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BRIEF COMMUNICATION

Improved Intracerebral Chemitrode for Chemical and Electrical Studies of the Brain

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KOVACS, D. A., J. G. ZOLL AND C. K. ERICKSON. *Improved intracerebral chemitrode for chemical and electrical studies of the brain.* PHARMAC. BIOCHEM. BEHAV. 4(5) 621–625, 1976. - An intracerebral chemitrode is described for infusion and recovery of solutes as well as stimulation and recording of discrete brain loci. The chemitrode consists of two insulated tubes in juxtaposition with implantable angular ends faced together and covered with a high porosity polycarbonate Nuclepore membrane so as to create a sealed, nonclogging, perfusion chamber. Electrical connectors are affixed directly to the tubes at the external end for stimulating and recording of the perfusion area. Experiments showing electrical stimulation of the chemitrode to produce chronic epileptic foci at the site of perfusion, infusion of drugs to produce changes in brain activity and extraction of ethanol following peripheral injection demonstrate the potential utility of the chemitrode in a wide variety of neurobiological problems.

Brain perfusion Electrical stimulation Kindled focus Epilepsy Infusion-withdrawal Ethanol Drug stimulation of the CNS

NUMEROUS cannulae have been reported for chemical and electrophysiological analysis and alteration of brain activity in experimental animals and humans [2–5, 10, 11, 13, 14, 16]. The most applicable designs consist of two tubes, one for infusion and one for collection, in either a concentric or side by side arrangement. Although the concentric or push-pull arrangement has received wide experimental use and can be constructed to small diameters for the study of very discrete areas, it has a number of serious restrictions: (a) the tip often becomes clogged (although there is apparently some disagreement here, [14]); (b) free flow from clogged tips is achieved usually only after brain damage including edema and contamination of the perfusate with blood and brain cells; (c) contamination of brain tissue from microorganisams in the perfusate; (d) cumbersome designs for adapting this tubing arrangement for electrophysiological studies, i.e., bipolar stimulation or recording at the tip. A concentric arrangement with the tip covered by a porous Silastic membrane has been reported for the infusion of anesthetic gases [11]. This, however, does not allow for communication of polar substances and ions and again is not amenable to electrical studies. We report the use of an improved modification [9] of the side by side cannula arrangement, Fig. 1. Termed a chemitrode, it differs from other designs, most notably the Delgado "dialytrode" [2] in the following ways: (a) easy assembly; and, (b) an essential angular tip configuration which allows for a rigid, thin (5–12 μ) porous Nuclepore membrane (0.1–0.6 μ pore dia. recommended) covering, e.g., polycarbonate. This membrane allows for rapid exchange of solutes by diffusion without the need for a specially constructed bag external to the end of the tubing as in the Delgado model. It also eliminates clogging of the tip and reduces tissue damage during perfusion. The compatibility of polycarbonate membranes in such biological situations as leucocyte [7] and epidermal [12] cell culture and for the

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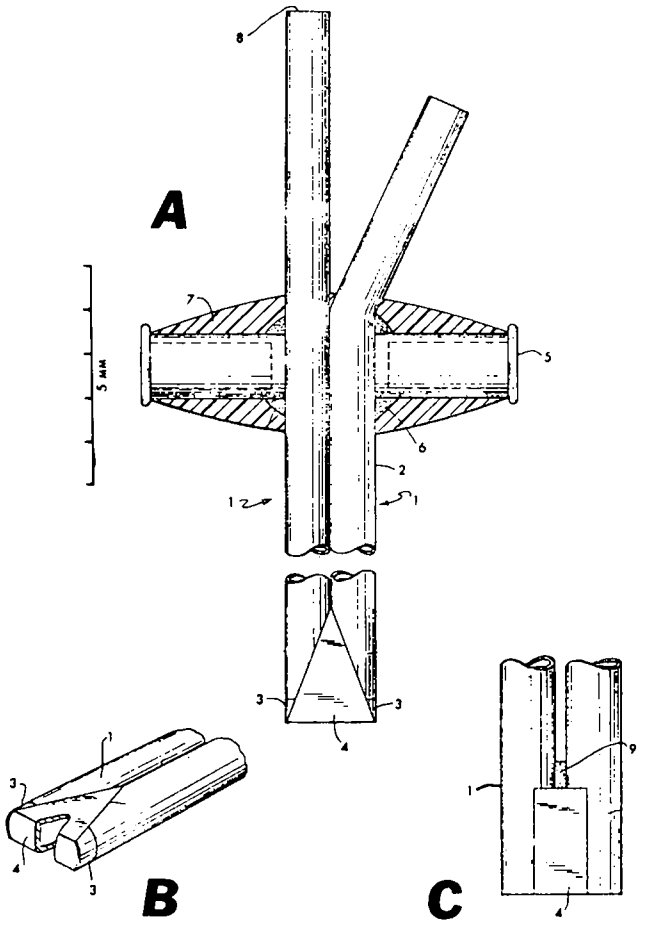

FIG. 1. (A) Side elevation of chemitrode. (B) Perspective view of the membrane covered end. (C) Example of another type of tip configuration. (1) adjacent 21 ga stainless steel tubing; (2) Epoxylite insulating compound; (3) uninsulated portion of tip to provide contact with tissue for recording or stimulating; (4) Nucleopore membrane covering angular tip; (5) Cambion electrical socket connector; (6) electrically conductive adhesive (Emerson and Cuming, Inc.); (7) dental acrylic cement; (8) perfusion port for tubing; (9) epoxy cement.

passage of metabolic products across the membrane [1] has been generally accepted. Direct attachment of electrical connectors to the tubing provides recording or stimulating capability at the site of perfusion. In this paper we present experiments which demonstrate the utility of this device as practical for rapid infusion or extraction by diffusion of solutes into or out of the brain as well as simultaneous bipolar stimulation or recording at the site of perfusion.

CONSTRUCTION

Chemitrodes were constructed from two equal lengths of 21 ga stainless steel tubing electrically insulated with medical grade Epoxylite 6001-M (Epoxylite Corp., Anaheim, Cal.) with the implantable ends of the tubes ground to equal angles (75°). The angle at the tip, though essential, can vary depending on the size and shape of the area of perfusion desired. The two tubes are placed adjacent to one another with the angular portions facing each other so as to allow for fluid communication. A bead of epoxy is applied to hold the two tubes together so as to create a border between the implantable and external portions. One tube is bent at its external end to allow for connection of perfusion tubing. A Nucleopore membrane (Nuclepore Corp., Pleasanton, Cal.) is affixed to the tip with fast drying (5-minute type) epoxy and allowed to cure one week at 50°C prior to implantation. The suggested procedure for applying this membrane is to lay the tubes flat on a surface and apply the epoxy on the exposed surface of the tip. The membrane which is cut so as to exceed the end is applied to the cemented edges and allowed to dry. The tubing is then rotated 180° so as to have the cemented portion of the membrane beneath the tubes and cement is applied to the exposed surface of the tip as before. The membrane is then wrapped over the tip and onto the now exposed side, pulled taut and allowed to dry. Thus the membrane provides, in combination with the tubing, a sealed chamber which does not exceed the general outline of the tubing.

To achieve electrical stimulation or recording of the perfusion area, a small area of Epoxylite insulation is removed at the tip and electrical terminals (Cambion No. 450–3703–01–03, Cambridge Thermionic Corp., Cambridge, Mass.) are mounted directly to each tube using electrically conductive adhesive (Eccobond Solder 57C, Emerson and Cuming, Inc., Canton, Mass.). These electrical terminals are then insulated with easily molded dental acrylic.

The external portion of the tubes providing ports for connection of polyethylene tubing for infusion and collection need not be insulated. These ports are kept free from foreign material by placing tight fitting caps over them. These caps are constructed from short lengths (5 mm) of stainless steel tubing, i.e. 18 ga caps for chemitrodes made of 21 ga tubing, tightly crimped at one end.

APPLICATION

Holtzman Sprague-Dawley female albino rats (200–280 g) were used for the experiments reported here involving ethanol. The remaining studies reported here were performed using female Carworth CFN strain albino rats weighing 200–280 g. Animals were housed in standard large metal or plastic rat cages with 2 or 3 animals per cage following surgery.

Chemitrodes and additional surface and depth recording electrodes were sterotaxically implanted in various brain regions under Innovar-Vet anesthesia. Indifferent stainless steel screw electrodes were chronically implanted in the skull over the nasal region. Animals were allowed a 2 week period of convalescence prior to experimentation. All experiments were conducted without anesthesia in freely moving animals. Perfusions were performed using a Harvard syringe pump with one syringe per chemitrode providing the head pressure. Pump flow rates were compared to the ratings posted on the pump by the manufacturer by filling a pipette and were found to be accurate. Simm's solution (Difco formula), an artificial cerebrospinal fluid, pH 7.2, was used for both infusion of drugs into the brain and extraction of chemicals from the brain milieu. Capacitance pulses to the brain via the chemitrode were made with a Grass S88 stimulator and SIU5 stimulus isolation unit. Voltage and amperage were monitored with a Textronix 4 beam oscilloscope.

Gas chromatography analysis of ethanol in chemitrode perfusates was carried out as follows: (a) Varian gas

chromatograph and Varian recorder; (b) column, Porapak QS, aluminum, 3/8 in. outside dia., 5 ft. long, 195°C; (c) detector, flame ionization, 230°C; (d) injector, 210°C; (e) carrier gas, Helium; (f) standard, external ethanol curve run prior to each day's perfusate analysis. Immediately after collection, perfusion samples were frozen at -15°C for up to one week prior to ethanol analysis. All perfusate samples appeared clear and free of contamination such as erythrocytes and leucocytes.

Chemitrode and electrode tip location and brain condition were determined either from hand sectioned fresh brain or in most cases from 10–15 μ thick paraffin or cryostat sections stained with methylene blue (Fig. 2).

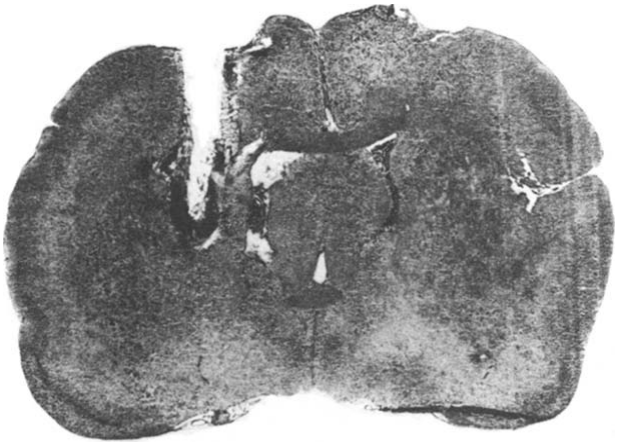

FIG. 2. Micrograph of rat brain showing portion of the chemitrode tract and condition of the brain following perfusion of the caudate nucleus for two hr; 0.6 μ pore Nucleopore membrane. Cryostat cut section, 14 μ thick, stained with methylene blue.

During the development of the chemitrode a number of important points were discovered from the autopsy of rat brains with chronically implanted chemitrodes: (a) necrosis and adherance of brain tissue will occur at the tip if the adhesive used to affix the membrane is not properly cured. Curing at 50°C for one week eliminated this problem. No EEG or behavioral abnormality was observed if the adhesive at the tip was properly cured. (b) For shallow implantations care should be taken to remove the brain meninges from around the point of entry to prevent ingrowth of this tissue around the chemitrode tip. Sterile surgical procedure is recommended. (c) With membranes of 0.6 μ pore and smaller chemitrodes can be implanted for at least six months with no or very minor leucocyte infiltration even after repeated perfusion. Membranes with pore sizes greater than 0.6 μ should not be used for implantations lasting over a few days because of leucocyte clogging. Chronically implanted chemitrodes should be flushed free of perfusion liquid following each experiment to eliminate leucocyte infiltration problems. If it is desired to leave liquid in the chemitrode for extended periods of time, e.g., for the administration of drugs, 0.4 μ pore or smaller Nuclepore membranes are recommended.

Results

Electrical stimulation. Chronic epileptic foci were produced in four rats by daily amygdala stimulation of chemitrodes by the kindling method of Goddard *et al.* [6], resulting in the observance of typical tonic-clinic seizures. Fig. 3 shows afterdischarges following a typical kindling stimulation of the chemitrode at 60 pps, 1 msec duration, 0.5 sec. train, 14V.

Infusion of drugs. Transient foci were produced in 6 rats by perfusion of a variety of epiletogenic compounds through chemitrodes in the ventral hippocampus. Fig. 4 A, B, C shows the effect of acetylcholine, 1 mg/ml, and Fig. 4 D, E, shows the effect of d-tubocurarine, 1 mg/ml, both at a perfusion rate of 0.039 ml/min.

Brain ethanol recovery. In 18 rats, five different brain regions (parietal cortex, n = 6; reticular formation, n = 3; amygdala, n = 4; caudate, n = 2; lateral ventricle over caudate, n = 3) were perfused via chemitrodes (0.6 μ pore membrane) with Simm's solution and ethanol was extracted following intraperionteal injection of 1.0 g/kg ethanol given as a 20% w/v ethanol in 0.9% NaCl solution. Location of the chemitrodes according to König and Klippel [8] were as follows: parietal cortex A 4, H 2.2, L 4.4; caudate A 6.8, H 0, L 2.2; lateral ventricle A 6.8, H 0, L 1.4; amygdala A 4, H–3.5, L 4.5; reticular formation A 0.5, H- 2, L 2. Perfusate samples were collected, 0.019 ml/min, for two hr following ethanol injection. Three 5 min samples were collected for the first 15 min, and 5 min samples were collected at 15 min intervals thereafter. Analysis by gas chromatography showed similar patterns from all regions

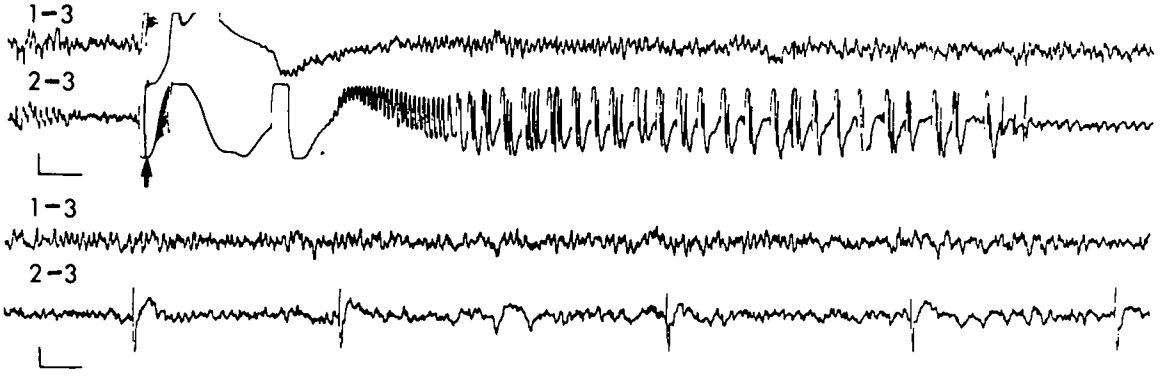

FIG. 3. EEG showing afterdischarges following stimulations of a chemitrode in the amygdala of a rat at the following parameters: 60 pps, 1 ms duration, 14 V, 0.5 sec train duration. Note that spiking does not spread to contralateral surface in these preconvulsive stimulations. (1) parietal surface contralateral to chemitrode; (2) chemitrode in amygdala; (3) indifferent. Calibration: 1 sec, 200 μV. Arrow indicates stimulus pulse.

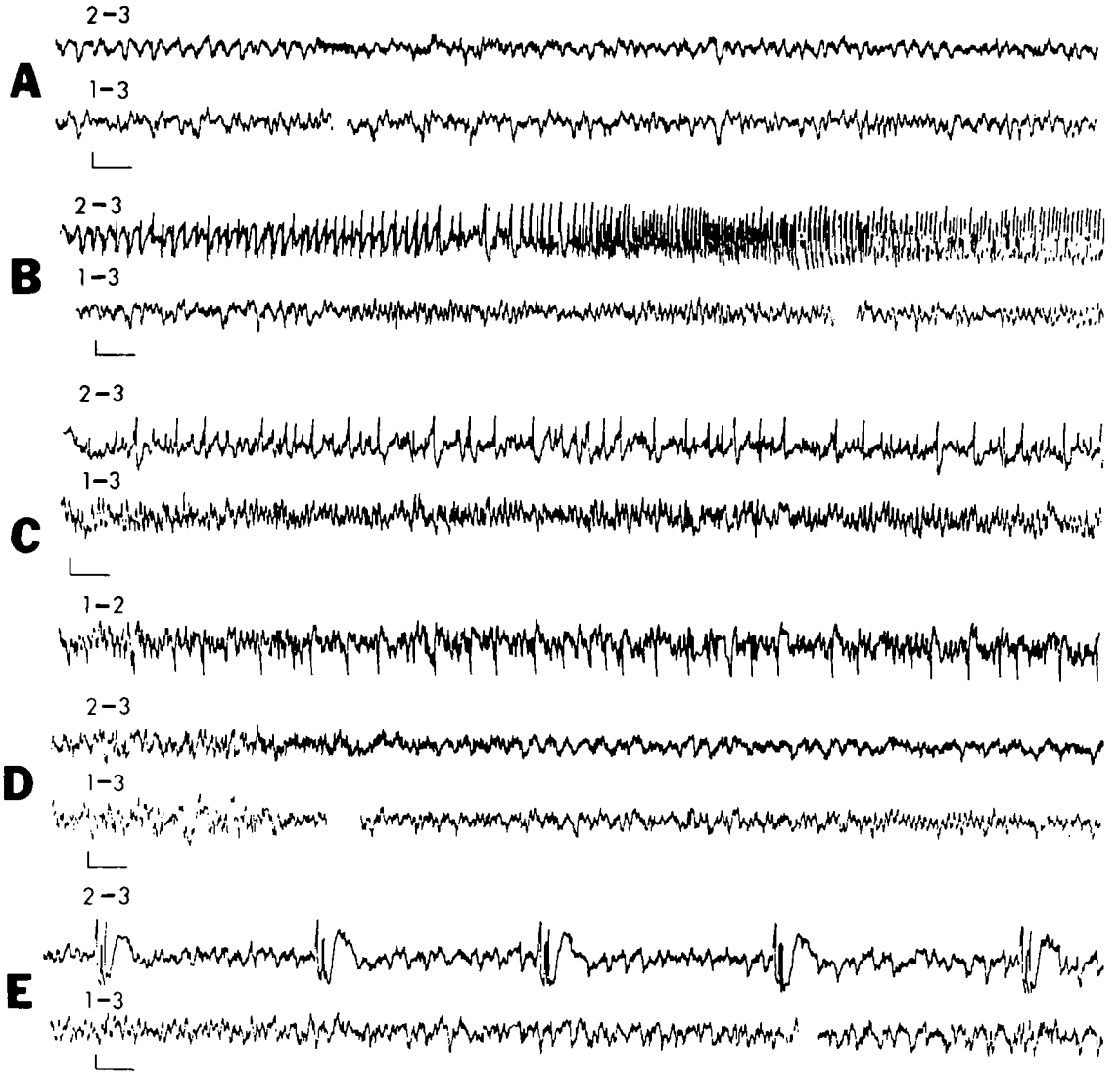

FIG. 4. Development of epileptiform spiking following chemitrode perfusion of the ventral hippocampus (0.039 ml/min) with acetylcholine chloride 1.0 mg/ml and d-tubocurarine 1.0 mg/ml. (A,D) Normal records prior to acetylcholine and d-tubocurarine perfusion, respectively. (B,C) Epileptiform spiking at chemitrode during acetylcholine perfusion. (E) Spiking at chemitrode during d-tubocurarine perfusion. (1) parietal surface contralateral to chemitrode; (2) chemitrode in hippocampus; (3) indifferent. Calibration: 1 sec, 200 μV.

(Fig. 5). The peak quantity of ethanol extracted from the different regions occurred 10–15 min after intraperitoneal injection. The relative quantities of ethanol in the perfusates measured at 15 min were, in descending order, amygdala, lateral ventricle, parietal cortex, and with no significant difference between them the reticular formation and caudate. Comparisons of the 15 min samples were tested for statistical significance by a *t* test for groups of unequal size. Homogeneity of variance among the samples from each brain region was supported by the S^2 max/S^2 min test for samples n = 5 or less [15].

DISCUSSION

The experiments reported in this paper demonstrate the importance of this chemitrode as a sturdy, easily constructed tool for the chemical and electrical investigation of discrete brain regions. Perfusions of drugs are shown to cause changes in neuronal activity which are restricted to the area of perfusion and to cause behavior alteration. The pattern and quantity of brain ethanol in various regions after intraperitoneal injection of ethanol shows the chemitrode's value in studying rapid changes in the chemical milieu of the brain. The chemitrode's value as a stimulation tool is shown in the production of chronic epileptic foci at its perfusion surface by kindling stimulations. In conclusion we mention our use of this chemitrode for the transfer by interbrain perfusion of epileptiform abnormality from seizure foci of donor rats to normal recipients (to be published). This suggests the feasibility of using this device in biochemical and electrophysiological studies of chronic brain diseases.

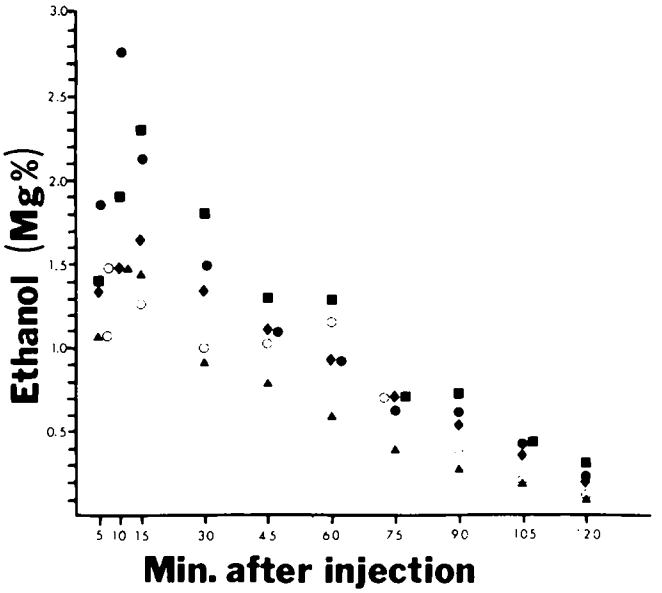

FIG. 5. Average ethanol recovery from 5 brain regions perfused with Simm's solution (pH 7.2, 0.019 ml/min for 5 min each time period) via chemitrodes (0.6 μ pore size membranes) following intraperionteal injection of 1 g/kg ethanol as a 20% w/v solution in 0.9% NaCl. ◆, parietal cortex, n = 6; ●, lateral ventricle over caudate, n = 3; ■, amygdala, n = 4; ▲, caudate, n = 2; ○, reticular formation, n = 3. Homogeneity of variance for each brain region was supported by the s^2 max/s^2 min test, $p>0.05$, for samples n = 5 or less [15].

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