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# A Modification of the Dialytrode for Simultaneous CNS Recording and Chemical Stimulation

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CORNISH, K. G. AND R. E. HALL. *A modification of the dialytrode for simultaneous CNS recording and chemical stimulation.* PHARMAC. BIOCHEM. BEHAV. **10**(3) 389–392, 1979.—Currently, chemical stimulation of the brain is done either with a single cannula that is inserted at the time the drug is delivered or with two cannulae that permit perfusion of the area of interest. The dialytrode is a push-pull cannula consisting of two concentric tubes, a porous membrane covering the tip of the tubing and a platinum wire loop that extends around the membrane and is used for EEG recordings. Studies with the dialytrode indicate that while the membrane is relatively impermeable to bacteria, molecules the size of neurotransmitters pass easily through the membrane. Therefore, the dialytrode is well suited for chronic chemical stimulation studies.

Dialytrode CNS recording Chemical stimulation

MYERS [1] has suggested that it is more meaningful to study the CNS by using chemical stimulation than with electrical stimulation. He pointed out that electrical stimulation excites both cells and fiber tracts in the area of the electrode while chemical stimulation activates only those areas receptive to it, i.e., the receptors on cell membranes. This then should be a more physiologic method for studying the CNS.

To date, chronic chemical stimulation has been complicated by the possibility of bacterial infection at the site of drug application and occlusion of the implanted cannulae after repeated use [1]. In an attempt to maintain patency of the injection tubing or prevent the admittance of bacteria along with the drug being studied, various alterations of the cannulae have been used along with a silastic membrane at the tip of the cannula [2,3]. These generally have low permeability especially to water soluble substances and do not permit the rapid administration (within a few seconds) of a test substance. Others have added electrodes to chematrodes to permit recording from and electrical stimulation of the brain [4,5]. The dialytrode presented here (initially described in part by Delgado *et al.* [4]) permits chemical stimulation while simultaneously recording the electrical activity at the site of stimulation without the problem of cannulae occlusion or infection resulting from bacteria injected along with the chemical being studied.

The dialytrode is a device that utilizes a porous polysulfone membrane attached to a push-pull perfusion cannula. The following is a detailed description of the methodology involved in its production, the modifications added to permit EEG recording and a short description of its use.

## METHOD

The dialytrode membranes are constructed of Udel Polysulfone P-3500 (Union Carbide Corporation, 270 Park Avenue, New York, New York 10017), 2-methoxyethanol (Methy Cellusolve or ethyleneglycol monomethyl ether) and dimethyl formamide (DMF). Permeability of the membranes is determined by the polysulfone-DMF/2-methoxyethanol ratio (P/M), with the porosity increasing as the P/M ratio is decreased.

The solution for making the membranes is prepared by dissolving solid polysulfone P-3500 in DMF in a covered container over low heat while stirring continuously. The concentration of P-3500 should be 15% w/w. After the polysulfone is in solution, 2-methoxyethanol is slowly added to produce a solution which has a final concentration of 12.5% 2-methoxyethanol w/w. Other concentrations may be used, i.e., 10%, 15% and 17%. Figure 1 shows the effect on the pressure flow relationship of altering the concentration of 2-methoxyethanol. The final dialytrode solution can be stored for several months if placed in a tightly sealed container and stored in a freezer. Care should be taken in the use of the solution, as humidity causes it to become cloudy and useless.

The diameter of the membranes is determined by the size of the glass dipping rod used in their production. These rods are made by pulling glass over a flame. When the glass cools, calipers are used to determine the section of the rod that has the desired outside diameter. The rod is then cut with diagonal cutters and the end ground and flame polished. Flaming should be done with care to prevent the formation of a small bulb at the end of the rod, which makes the removal of the membranes difficult.

The actual production of the membranes involves dipping the glass rod into the polysulfone, DMF, 2-methoxyethanol solution which is cooled on ice (no water). The cooling increases the viscosity of the fluid, thus facilitating uniform

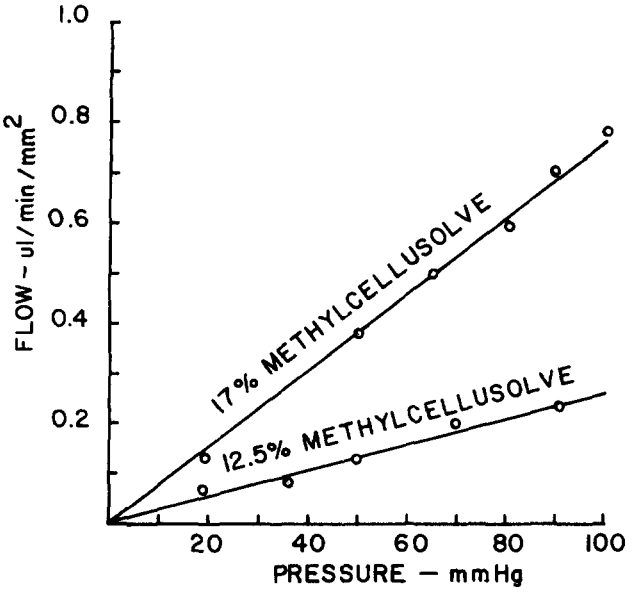


FIG. 1. Pressure-flow relationship of dialytrode membranes composed of 17% methylcellusolve or 12.5% methylcellusolve.

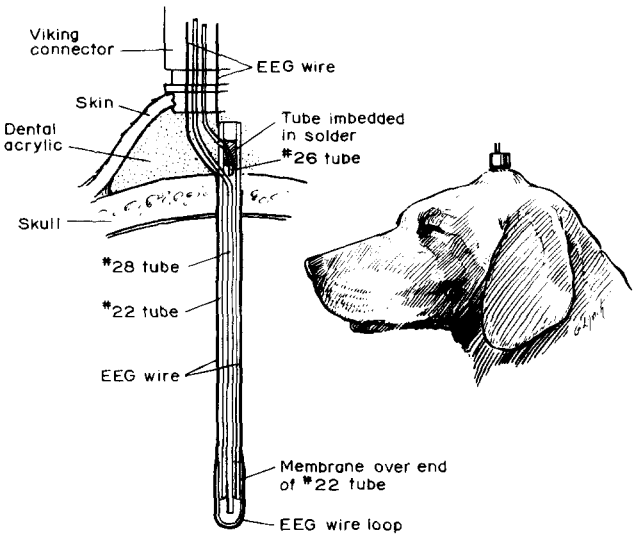


FIG. 2. A diagrammatic representation of the dialytrode membrane connected to the push-pull perfusion cannula. Also shown is the platinum (EEG wire loop) electrode. This is the assembly used for chronic implantation in the dog.

membrane formation. The rod is slowly, evenly withdrawn from the solution, then rapidly immersed in an ice water bath where it allowed to solidify for 1–2 sec. While the membrane is solidifying in the ice bath, the solvents diffuse into the water. The membrane is then rapidly withdrawn from the bath, cut to the desired length and placed in a tepid water bath while there are still solvents present within the membrane; the solvents facilitate the removal of the bag from the glass rod. If the membrane is left too long in the ice bath, it will solidify completely and be difficult to remove from the glass rod. The removal of the membrane can be achieved by slipping it from the glass rod by pushing on the excess polysulfone on the upper part of the glass rod. The entire removal should be done slowly and underwater (room temperature). If the removal is done properly, the cut portion of the membrane will slowly sink to the bottom of the container. If it floats, it should be discarded; membranes that float have low porosity. Once the membranes have been prepared, they should be stored underwater and refrigerated.

The completed membranes are then placed on No. 22 ga tubing that has an inner cannual of No. 28 ga tubing which extends 0.5 mm below the tip of the No. 22 ga tubing. Forty mm above the tip of the dialytrode membrane, the No. 22 ga. tubing is tapped by No. 26 ga tubing. The membranes are attached to the No. 22 ga tubing with silicone rubber adhesive (General Electric RTV 112), which is applied as a thin coat to the tubing and membrane except for the last 1–1.5 mm of the membrane. The adhesive serves to insulate the tubing as well as to fasten the membrane. A piece of platinum wire 0.007 inches in diameter (Medwire Corporation, 121 South Columbus Avenue, Mt. Vernon, New York 10553), from which the teflon has been removed, is placed lengthwise along the entire assembly such that it produces a loop around the tip of the membrane. This serves to protect the membrane and to act as a second electrode, the stainless steel tubing inside the membrane being the other. This is used as a bipolar electrode for recording electrical activity in

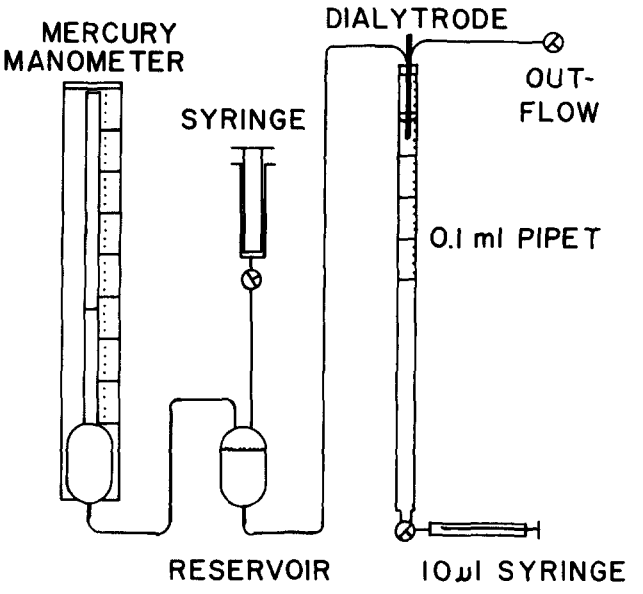


FIG. 3. Apparatus used for the calibration of dialytrodes. Pressure was applied to the reservoir (containing Ringer's solution) and flow through the dialytrode determined as µl/min/mm Hg.

the implanted area during chemical stimulation periods. The wire is fastened to the No. 22 ga tubing with silicone adhesive that has been diluted 50:50 with xylene. Two very thin coats are used over the entire length, except for the membrane. Gold Viking (Viking Industries, Inc., 9324 Topanga Canyon Boulevard, Chatsworth, California 91311) miniature male connector pins are soldered to the platinum wire as well as to a stainless steel wire that has been fixed to the tubing above the No. 26 ga tap. The entire assembly is shown in Fig. 2.

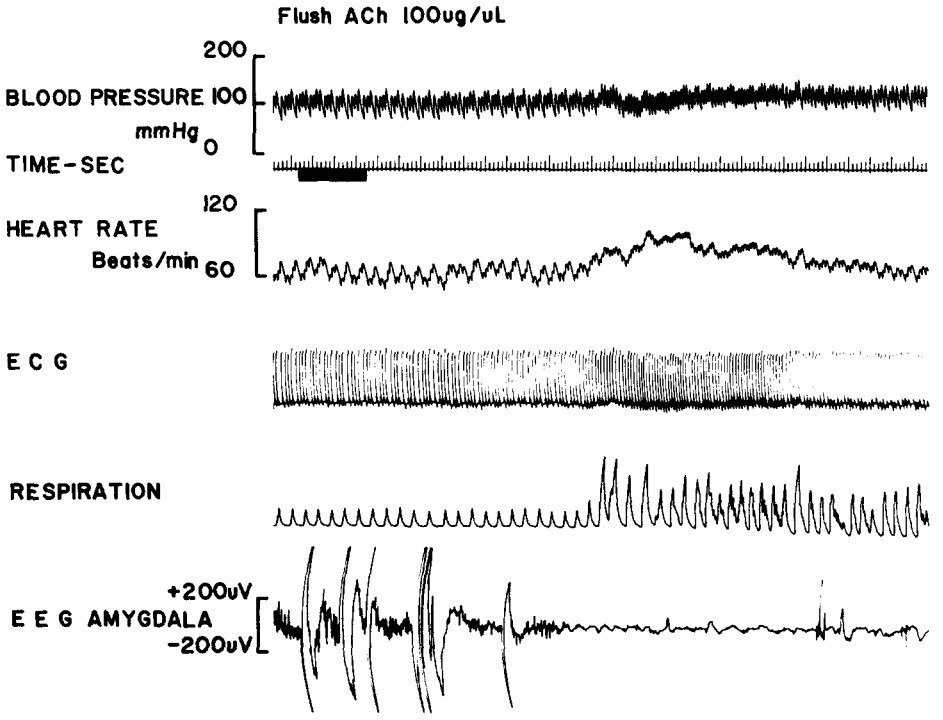


FIG. 4. The effect on blood pressure, heart rate, ECG, respiration and amygdaloid EEG of flushing into the amygdala, Ringer's solution containing 100 $\mu$g/$\mu$l acetylcholine.

Due to the hydrophobic nature of the polysulfone, drying causes complete loss of permeability. There are two ways to restore permeability either partly or completely. The use of 5–10% isopropyl alcohol solutions will partly rehydrate the pores; however, the most satisfactory method is to steam autoclave the dialytrodes while submerged in distilled water. Thirty to sixty minutes of autoclaving are required to restore permeability. As it was necessary in our procedures to have sterile dialytrodes, autoclaving was done just prior to surgery.

Calibration of the dialytrode is done prior to implantation with the apparatus shown in Fig. 3. With this set-up pressure is applied to the fluid inside the dialytrode and the flow across the membrane measured with the 10 $\mu$l syringe shown. The pressure is maintained for periods of 10–20 min to insure accuracy at low flow rates. During the calibration a solution of aniline blue dye was flushed into the dialytrode. This permitted visualization of that part of the dialytrode involved in fluid exchange. Initially calibration of the membranes was performed colorometrically with the aniline blue but since no difference was observed between the colormetric and volumetric calibrations, the volumetric method was routinely used.

Membranes prepared by using 12.5% methylcellusolve had flow rates of 0.0465 ± 0.01 $\mu$l/min/mm Hg. The dialytrode tubing without an attached membrane had a flow rate of 0.846 $\mu$l/min/mm Hg. After implantation for one month, the recalibration of one membrane maintained a flow of 0.049 $\mu$l/min/mm Hg. Since most membranes were damaged during the autopsy procedure, recalibration was not routinely done.

When the dialytrode is implanted, guide holes are first made in the brain that extend to within 0.5 mm of the site to be studied. Even with this precaution, the delicate membranes are occasionally damaged during implantation.

## RESULTS

The dialytrodes were used by applying slight negative pressure with a 1 ml syringe to the outflow tubing while injecting either Ringer's solution or a drug dissolved in Ringer's. When an adequate volume had been flushed through the dialytrode to insure drug placement at the dialytrode tip, the outflow tubing was occluded and 1 $\mu$l of fluid was injected with a 10 $\mu$l syringe connected to the inflow tubing. Injections were usually made over a 10–30 sec interval. After drug delivery, the dialytrode was flushed with Ringer's solution. Figure 4 shows the responses observed in a chronically prepared dog in response to filling the dialytrode (located in the amygdaloid) with Ringer's solution containing 100 $\mu$g acetylcholine/$\mu$l. The recorded parameters are blood pressure, heart rate, ECG, respiration and the EEG activity as recorded from the tip of the dialytrode. The large spikes at the beginning of the EEG tracing are artifacts resulting from handling the dialytrode tubing. In this example, the response to ACh was due to its passive diffusion out of the dialytrode since no volume was actually injected.

Prior to sacrificing the animal, the dialytrode was filled with aniline blue and 1 $\mu$l was injected. Upon histologic examination, it was determined that most of the aniline blue was located within 0.5 mm of the membrane. This is in agreement with the results reported by Myers [1].

## DISCUSSION

Our experience with the dialytrode indicates that

molecules the size of putative neurotransmitters pass easily through the dialytrode membrane into the brain. Delgado [4] found that radioactively labeled gamma amino butric acid not only diffused passively out of the dialytrode but could also be recovered in the effluent from the dialytrode.

We have successfully implanted dialytrodes bilaterally into 16 dogs, some of which were used for as long as two months at which time the experiments were terminated. Histologic examination of the dialytrode sites show gliosis but no indication of infection.

When Delgado *et al.* [4] described the dialytrode and its use, they emphasized that the membranes served as microfilters to prevent the passage of bacteria into the brain. As it is described here, the dialytrode is a modification of the push-pull cannula that can be implanted chronically permitting long-term studies involving chemical stimulation with simultaneous EEG recordings from the site of chemical stimulation. Since the membrane prevents the passage of bacteria, infection at the point of stimulation is no longer of concern.

ACKNOWLEDGMENTS

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