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Perfusion Techniques

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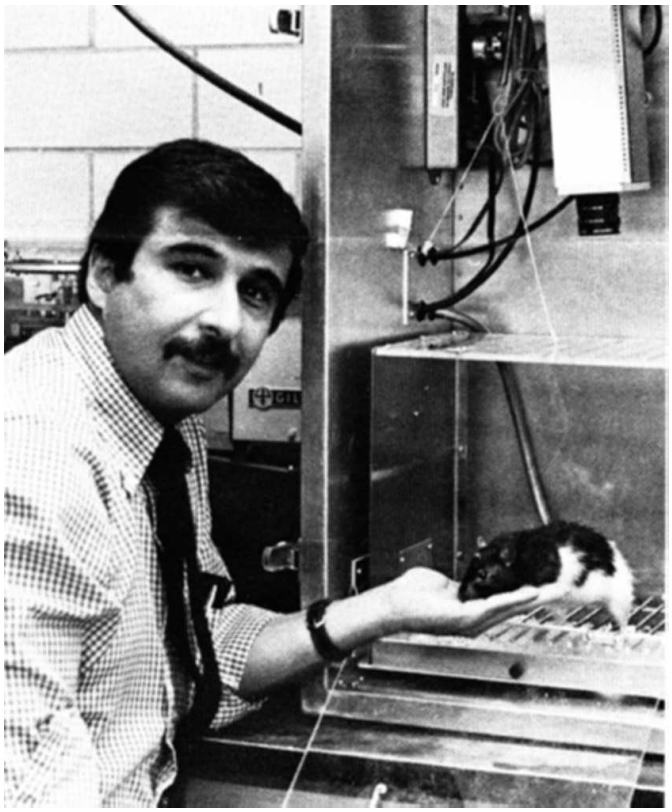
Since our initial interest in utilizing push-pull brain perfusion techniques in conjunction with objective behavior analysis, so that drug-neuro-chemical-behavior studies could be performed concurrently,[1] we have tried to devise a reliable and versatile system. For obvious reasons, it was felt that all stainless steel devices were most desirable, especially for chronic studies in which experimental subjects would be used over extended periods of time. To this end, we have recently started making our own caps and modifying commercially available stainless brain injection cannulas for use as bases. The technique is quite simple and adaptable for many types of experiments. This short description outlines the construction of our cannulas, the per-fusion system we find most reliable, and one method of analyzing perfusate for the catecholamines and their metabolites after either pulse labeling brain with a trace dose of transmitter or continuous perfusion of brain with a solution containing trace quantities of these substances. Since most of ,the experiments performed to date have involved the push-pull perfusion of the lateral ventricular space of rats, construction of a relatively larger cannula is described. We have successfully perfused lateral ventricles, as well as solid tissue, with proportionately smaller devices, indicating that alterations in tubing size can be made easily without affecting function to any significant degree.

We modify David Kopf cannula bases as follows: First, a substantial amount of the skirt is trimmed from the base and the protrusion from the hole in the bottom is filed off. Then a length (approximately 2.5 cm) of 20 gauge thin wall stainless steel needle tubing is secured with Loctite[2] retaining compound (No. 35) just inside the wall at the bottom of the base with most of the tubing extending through the hole in the base. The tubing is then trimmed to extend 6 mm beyond the base. Silicone (usually 2) O-rings[3] (size 32-55) are fitted into the base. Styluses and infusion or perfusion caps are made from 3s inch No. 6-32 socket head stainless steel screws.



An appropriate sized hole is drilled through the center of each screw and a piece of appropriate size stainless steel wire or tubing is cemented with Loctite into the hole. The wires are then cut otf so that they extend just beyond the tip of the thin wall stainless tubing when screwed down firmly within the base. An infusion cap is made by cementing a piece of 23 gauge thin wall tubing inside the drilled out screw. The infusion cap is cut to extend 0.5 mm beyond the base. Perfusion caps are made by cementing a 23 gauge thin wall pull tube and a 30 gauge push tube (the top of which is reinforced by cementing over it a piece of 23 gauge thin wall tubing) inside a drilled out screw. The pull tube extends no more than 0.5 mm beyond the bottom of the screw; the 30 gauge part of the push tube is cut off so that it extends 0.5-0.75 mm beyond the 20 gauge thin wall tubing cemented to the base when screwed down firmly. Both tubes are cut off 0.5 cm above the screw top and the pull tube is bent away from the push tube. This separation of the two tubes is neces-

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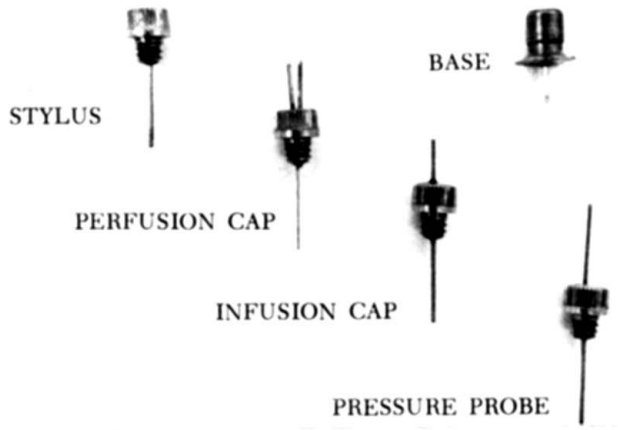

FIGURE 1

FIGURE 2

sary so that silicone rubber tubing can be slipped over both tubes for perfusing. The pressure probe is modeled after one described by Dismukes and Snyder[4]. A piece of 23 gauge thin wall tubing is secured in a drilled out screw. Using a wire cutter, the tubing is crimped closed and cut off so that it protrudes 1 mm beyond the end of the base. Using a fine ruby stone a slit is made just above the crimped end. See Figures 1 and 2 for parts and assembled bases and caps described.

The cannula base is implanted into the lateral ventricle or specific brain regions of an adult rat, using stereotaxic coordinates for reference. The animal is initially injected with 45 mg pentobarbital/ kg body weight and is subsequently maintained under anesthesia with methoxyflurane, if necessary. The fur is then clipped from the top of the animal's head, and he is mounted in a David Kopf rodent stereotaxic unit, with the incisor bar 5 mm above the center of the ear bars. An incision is made from between the eyes back to behind the ears, the skin is retracted, and the skull is scraped free of tissue. The coordinates of the lambda and bregma are noted and for ventricular implantation, a hole 0.5 mm posterior and 1.7 mm lateral to the bregma is made with a centering bit mounted in the Kopf stereotaxic hand drill attachment. Two-three other holes are drilled on the left or right side of the skull contralateral to the hole for cannula implantation, for anchor screws. The holes are then re-drilled with a .035 inch drill to accommodate the screws and cannula base. The anchor screws (No. 0-80, }s inch) are screwed securely (approximately
1 mm) into the skull. The dura is pierced with a sterile needle and the cannula base with the pressure probe in place is lowered into the brain while attached to a standard Kopf electrode holder. A piece of PE-50 tubing which has been attached to the top of the pressure probe and filled with sterile saline to a height of about 5 cm above the base is clamped on top. When the tip of the probe is lowered into tissue, the clamp is released and the miniscus of the saline column is observed with further lowering of the cannula. When pulsation of the saline column corresponding to the animal's respiration is observed, the tip of the cannula is in the ventricle. A mound of dental acrylic cement is built up and around the base and the anchor screws, securing the base to the skull. After the cement has hardened, the pressure probe is removed from the base and replaced with the stylus and the wound is sutured closed around the base. After at least one week to recover from the surgery, with food and water available ad lib, the animal is deprived to 80% of his free-feeding weight and shaped on an operant schedule of reinforcement. Most of the perfusion work to date has been with ratio schedules, although interval schedules have proven amenable to perfusion studies. He is then habituated to having the perfusion tubes and cap attached to the cannula base during operant sessions.

It should be kept in mind that most of our studies of effects of drugs on metabolic disposition of transmitters were performed upon animals engaged in complex behavior maintained by operant schedules of reinforcement, while their brains were being perfused. Acute or chronic studies of conscious animals are obviously possible, without having to invest the time in behavior analysis. However, the animals' attendance to the bar-pressing task decreases random circling and exploratory behavior which may lead to twisting or tangling of the tubing. Addition

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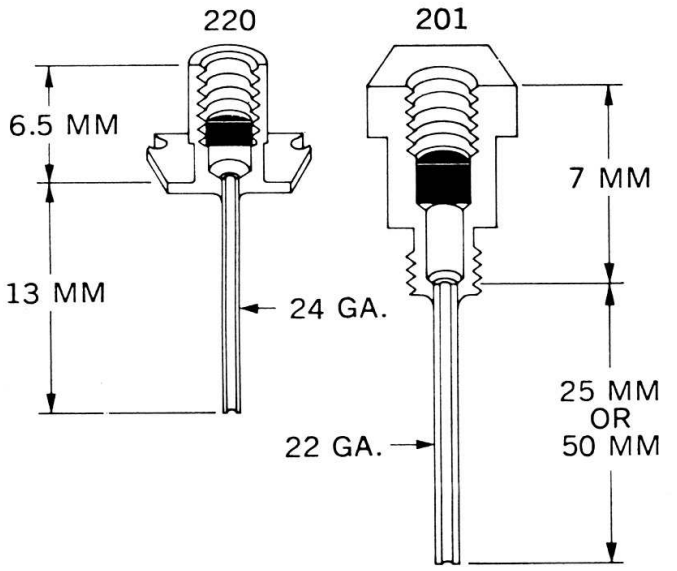

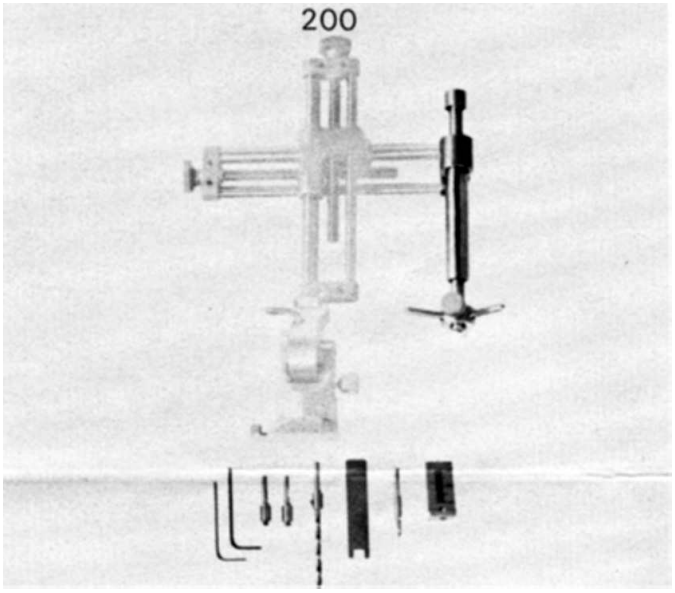

200 CANNULA INSERTION KIT

The 200 insertion kit is used for placing cannula on precise stereotaxic coordinates. The positioning, drilling and inserting tool is easily attached in place of the electrode holder for any of the current model screw driven carriers or manipulators.

201 and 220 CANNULA (Stainless Steel)

The 220 Cannula is designed for use in rats and other smaller animals. The 201 is designed to be used in cats, monkeys, rabbits and dogs.

Both the insertion kit and cannula are available as shown. Parts for the insertion kit may be purchased separately, and cannula hubs as described in Dr. Sparber's article, may be purchased separately.

NOTICES

In the April, 1975 Carrier, page 6, line 7, ... should read ... It does not have any built in facility for automatic application . . . and line 10 should read ... It is not suitable for systems involving more than three or four iontophoresis barrels.

The following laboratory and/or research manuals are now available from the publisher's:

Experimental Neuropsychology, Benjamin L. Hart, Ph.D., University of California, Davis. Published by W. H. Freeman and Company, San Francisco, Calif.

Neuroscience, James E, Skinner, Ph.D., Baylor College of Medicine, Houston, Texas. Published by W. B. Sounders Company, Philadelphia, Pa.

Principles of Research Methodology in Psysiological Psychology, William G. Webster, Carlton University, Ottawa, Canada. Published by Harper and Row, New York.

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ally, the complex behavior affords the experimenter the option of implying that physiological conditions are maintained under these circumstances.

We are currently using a four channel peristaltic pump (Gilson Minipuls II)[5] (see figure 3) to control both infusion and perfusion. The pump is connected to the perfusion cap by silicone tubing (Dow-Corning Silastic).[6] This tubing offers the advantage of being autoclavable and can stretch considerably without ripping or changing perfusion characteristics. We do not use polyvinyl chloride (Tygon). It seems that there may be alterations in responsiveness of the CNS to amines, like dopamine, after perfusing them a few times with a system containing polyvinyl chloride tubing. One piece of tubing connects the push tube of the cap through a 0.060 inch manifold tube in one channel of the pump to a reservoir containing perfusion medium. Another piece of tubing connects the pull tube of the cap through a manifold tube in a second channel in the pump (in the opposite direction) to a miniature fraction collector. Enough tubing is left between the cannula and the pump to allow the animal free movement in the Skinner box when he is attached to the perfusion cap. The two pieces of tubing between the perfusion cap and the pump are cemented together with Silastic Medical Adhesive. This strengthens the tubing and keeps them from becoming tangled. For infusion, a single piece of tubing is connected through a third channel of the pump to the infusion cap.

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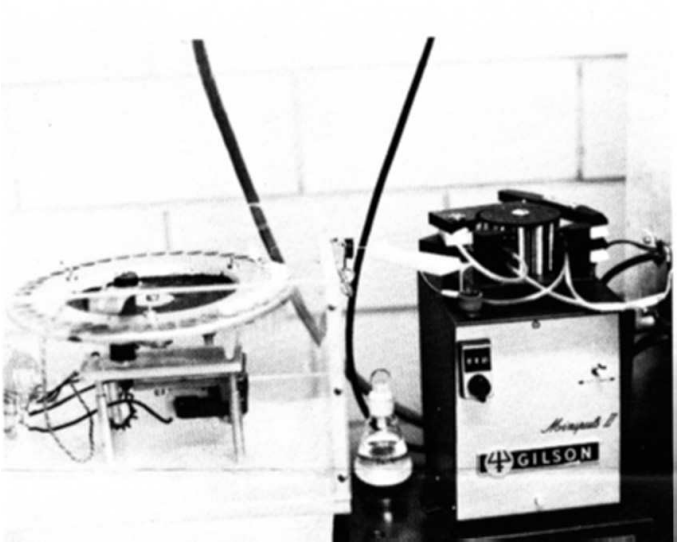

FIGURE 3

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On the day of a perfusion session, the animal is infused over a 5 minute period with 5-10 ul of labeled compound through the infusion cap. Following the infusion, the infusion cap is replaced with a stylus, and the animal is returned to his home cage. After an appropriate time interval, the animal is connected to the perfusion apparatus and put into the operant chamber where reinforcement is available on a fixed ratio schedule of reinforcement. When the animal receives his second reinforcer, the perfusion pump is started. The perfusion is carried out with appropriate medium, at a rate of 10-15 ul/min. After at least 3 samples are collected, the animal is injected i.p. with saline or drug solution f 1 mg/kg; e.g. when 5 minute samples are collected, the injection occurs in the first half of sample number 4).

The 5 minute samples are collected into polyethylene microsample tubes containing 10 ug each of 13 cold carriers in 10 ul of 1 N formic acid. When we infuse radiolabeled catecholamines, we include the following precursors and metabolites as cold carriers for subsequent TLC separation: DOPA, DA, DOPAC, HVA, 3-MT, NE, NM, VMA, DOMA, DOPEG, MOPEG, DOPET, and HVET. After approximately 45 minutes of perfusion, the behavioral session is terminated, the perfusion cap is removed and replaced with the stylus, and the animal is returned to his home cage. The perfusion pump is left operating until the entire perfusate has been collected in sequential 5 minute samples. Ten (10) ul aliquots of each sample are counted in appropriate liquid scintillation cocktail and corrected for quenching by use of an external standard. We further analyze the sample before injection (e.g. No. 3) and several samples after injection (e.g. No. 4, No. 6, No. 8). Two 10 ul aliquots of each of these samples are spotted on microcrystalline cellulose TLC plates. One aliquot is spotted in the lower-left hand corner of the plate 2.5 cm from the edges (origin), and the other aliquot is spotted in the upper right-hand corner where it will not be exposed to the solvent systems, except their vapors. The plates are then developed 13-15 cm in each direction in the two-dimensional chromatographic system described by Fleming and Clark[7]

We allow 0.5 hour drying time under cool air between solvent systems. After developing, the separated compounds are visualized with p-nitro-aniline spray and resulting spots (plus the origin) are scraped and oxidized in a (BMO) biological material oxidizer and collected as tritium water. All samples are counted to a 3% counting error and dpms are calculated using the correction factor for the BMO and counting efficiency. Standard plates are also run and analyzed. An aliquot of the labeled compound used for infusion is spotted in sterile saline or other medium along with, cold carrier in formic acid and run through the separation and oxidation procedures. These standard plates are then used as blank plates and relative amounts of blank or background counts on appropriate metabolites and precursors are subtracted from dpms observed after perfusing. The data thus collected can be examined in several ways. We routinely determine radioactivity in each sample for the entire perfusion session. As mentioned previously, several samples are analyzed for individual metabolites.

We have more recently perfused brain with trace concentrations of radiolabeled dopamine. This enabled us to examine effects of drugs upon this transmitter. It was necessary to perfuse instead of infuse DA, since its rapid metabolism and elimination from brain made it difficult to infuse trace doses and follow metabolism for extended periods of time (i.e. 0.5-1 hour). We have observed significant amounts of HVA and DOPAC in perfusate within 10-20 minutes of initiation of perfusion. No neutral DA metabolites were observable. It now appears that? t presentation to various brain structures with varying concentrations of transmitters, drugs, hormones, etc. is feasible with this technique.

FOOTNOTES

1. Sparber, S. B., Neurochemical changes associated with schedule-controlled behavior. Fed. Proc. 34(9); 1802-1812, 1975.

2. Loctite Retaining Compound 35 Catalog # 35-31
Lockquic Primer Grade T Cut #47-56
Loctite Corporation Newington, Connecticut 06111

3. O-rings Silicone Compound 19711
Precision Associates, Inc. 740 Washington Avenue N. Minneapolis, Minnesota 55401

4. Dismukes, K. and Snyder, S. H., Histamine turnover in rat brain. Brain Res. 78:467-481, 1974.

5. Gilson Medical Electronics, Inc. P.O. Box 27 3000 W. Beltline Highway Middleton, Wisconsin 53562

6. Tubing Silastic Medical-Grade Tubing Dow Corning Corporation Medical Products Midland, Michigan 48640

7. Fleming, R. M. and Clark, W. G., Quantitative thin-layer chromatographic estimation of labeled dopamine and norepinephrine, their precursors and metabolites. J. Chromatog. 52:305-312, 1970

ACKNOWLEDGEMENT

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