The Dialysis Electrode—a New Method for *in vivo* Monitoring

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A new type of enzyme electrode, the 'dialysis electrode', based on the enzyme glutamate oxidase, is used for the continuous measurement of levels of the neurotransmitter glutamate in the brain of a freely moving rat.

The use of *in vivo* enzyme electrodes allows the measurement in real time of the concentration changes of important neurochemicals. In previous work we have used an implanted glucose electrode to monitor glucose in the extracellular fluid in the brain of a freely moving rat.[1,2] We report here a novel enzyme electrode, the 'dialysis electrode', which combines the advantages of *in vivo* monitoring using voltammetry[3] with those of microdialysis.

In conventional microdialysis[4] one slowly passes a solution through a short length of dialysis membrane implanted into the tissue of interest. Target molecules diffuse across the membrane and are carried out of the probe by the flow for conventional analysis in the laboratory. In the dialysis electrode, shown in Fig. 1, we insert an electrode into the tip of the probe. The electrode is surrounded by a chosen enzyme, which is introduced by slow perfusion. This arrangement achieves the good sensitivity, time resolution and continuous output of an implanted electrode, with the added advantage that fresh enzyme can be introduced and, as described below, mixtures of enzymes can be used. We describe the use of this dialysis electrode to monitor changes in the concentration of the important neurotransmitter, glutamate, in the brain of a freely moving rat. Hitherto, it has not been possible to monitor such changes.

The solution in the probe and upper compartment (Fig. 1) is changed using a CMA/100 microdialysis pump. Throughout, the concentration of chloride ion was 0.14 mol dm^{-3} and all potentials are reported with respect to this Ag/AgCl reference electrode. The sensing electrode is made from Teflon-coated platinum wire with the Teflon removed to expose 4 mm of platinum at the end. This is coated with Nafion to reduce interference from ascorbate. Currents were measured using a low-noise, low-damping potentiostat of our own design.

To measure glutamate the dialysis electrode was surrounded by the enzyme glutamate oxidase (EC 1.4.3.11).[5] Hydrogen peroxide was measured at a potential of 650 mV where kinetic analysis showed that the diffusion of glutamate through the dialysis membrane was the rate-limiting step. A linear calibration plot was obtained *in vitro* in the concentration range of interest for *in vivo* measurements. This is shown in Fig. 2.

A major problem with the electrochemical measurement of glutamate in the brain is that ascorbate is also oxidised by the electrode. The glutamate concentration[6] is typically of the

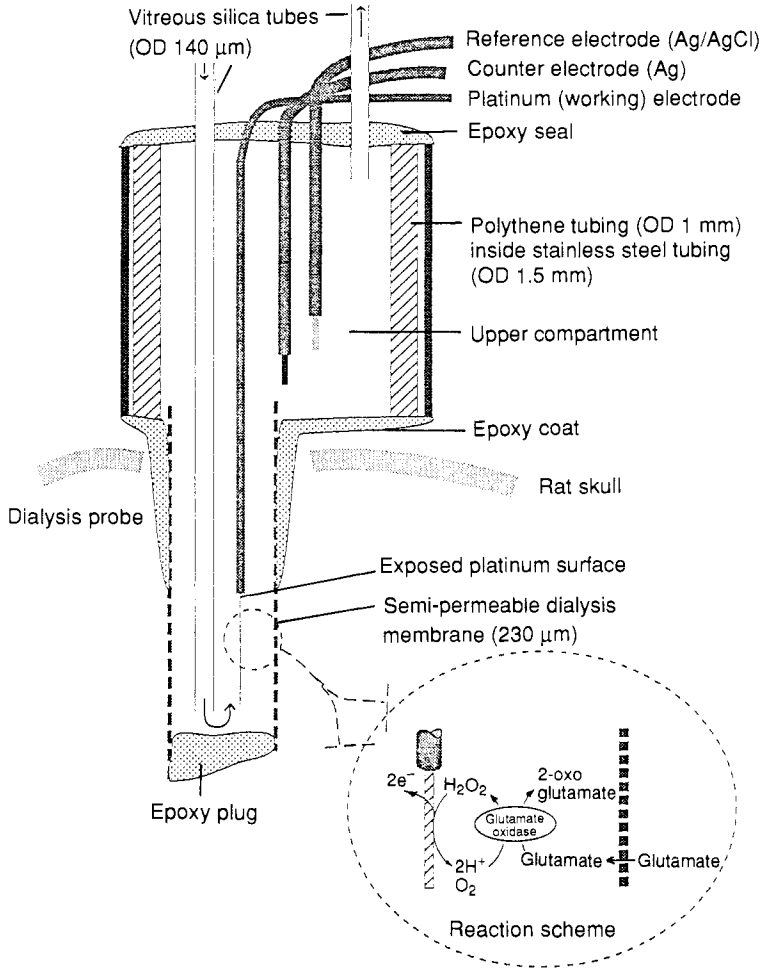

Fig. 1 A dialysis electrode for measuring glutamate

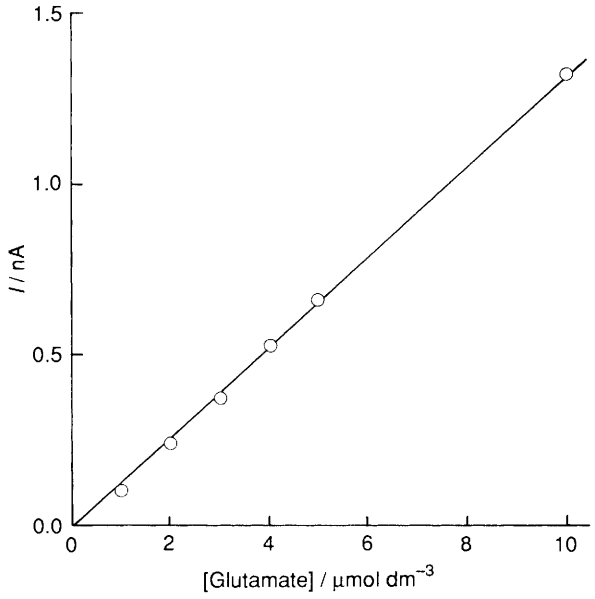

Fig. 2 Calibration plot for the glutamate dialysis electrode

order of 5 to 10 μmol dm^{-3}, whereas ascorbate[7] is present at a much larger concentration of 250 to 400 μmol dm^{-3}. Hence, despite the Nafion coating, the electrode response is dominated by the current from ascorbate and not by that from glutamate. In the upper trace of Fig. 3 we show the response of a dialysis electrode implanted in the dorsal striatum of a freely moving rat. At time zero a mild behavioural stimulus is given to the rat by placing a paper clip on its tail.[8] This mild tail pinch (TP) gives a current rise for the duration of the pinch. At this stage the probe contains Ringer's saline solution but no enzymes and the current is caused by the rise in ascorbate concentration during the tail pinch. When the paper clip is removed the ascorbate concentration returns to its base level, agreeing with our previous findings.[3] We next introduce the enzyme ascorbate oxidase (AA Ox, EC 1.10.3.3) into the probe. In the middle trace of Fig. 3 we see that the basal current is now much lower, and no rise in ascorbate can be measured during tail pinch—the enzyme is oxidising all the ascorbate before it can reach the electrode. Finally, we introduce glutamate oxidase (Glut Ox) alongside the ascorbate oxidase to give the lower trace of Fig. 3. First, we see that the basal current has increased by ≈ 1 nA owing to the resting glutamate levels in the brain. Secondly, during tail pinch the glutamate level rises and again, when the paper clip is removed, the glutamate concentration returns to its base level. The similarity of the ascorbate and glutamate responses to tail pinch agrees with our suggestion that ascorbate is released during the reuptake of neuronally released glutamate[6].

The results in Fig. 3 illustrate the great advantage in a dialysis electrode of being able to control the local environment of the implanted electrode. By using different enzymes we can measure in real time the concentration of an analyte of choice in the same animal, and with the same probe. By using an enzymatic filter we can remove ascorbate interference, and we can check this at any time *in vivo*. Inactive enzyme can easily be replaced. Finally, because measurements are made when there is no flow through the probe, disturbance of the brain tissue is minimised. Indeed, when not in use we now remove all enzymes to prevent any depletion.

The general method described here for glutamate is applicable to measuring a wide range of target analytes in real time. We have already obtained good results for glucose and acetylcholine *in vitro*.

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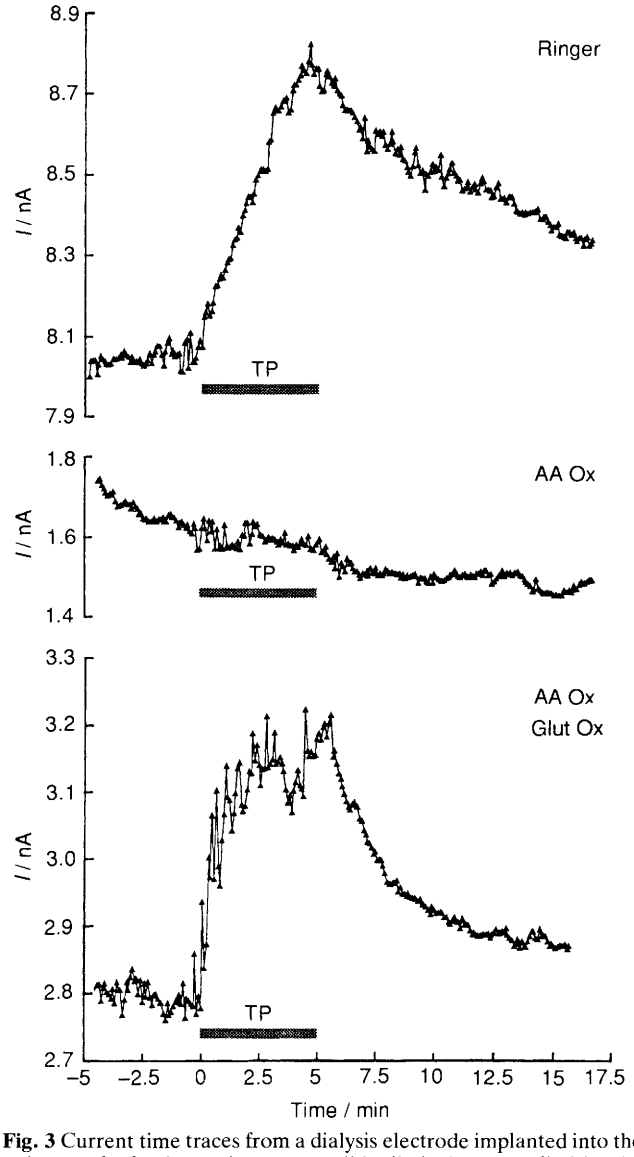

Fig. 3 Current time traces from a dialysis electrode implanted into the striatum of a freely moving rat. A mild tail pinch was applied for the time TP. Upper trace: Ringer's saline only in probe. Middle trace: The enzyme ascorbate oxidase in the probe. Lower trace: The enzymes ascorbate oxidase and glutamate oxidase in the probe. All values are an average over 5 s of a continuous signal.

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