A double-barrelled Pt-microelectrode for simultaneous measurement of PO_2 and bioelectrical activity in excitable tissues

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Summary. A technique for making a double-barrelled PO_2-microelectrode is described. It has been used to measure simultaneously the tissular PO_2- and the bioelectrical activity in the retina and the brain cortex.

To record rapid or localized changes in oxygen tension of a tissue, it is often desirable to use a PO_2-electrode with a tip only a few µm in diameter. In some tissues, such as the brain, there occur extracellular DC-potential changes which may alter the polarizing voltage and thereby the polarographic current, since Pt-microelectrodes have current-voltage curves with no true plateau. The artefact can be avoided if the current reference electrode is placed very close to the tip of the Pt-electrode[2]. An alternative method consists of dividing the PO_2-reference system into a current-free microelectrode close to the Pt-electrode, and a current-carrying macroelectrode at a remote inactive point. The current-free microelectrode is connected to a voltage-follower whose output, the local bioelectric potential, is used as the reference for the polarization voltage. In this way, the voltage between the Pt-tip and the bordering tissue is clamped to a constant value[3]. The present paper describes a technique for combining the Pt-electrode and the current-free reference electrode in a double-barrelled microelectrode that works on the same principle but is easier to use.

Materials and method. The tip of a Pt-wire (Pt 90% + Ir 10%) was etched by conventional techniques[4] to the form shown in figure 1 B, so as to fit into the taper of the

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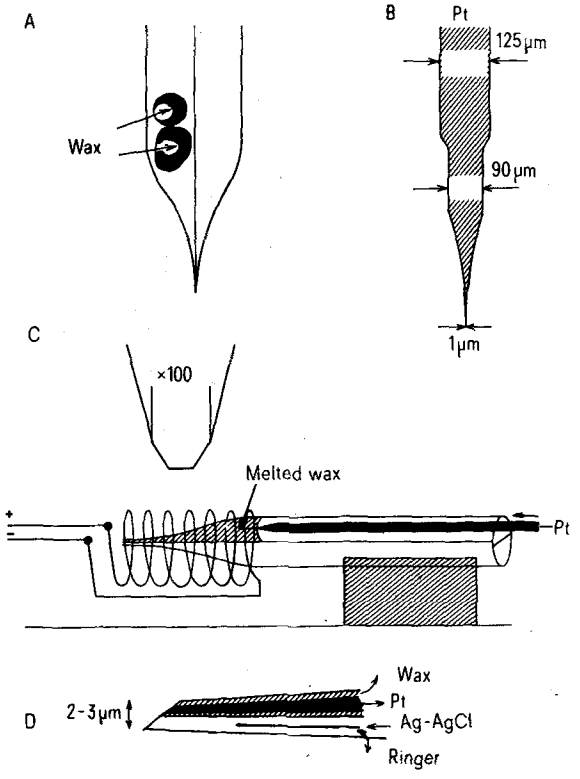

Fig. 1. Method for construction of the PO_2-microelectrode. *A* The micropipette pulled from a capillary of 'theta' glass. Pieces of wax have been inserted in one barrel. *B* The Pt-wire etched to fit in the micropipette. *C* Insertion under microscopic observation of the Pt-wire down one barrel of the micropipette. The pieces of wax are melted by a heating coil. *D* Section through the tip of the finished electrode after bevelling.

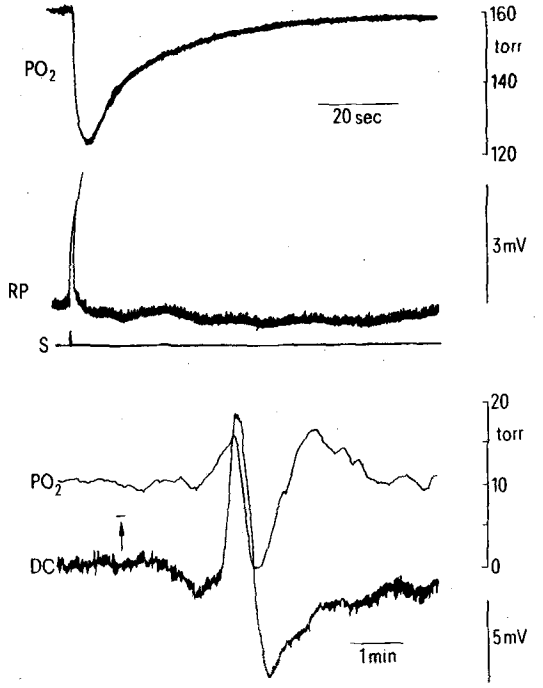

Fig. 2. Experimental application of a double-barrelled PO_2-microelectrode. *A* Simultaneous recording of local PO_2- and extracellular receptor potential (RP) following 50 msec flash light stimulation (S) of the honeybee drone retina. *B* Change of local PO_2- and DC-potential (DC) during a spreading depression in the cerebral cortex of the rat. The wave is selected from a series of repeated ones caused by placing a KCl-crystal on the cortical surface (e.g. see Lehmenkühler et al.[5]).

insulating micropipette. A micropipette was pulled from borosilicate 'theta' capillary (Hilgenberg Glas, D–3509 Malsfeld, Federal Republic of Germany) with a horizontal puller (Narishige) using low heat and a strong pull in order to obtain a tip of about 1.5 µm and a short taper. A few small pieces of elephant wax (m.p. 100 °C) were dropped down one barrel so as to lodge at the beginning of the taper (figure 1 A). The micropipette was placed on the stage of a microscope and a spiral heating element (Pt 50% + Ir 50%) was positioned over the tip as shown in figure 1 C. With a minimum of heat, the wax was melted so that it flowed to the tip of the barrel but did not enter the other barrel. Using, as a guide, a small funnel made from a Pasteur pipette, the Pt-wire was advanced down the barrel with a micromanipulator. The wax was then resoftened and the Pt-wire pushed to the extreme tip of the micropipette. Next, the electrode tip was bevelled on a plate of 0.3 µm rugosity (Stähli, CH–2542 Pieterlen, Switzerland) with the Pt-containing barrel lowermost to make a bevel 2–3 µm wide (figure 1 D). The back of the other barrel was broken for 2–3 mm backwards, filled with Ringer's solution and a Ag–AgCl-wire introduced. The wires were sealed in with wax, and the electrode was then ready for use. Experiments showed that it is not necessary to cover the tip with a membrane, although technically this is feasible. The electrical set-up used for the microamperometric measurement of PO_2 and the simultaneous recording of the bioelectrical activity reference barrel has been described[3].

Results and discussion. When immersed in an air-equilibrated Ringer's solution and polarized by −600 mV, these PO_2-microelectrodes passed currents of 20–160 pA (20 microelectrodes). The current was fairly stable over several days (± 10 pA maximum variation) even if the microelectrode was kept immersed in Ringer or used repeatedly in experiments. We attribute this stability to: a) the hydrophobic properties of the wax insulation in the tip; b) the well-defined geometry of the exposed surface of the Pt, produced by the bevelling; c) the absence of a membrane whose alteration during experiments or over days of storage in water could cause a change in O_2-current.

Figure 2 shows examples of the application of electrodes in 2 different excitable tissues, the retina of the honeybee drone and the cortex of the rat brain. The bioelectrical activity was recorded simultaneously by the reference barrel. In conclusion, a double barrelled PO_2-microelectrode has been developed that permits accurate and reproducible measurements of PO_2-variations, and simultaneous recording of bioelectrical activity in excitable tissues.

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Visual responses in the central nervous system of the scallop *Pecten ziczac*[1]

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Summary. Electrical activity recorded from the lateral lobe of the visceroparietal ganglion in the scallop Pecten ziczac reflects predominantly the visual response to onset of light stimulation, suggesting that shadow reflex mechanisms likely occur elsewhere within the central nervous system.

Action potentials recorded from the optic (pallial) nerves of the scallop and related lamellibranch molluscs appear in response to both the onset and termination of a light stimulus[2–4]. These functionally distinct responses arise from separate retinal layers of the eye[2–6]. Depolarizing receptors in the proximal retina produce the 'on' response whereas hyperpolarizing receptors in the distal retina are inhibited by light and produce the 'off' response[7]. A mechanism in which primary visual receptors independently trigger activity signaling a decrease in light intensity, i.e., an 'off' response not dependent on peripheral synaptic interactions, appears to be unique to bivalve molluscs[3, 8].

Shadow response reflex behavior has evolved to varying degrees in nearly all bivalves. In the scallop, this response is believed to be the direct result of 'off' fibre discharges[3, 9] since images are formed only on the distal retina and since 'off' fibre activity corresponds with the shadow reflex. In the present experiments, visual responses have been recorded in the visceroparietal ganglion as well as in the optic nerves.

Methods and materials. Sand scallops, Pecten ziczac, were collected from Harrington Sound, Bermuda, and kept in circulating natural seawater. A semi-intact scallop preparation was used in which only the lower (right) valve was removed. In P. ziczac, most of the pallial eyes are contained in the upper mantle. The visceroparietal ganglion and pallial nerves were exposed on the ventral surface of the adductor muscle. Ganglionic and pallial nerve activity was recorded extracellularly, using either suction electrodes or wire hooks insulated with oil[11], amplified, and filmed directly from an oscilloscope. The light stimulus (maximum intensity 19.5 W/m² at 3 cm) was delivered through a fibre optic light pipe and monitored by a photocell. Animals were initially dark-adapted for 10 min and then subjected to sequences of on–off light stimuli. Following 30 sec of light adaptation, sequences of off–on stimuli were then presented. All experiments were conducted at 20 °C.

Results and discussion. The following data are representative of the results obtained from experiments on 33 animals. Simultaneous recordings from the pallial nerve and the lateral lobe of the ganglion are illustrated in figure 1. Following dark adaptation (A), both 'on' and 'off' discharges appear in the nerve. Activity in the ganglion, however, is associated exclusively with 'on' responses in the nerve. After light adaptation (B), the 'on' response in the optic nerve is diminished along with an increase in latency whereas the 'off' response is potentiated: response frequency and duration increase,