-sensitive dyes to ascertain how selectivity for stimulus orientation is mapped across the surface of striate cortex in the macaque monkey. Their study provides an intriguing and unexpected hint about modular organization of the cortex. In addition, it emphasizes the feasibility of the optical approach for attacking a host of interesting issues concerning cortical function.

It is well known[2] that cells in the striate cortex are arranged in orientation columns, in which aggregates of cells aligned orthogonal to the cortical surface all respond best to the same stimulus orientation (except for cells completely lacking orientation selectivity). Parallel to the cortical surface, orientation preferences tend to shift gradually and smoothly, with only occasional discontinuities in the progression. A major unresolved issue has been the relationship of this orderly array of orientation columns to two other basic features of striate cortex: a system of ocular dominance stripes that is most clearly delineated by a sharp segregation of geniculocortical inputs from the two eyes within layer 4; and a patchwork array of cytochrome oxidase-rich 'blobs', which are aligned with ocular dominance stripes and whose constituent cells generally lack orientation selectivity[3].

What has clearly been needed to sort out these relationships is a means for monitoring the two-dimensional pattern of visually evoked cortical activity with high spatial resolution. The 2-deoxyglucose technique, in which activity-dependent metabolism is measured autoradiographically, has proven helpful in this respect[4]. But it suffers from being a one-shot approach, in which only a single stimulation condition can be tested in any given cortical region. The technique of *in vivo* optical monitoring of neural activity using voltage-sensitive dyes is in principle much more flexible, because it has vastly superior time resolution and allows repeated measurements using various stimuli. First developed and tested on invertebrate preparations by Cohen and collaborators[5], voltage-sensitive dyes have recently been successfully applied to the mammalian somatosensory and visual cortex [6,7].

Several methodological refinements introduced in the study of Blasdel and Salama[1] yield a spectacular increase in the spatial resolution of the technique. Most importantly, the authors used a video camera instead of the standard photodetector array, and monitored a very slow visually evoked response, thereby gaining spatial resolution in exchange for an affordable loss of temporal resolution. In addition, they used a clever combination of stimulation paradigms and image-processing techniques to generate detailed false-colour maps of how orientation preferences are mapped across the surface of the cortex. The basic validity of this analysis was confirmed by direct comparisons between optical and electrophysiological determinations of ocular dominance and preferred orientation at selected sites.

Blasdel and Salama find that regions of similar orientation preference generally occupy small patches in the orientation map. These patches range from nearly circular to highly elongated in shape, with a length typically less than 1 mm. The alignment of iso-orientation domains is not strongly correlated with ocular dominance stripes nor with cytochrome oxidase blobs, an outcome which is consistent with previous deoxyglucose-based results[4]. It runs counter to more organized patterns that have been hypothesized, such as an orthogonality between ocular dominance and orientation stripes[7] or a radial or circumferential arrangement of iso-orientation domains centred around cytochrome oxidase blobs[8].

A particularly striking feature of the orientation maps is a pattern of 'fractures', lines or narrow strips along which the preferred orientation changes very rapidly. The fracture lines form a tightly interlaced network containing numerous loops and rings that are variable in size and shape, but are generally much less than 1 mm across. Blasdel and Salama raise the intriguing suggestion that these fracture lines form the outlines of discrete 'modules' within the cortex. However, they also note several potential complications relating to the fracture lines, and these deserve careful evaluation.

One of the complications arises from the fact that the maps display a well-defined orientation at all points, even in regions which post-mortem histochemistry shows to be superimposed on the cytochrome oxidase blobs. This is surprising in view of the previously mentioned complete absence of electrophysiologically assessed orientation selectivity in most blobs[3]. One possibility is that orientation selectivity might indeed be present in the blobs, but restricted to the most superficial cells (say, in layer 2), which presumably dominate the optical signals. Alternatively, the apparent orientation selectivity within the blobs might arise from spatial blurring of the optical signal, for example, as a result of light scattering within the cortex or as a reflection of the extent of dendritic arborizations. By this hypothesis patches whose cells genuinely lack orientation selectivity would nonetheless show an apparent orientation preference throughout, by virtue of spatial averaging of the orientations of nearby sites within the blur circle. Fracture lines associated with blobs would then no longer represent an abrupt discontinuity in a parameter that has a well-defined value at each point, but rather a mismatch in the extrapolated orientation in going from one side of a blob to the other.

Another puzzling feature is that not all orientations are equally represented within either of the two orientation maps illustrated by Blasdel and Salama. In particular, one oblique orientation (light blue in their false-colour representations) appears to be notably sparse, generally occurring as very narrow strips. Nonlinearities in the colour reproduction process may contribute to this impression but are unlikely to provide a complete explanation, because many of the fracture lines run directly along the thin strips associated with the under-represented orientation. There may be pronounced anisotropies in the cortical representation of orientation, analogous to those suggested from electrophysiological recordings[9]. Yet another possibility is that the apparent under-representation was a consequence of slightly reduced efficacy of a particular stimulus orientation, conceivably because of astigmatism of one or both eyes. A final complication to the fracture story is that some of the fracture lines (more than seems likely by chance association) appear by visual inspection to parallel closely the vascular pattern (including some of the smaller blood vessels) that is shown in a figure accompanying the study. This may reflect yet another way in which non-neural factors can subtly bias the orientation estimates derived from optical measurements.

The question of whether striate cortex contains well-defined modules is fundamental to our understanding of cortical organization. Hubel and Wiesel[2,4] laid the

groundwork for this notion with the suggestion that any small region of cortex, about 1–2 mm on a side, contains a set of 'hypercolumns', that is, all the neural machinery needed for the analysis of orientation and ocular dominance in any small portion of the visual field. The idea received a major boost with the discovery of the cytochrome oxidase blobs, which clearly denote an anatomically distinctive two-dimensional repetitive pattern, but which leave open the question of whether hypercolumns have well-defined starting and stopping points.

The orientation fracture lines described by Blasdel and Salama are attractive candidates for hypercolumn boundaries. But the relationship is not entirely straightforward, as most of the modules delineated by fractures fail to enclose a complete set of orientations, and do not all contain a representation of both ocular dominance stripes. A more precise understanding of this relationship must await resolution of the technically based concerns described above about the origins and interpretations of some of the orientation fracture lines.

These concerns are amenable to experimental analysis, and clarification of the key issues should not be long in coming. Further refinements may also be anticipated for dealing with various technical limitations of the optical technique relating to signal-to-noise problems, image blur and the analysis of signal distribution within the third dimension (depth) of the target tissue.

In the meantime there is good reason to celebrate the successful application of voltage-sensitive dyes to basic questions of cortical organization. Blasdel and Salama have made clear that optically based activity monitoring techniques will become a powerful component in the ever-expanding repertoire available to neuroscientists interested in structure–function relationships within the brain. Application of this approach to questions of information processing, development and plasticity in sensory and motor areas of the cortex will keep many laboratories busy for years to come. □

1. Blasdel, G. & Salama, G. *Nature* **321**, 579 (1986).
2. Hubel, D.H. & Wiesel, T.N. *Proc. R. Soc.* B**198**, 1 (1977).
3. Livingstone, M.S. & Hubel, D.H. *J. Neurosci.* *4*, 309 (1984).
4. Hubel, D.H., Wiesel, T.N. & Stryker, M. *J. comp. Neurol.* **177**, 361 (1978).
5. Grinvald, A., Cohen, L.B., Lesher, S. & Boyle, M.B. *J. Neurophysiol.* **45**, 829 (1981).
6. Orbach, H.S., Cohen, L.B. & Grinvald, A. *J. Neurosci.* **5**, 1886 (1985).
7. Grinvald, A., Gilbert, C.D., Hildesheim, R., Lieke, E. & Wiesel, T.N. *Soc. Neurosci. Abstr.* **11**, 18 (1985).
8. Braitenberg, V. & Braitenberg, C. *Biol. Cybern.* **33**, 179 (1979).
9. Mansfield, R.J. *Science* **186**, 1133 (1974).

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Molecular biology

Why do disused proteins become genetically lost or repressed?

from Jared M. Diamond

ARTICLES in these columns typically focus on recent discoveries. But progress also depends on exposing areas of ignorance. Hence this article concerns our failure so far to resolve a fundamental problem of biology: why disused proteins become phenotypically repressed and are eventually genetically eliminated[1–5].

Evolution consists of the acquisition and loss of traits. Most biologists since Darwin have agreed that the acquisition of functionally significant traits is caused by natural selection. But why do disused, formerly significant traits become lost? (Think of the loss of eyes in cave animals, or the loss of flight in birds on predator-free islands.) Two answers have been suggested, both stemming from Darwin's work: disuse or natural selection. Most mutations destroy a function rather than improve it. Thus, if a character becomes selectively neutral through disuse, mutations will gradually remove the character even in the absence of positive selection to eliminate it. On the other hand, any character costs something to build and maintain. Thus, a disused character may be selectively disadvantageous, not merely neutral; selection may eliminate the character to recoup the wasted costs. The cost most often mentioned in this context is biosynthetic energy.

The traits whose costs could be measured most easily are single enzymes or biosynthetic pathways. Consider auxotrophic mutants, which have lost the ability to synthesize some essential solute and can grow only if that solute is provided exogenously. Lwoff[6] proposed that there should be positive selection for such mutants in the presence of the solute because the mutants save unnecessary biosynthetic costs incurred by the wild strain. Zamenhof and Eichhorn[7] confirmed that His^- and Trp^- auxotrophs of *Bacillus subtilis* are selected over the wild-type strain in the presence of His and Trp, respectively. Similar findings apply to Tyr^- and Trp^- auxotrophs of *Escherichia coli*[1,8]. Few attempts have been made to compare the saved biosynthetic costs of the auxotroph at the molecular level with its selective advantage at the population level, and thereby to decide whether the wasted energy hypothesis suffices to explain this genetic loss of a disused trait. The rare attempts that have been made cast doubt on the energy hypothesis[1,2].

The most detailed comparison is one in which Dykhuizen[1] used a chemostat to study *E. coli* mutants differing from the wild type only in their inability to synthesize tryptophan (Trp). Dykhuizen first calculated the energy needed by wild-type *E. coli* to synthesize various amino acids, assuming maintenance costs to be negligible compared with the costs of growth[9]. Amino-acid synthesis was estimated to cost 40 per cent of the total energy budget, Trp, in particular, 1.25 per cent. In a Trp-containing medium, the synthesis of this amino acid is repressed to a level where it costs only 0.01 per cent of the energy budget. The energy to synthesize the Trp-synthesizing enzymes themselves (in the absence of turnover) was estimated as only 17 per cent of this cost of Trp synthesis. Thus, if the sole advantage of the auxotrophs over the wild type in a Trp-containing medium were the saved synthetic energy, the auxotrophs should have had an insignificantly higher growth rate, by 0.01 per cent per generation (independent of generation time). In fact, their growth rate advantage was far greater — around 10 per cent per generation — and it increased with generation time.

Because these calculations of saved energy involve many assumptions, Dykhuizen next compared the selective advantage (over the wild type) of a mis-sense mutant that makes Trp-synthesizing enzymes but not the protein against a polar nonsense mutant that makes neither the enzymes nor Trp. By the saved energy hypothesis the polar nonsense mutant should have had a 17 per cent greater selective advantage than the mis-sense mutant (because energy savings are 17 per cent greater), but the two mutants were found to have the same advantage. Furthermore, mutants can still synthesize Trp from anthranilate via indole, although at some biosynthetic cost. Hence the selective advantage of the mutants over the wild type should decrease from a Trp to an indole to an anthranilate medium, and Dykhuizen, from the biosynthetic costs of each step, predicted relative advantages of 1.00:0.83:0.67. The actual relative advantages were the same for indole and anthranilate, and were lower than predicted (1.00:0.50:0.50).

Most puzzling of all, the advantage of mutants over the wild type was apparent only when they were grown together; the mutants and wild type had the same growth rates when grown separately. One can think of many uncertainties in these cost estimates: assumptions in the energy calculations that would affect the numbers; features of cell physiology that could