matters arising

Bicuculline and visual responses

DUFFY *et al.*[1] proposed that the influence of intravenously administered bicuculline on the receptive field properties of neurones in the visual cortices of cats which have experienced monocular deprivation indicates the "active inhibition of the relatively intact input from the amblyopic eye" by information arising from the normal eye.

In investigations of this type, it hardly seems necessary to expose unanaesthetised, paralysed animals (admittedly locally anaesthetised "to avoid possible confounding" of the results) to bicuculline administered systemically, particularly when its action "was often complicated by its potent convulsive effects". The microelectrophoretic administration of bicuculline or other GABA antagonists near physiologically identified neurones in various regions of the visual system would be likely to provide more definitive evidence regarding the involvement and localisation of GABA-mediated inhibitory mechanisms in binocular vision, and their perturbation as a consequence of monocular deprivation, with considerably less distress to the experimental animal.

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[1] Duffy, F. H., Snodgrass, S. R., Burchfiel, J. L., and Conway, J. L., *Nature*, **260**, 256–257 (1976).

DUFFY ET AL. REPLY—Curtis has raised[1] at least two issues. He seems to imply that the use of locally anaesthetised animals is improper or unwarranted and, second, he raises the issue of the proper place of iontophoresis in pharmacological studies of the amblyopic animal.

We feel that experimenters have an obligation to minimise an animal's discomfort. No procedure, however, can successfully claim total freedom from trauma. General anaesthesia is probably the best in this regard and should be used whenever possible. General anaesthetics do act on the brain and, in our experience, modify the receptive field characteristics in the visual and somatosensory system. Furthermore, anaesthetic agents may interact with other experimentally administered drugs, thereby confounding experimental findings and possibly requiring additional experimentation. Since most neurophysiological and neuropharmacological investigations result in an animal's death, we feel that experimenters have an equal obligation to minimise the number of animals used.

In the course of behavioural studies of amblyopic cats, we have administered bicuculline to a number of awake and unrestrained animals. Bicuculline seems to have a sedative effect at low and medium dosage levels. At higher levels, epileptic activity in the EEG and convulsions occur quite suddenly and result in an immediate loss of consciousness. Cats do not seem unduly distressed at any time. For all these considerations, we felt that the locally anaesthetised preparation was best for our initial experimentation.

We agree with Curtis that iontophoresis is especially useful for discerning the location of a given drug effect within the nervous system, but feel that it has some problems as an exploratory technique. For example, a negative iontophoretic study with bicuculline in amblyopic cats would not have great meaning unless many regions were sampled which would necessitate the use of more animals. We would also point out that when the data obtained with iontophoretic and intravenous drug administration are in apparent conflict, it is by no means certain that the iontophoretic data give a correct perspective. For example, we cite the recent work of Ben-Ari and Kelly[2] where iontophoretic, but not intravenous, fluphenthixol blocked the response to iontophoretically applied dopamine. We do plan to make use of iontophoresis in the future and expect that it will be useful in elucidating the detailed mechanism of effect.

Finally we agree that intravenous bicuculline is a toxic agent and have been seeking a safer agent with a longer duration of action. We believe that intravenous administration of ammonium salts may meet these requirements.

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[1] Curtis, D. R., *Nature*, **263**, 531 (1976).
[2] Ben-Ari, Y., and Kelly, X., *J. Physiol., Lond.*, **256**, 1 (1976).

Mammalian cell growth regulation

HOLLEY has proposed that mammalian cell growth regulation in culture is regulated primarily by the depletion of diffusable resources, especially polypeptide factors; that the responsiveness of cells to such factors is density dependent; and that this responsiveness changes in a characteristic manner following transformation.

If this theory is correct, the following must be true: (1) that normal and malignant cells differ demonstrably in their growth regulatory policies; (2) that growth control involves a conventional density dependence; (3) that growth inhibition at high density is not merely the result of culture starvation by careless investigators; and (4) that mechanisms other than diffusable substances contribute little to growth regulation.

It may fairly be stated that there is no *in vitro* cell characteristic which has ever been rigorously demonstrated to correlate with and be diagnostic of the malignant state for a broad spectrum of cell types. The following *in vitro* indices are not broad spectrum malignancy correlates: saturation density, multilayering, contact growth inhibition, growth rate, and growth in low serum[2-10]; cell adhesiveness and surface charge[11-14]; lectin agglutinability[15-18]; fibrinolytic activity[19,20]; cell morphology and karyotype[8,9,21,22]. A recalculation of chi-square values for the data of ref. 23, assuming 3T3 cells to be malignant because of their production of tumour angiogenesis factor[24] and *in vivo* tumorigenicity[2], casts grave doubt on the lack of anchorage dependence as a valid tumorigenicity index. Similarly, the satus of the LETS–SF complex is in doubt because many studies of it have used as "normal" controls either cell lines of untested tumorigenicity or lines known (3T3 (ref. 2), BHK21 (refs 9 and 25) or suspected (Wi38 ref. 24)) to be tumorigenic. Holley's argument about growth policy differences between normal and malignant cells is clearly without foundation.

Holley contends that density-dependent growth regulation is "due to a quantitative increase in the requirements for macromolecular growth factors as cell density increases." His only basis for this statement is the citation of three references which speculate about growth regulation by the density-dependent