Letters to the Editor
A METHOD OF MICROPERFUSION WITH OXYGENATED SALINE AS APPLIED TO AN INSECT BRAIN

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For electrophysiological studies in the fly's optic lobe, a procedure was required to perfuse the brain at controllable rates with oxygenated solutions, while recording intracellularly from visual interneurons. The cornea of the compound eyes has to be left in air, in order not to disturb the optics, and to allow physiological stimulation. When the head capsule is opened to get access to the brain, air sacs and major tracheal trunks tend to collapse by either of two causes: surface tension may flatten tracheae at too low fluid levels and hydrostatic pressure may flatten tracheae at too high levels. Only within a small range of saline levels (< ± 30 um) were main tracheae and air sacs found to remain inflated in the dissected head. Therefore the saline level had to be precisely controlled and stabilized within a few microns.

Fig. 1 shows a simple set-up which satisfies these requirements, and which has proved to work very reliably: saline is pumped by a small, servo-controlled

Fig. 1. Microperfusion set-up. The fly faces downwards; the brain (shaded) is exposed from the back; body not shown. P1, P2: peristaltic pumps. HR, hydrostatic reservoir (± 1 ml) for oxygenation with level mark LM and syphon SY. C1, C2: micropipettes in grounded holders; C2 is bevelled. ST: flexible silicon tubing. DV: damping volume.
peristaltic pump (P1: 0-20 µl/s) into a hydrostatic reservoir (HR), where it is bubbled with premoistened oxygen. Solutions can be quickly exchanged by emptying the reservoir HR through the syphon SY and refilling it from the top, up to the adjustable steady state level mark LM. The flow rate from HR into the preparation is determined by P1, because the level in HR rises to an equilibrium level Ah which is determined by the friction in capillary Cl. Saline is removed from the preparation by the pump P2 (0-50 µl/s), which operates at a somewhat larger rate than P1. It is connected to the preparation via a damping volume (DV: 10 ml) to reduce pump pulsation, and via the capillary C2. Both capillaries are carried by grounded microelectrode holders to prevent electrical interference, and are connected to the preparation by highly flexible silicon tubing (ST: 1 mm i.d.) to prevent mechanical interference. If capillary C2 is just a broken micropipette, the whole system does not work properly, because the meniscus which forms when touching the saline is too large; it forms and breaks abruptly, causing intolerable mechanical interference. If however, the tip of C2 is shallowly bevelled, the meniscus which forms upon just touching the fluid surface is very small, and increases with immersion depth (Fig.2). At the same time pump P2 aspirates air through the exposed upper end of the bevel, and minute bubbles form inside the micropipette. Suitable capillaries are easy to make by cutting micropipettes with a writing diamond at about 200 µm o.d., and by dry bevelling on lapping film of about 10 µm particle size (3M Corporation) with a grammophone. The rate of saline removal depends upon the size of the meniscus, i.e. upon the depth of

Fig.2. Automatic rate control of saline removal by immersion-dependent size of the meniscus at the bevelled capillary C2.
immers ion, and stabilizes at a level which is determined by the rate of influx. Smooth operation and precise levelling is achieved if, for a given flow rate, the pump rate of P2 is adjusted such that a continuous stream of bubbles moves through capillary C2. Once adjusted, the perfusion is dynamically stable, and requires no further attention or adjustment for hours.

With this procedure, it is possible to maintain the fly's brain (< 5 µl) alive for more than 24 h in a perfused volume of only 15 µl, and to record intracellularly from fibres as small as 2 µm. The same procedure should be applicable wherever small, open volumes have to be precisely perfused, and mechanical or electrical artifacts cannot be tolerated.

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