Pharmacology Biochemistry & Behavior, Vol. 18, pp. 791–795, 1983. © Ankho International. Printed in the U.S.A.

New Multi-Cannula Pedestal Device for Micro-Injection of Drugs into Brain Tissue or Cerebral Ventricle

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Received 7 December 1982

HEPLER, J. R. AND R. D. MYERS. *New multi-cannula pedestal device for micro-injection of drugs into brain tissue or cerebral ventricle.* PHARMACOL BIOCHEM BEHAV **18**(5)791–795, 1983.—A new multiple-cannula-pedestal system for micro-injection of drugs directly into either brain tissue or cerebral ventricle is described. Its features include ease of construction from commonly available materials, no specialized machining required, durability and economy. A special aspect of the cannula system is a protective cap containing a bolt which threads onto a nut fixed within the pedestal base. Since the cap cannot be dislodged, potential damage to indwelling stylets and exposed guide tubes is prevented. Moreover, an aseptic preparation is therefore provided so that test compounds can be infused repeatedly over a prolonged period. Finally, the protective caps are interchangeable and the pedestal base itself can be re-cycled for usage in different animals.

Micro-injection of drugs Brain cannulation Cerebroventricular administration Cannula system
Neuroactive chemical Chemical stimulation of brain Drug delivery procedure

DURING the last 25 years, a large number of cannula devices have been designed for the administration of a drug or other chemical compound to a specific region or structure of the brain [10]. In most cases, the design of the cannula system has permitted repeated injections directly into the brain's parenchyma or cerebral ventricle of the unanesthetized animal [9,15], with durability for chronic usage being a principle hallmark of the device [11]. Many of these cannula systems are relatively easy to construct of inexpensive materials that are readily available [1, 3, 6, 7, 13]. Other systems incorporate specialized features including dual guide cannulae for bilateral micro-injection [4,14], ruggedness under circumstances of multiple housing of animals [5], their adaptation to different species [8] and procedures to prevent de-cannulation [16].

Although the purpose and principles of the cannula system described in this paper correspond to those of the past, recently it has become apparent that additional factors can enter into the experimental requirements for the long-term use of an intracerebral cannula [11]. For example, it is well known today that typical laboratory rats can be susceptible to a variety of different bacterial infections, presumably because of extensive genetic inbreeding. In fact, introduction into cerebral tissue of bacteria or other foreign matter can often cause localized adhesions and other pathological sequelae [2] which subsequently interfere with the outcome of a series of micro-injections given repeatedly over time [11].

The present cannula system employs a pedestal fitted with a protective cap which cannot be dislodged by the animal. In this case, the guide tubes for insertion of the injector needle are maintained in a sterile and particle-free condition. The cannula system incorporates ease of assembly, using commonly available material, with durability and no need for specialized machining. In addition, the protective caps are interchangeable, and the pedestal base, when retrieved from the animal, can be re-used in a different animal. Finally, the multiple cannula array can be positioned within the pedestal at different points of lateral and coronal separation so that a given compound can be infused at stereotaxically distinct sites.

MATERIALS AND CONSTRUCTION

Although adaptable for use in most species, the cannula system as described here is intended for implantation in the laboratory rat. The guide cannulae can be separated laterally by a variable number of millimeters, but in this prototype example, the distance selected was 3.0 mm between each. Further, the depth of the implant can also vary, and in this example a length is illustrated for cannulation of the rat's cerebral ventricle.

Pedestal Assembly

Each guide tube is cut (Fig. 1, step 1) from thin-walled

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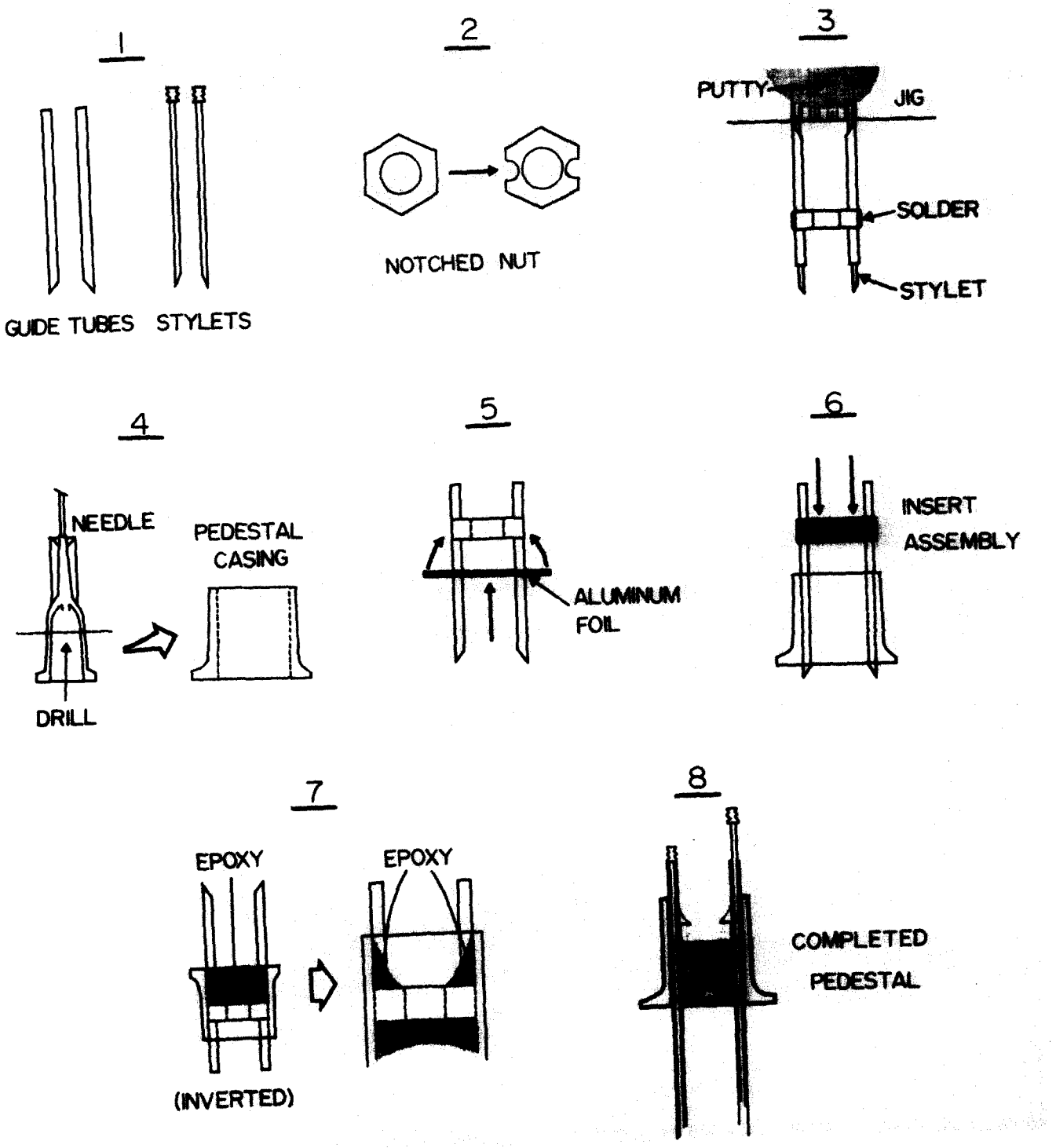

FIG. 1. Eight principal steps illustrated schematically for the construction of the cannula-pedestal base. The dimensions, materials and other details for each of the components are described in the text. In step 3, Epoxy Steel can be substituted for solder.

stainless steel tubing (Perfektum Popper) to a length depending upon the particular structure in which an infusion or a micro-injection is to be made. For example, a 23 gauge guide cannula is cut to a length of 15.0 mm for experiments in which an intracerebroventricular infusion is to be given. The internal tip of the guide tube in either case is beveled on a carborundum wheel to an angle of 45° to 60°. An indwelling stylet is cut from 27 gauge regular-walled tubing and beveled to a point so that their lengths are identical (Fig. 1, step 1).

A stainless-steel #1-64 nut (Star Stainless Screw) is used

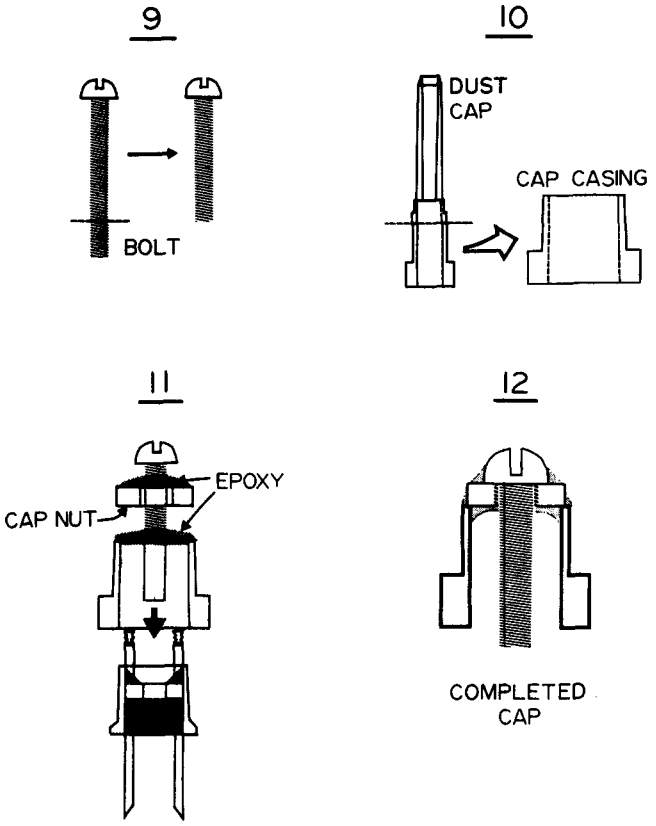

FIG. 2. Schematic diagrams illustrating steps 9 through 12 for the construction of the protective cap which fits onto the cannula pedestal depicted in Fig. 1.

(Fig. 1, step 2) not only to determine the spacing between two bilaterally positioned guide tubes but also to serve as the receiving and fixing nut for the bolt contained in the protective cap. The nut itself is placed in a small vise, and the opposing sides of the hexagonal nut are notched with an ordinary hacksaw blade (Fig. 1, step 2). Using a plastic jig with parallel grooves cut at 1.0 mm intervals to accommodate stainless steel stylet tubing (Fig. 1, step 3), the specific mm separation of the guide cannulae is selected.

After individual stylet tubes are placed in the grooves of the jig, they are held in place with pliable putty. Then the cannulae to be fixed on either side of the nut are slipped onto the guide tubes. The notched nut is positioned approximately 4.0 mm from the external tips of the guide cannulae (Fig. 1, step 3) in such a way that it is precisely perpendicular to the cannulae themselves. A small amount of 10% hydrochloric acid flux is painted onto the adjacent surfaces of the nut and guide tubes to be joined. Next a stainless-steel non-flux solder (60–40 tin-lead) is applied to the joints, using a fine-tipped soldering iron, so as to hold both the nut and the guide tubes firmly in place (Fig. 1, step 3). Excess solder accumulated at the joint is removed carefully by grinding on a carborundum wheel.

As an alternative to the solder, an epoxy cement designed especially for metal-to-metal bonding can be used. In this case, Epoxy Steel (Magic America Chemical Corp.), when applied to the cannula-nut interface, will dry to hardness in two hrs if low heat is applied to speed the curing process.

The pedestal casing is comprised of the base of a disposable syringe needle. A number 11 drill-bit attached to a hand-held power drill is used to drill out the internal portion of the needle casing. After the needle portion is clipped away (Fig. 1, step 4), the needle end of the base is ground down by a carborundum wheel until the height of the casing is 6 mm. Thus, the inside diameter of the pedestal casing will readily accommodate the soldered nut-guide tube assembly.

The internal portion to be implanted of both guide tubes is pushed simultaneously through a sheet of aluminum foil (Reynolds Wrap) (Fig. 1, step 5) until the foil covers the nut. The aluminum foil is then cut carefully by scissors in a circle so that its circumference is slightly larger than that of the nut. At this point, the entire assembly is inserted through the top of the pedestal casing (Fig. 1, step 6) so that the external tip of each guide tube extends precisely 2.0 mm above the top of the casing. The internal portion of the guide tubes thus extends 7.0 mm beyond the bottom of the pedestal casing (Fig. 1, step 6), so that the tips would just reach the dorsal edge of the cerebral ventricle.

Once the guide tube assembly has been properly positioned within the confines of the pedestal, the casing is inverted (Fig. 1, step 7). Then its internal cavity is filled either with rapidly setting epoxy resin cement (e.g., Davcon "5 Minute") or, if time permits, a longer setting (24-hour) cement such as Araldite (Ciba-Geigy). After the epoxy cement has set and dried, the entire pedestal is inverted again (Fig. 1, step 7). Additional epoxy cement is applied very carefully with a fine stainless steel needle to the internal space (Fig. 1, step 7) between the wall of the casing and the external surface of the nut. This provides a secure and complete bond at the exposed junction of the nut and casing. Special care should be taken to ensure that the cement does not occlude the internal threading of the receiving nut.

This completes the assembly of the pedestal base (Fig. 1, step 8).

Cap Assembly

The main feature of the cap is that it is secured to the pedestal base by an internal bolt. The threaded shaft of a 0.5 inch #1-64 flat-headed stainless-steel bolt is cut with a fine-bladed hacksaw so that the overall length beneath the head is 9.0 mm (Fig. 2, step 9). Rough fettlings are removed by careful grinding or emery cloth.

Next the length of the dust cap of a disposable syringe needle is reduced to approximately 9.0 mm by grinding on the carborundum wheel (Fig. 2, step 10), with special care taken to ensure that the top portion is perpendicular to the sides. A #2-56 stainless-steel nut is next slipped over the threading of the smaller #1-64 bolt (Fig. 2, step 11) and the two are then inserted into the cap casing. When properly positioned, the exposed threading of the #1-64 bolt extends inside the cap casing, whereas the internal face of the #2-56 cap nut rests evenly on the external top surface of the cap. In this way, the #2-56 cap nut serves as a spacing washer (Fig. 2, step 11).

Prior to the application of cement to the bolt, a test-fit is made so that the bolt, when inserted into its pedestal (Fig. 2, step 11), will catch the threads of the indwelling receiving nut. A jeweler's screw driver is used to adjust the position of the bolt. Further adjustment to the depth of the bolt can be made

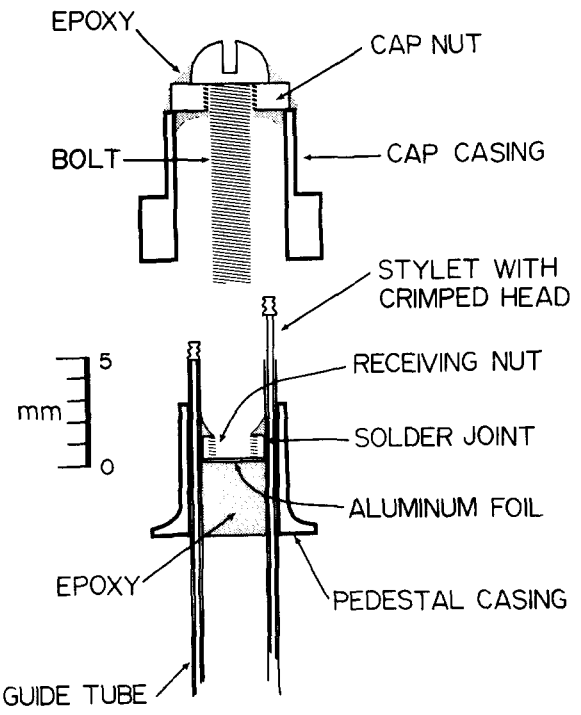

FIG. 3. Components of the cap and cannula pedestal with dimensions according to the millimeter scale (left). The schematic diagram illustrates the position of a stylet (right) as it is removed from the guide tube.

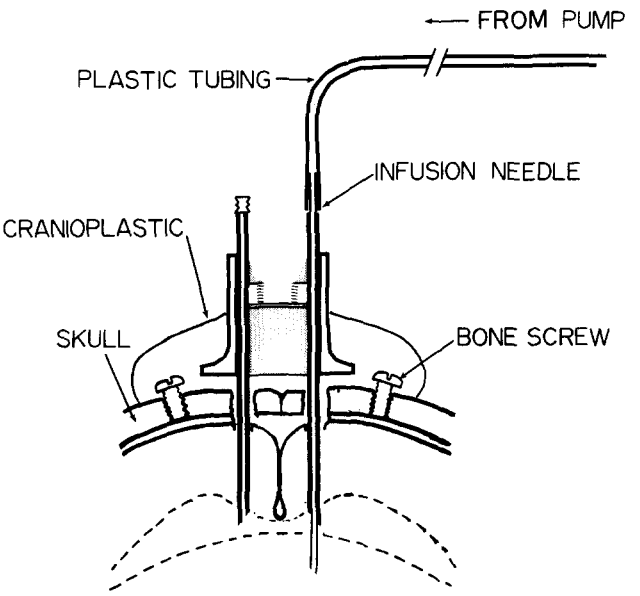

FIG. 4. Pictorial diagram of cannula device affixed to the cranium of a rat by cranioplastic cement and indwelling bone screws. An infusion needle has been lowered through the guide tube, after removal of the stylet (Fig. 3), into the brain's parenchyma for a tissue micro-injection.

simply by grinding off a small portion of the external portion of the cap.

Once it is clear that the bolt and nut will fit together properly (Fig. 2, step 11), epoxy resin cement is carefully and liberally applied with a fine wire to both the inner face of the nut and the bottom of the screw head as well as to the top of the cap (Fig. 2, step 11). Before the cement is permitted to set, the cap is screwed onto the corresponding pedestal base and inverted immediately so as to prevent any back-flow of the epoxy into the pedestal. A 1.0 hour period of drying of the cement concludes the cap assembly.

Figure 3 illustrates such a completed cap as it is ready to be inserted into its corresponding pedestal. The scale of Fig. 3 provides the millimeter references for both the spatial relationships and dimensions of each of the components.

Construction Hints

In the assembly of the pedestal base, it is essential that the receiving nut is perfectly parallel to the base, so that the bolt is readily threaded onto it. A slight angulation of the nut would limit the rotation of the bolt to only one turn or less, consequently favoring dislodgment of the cap. To secure the cap firmly to the base, the cap's bolt has to enter the pedestal receiving nut by two or three turns.

When the joints between the guide cannula and the grooves of the receiving nut are either soldered or cemented with Epoxy Steel, it is important to ensure that the bond is complete. In the case of soldering, this is readily accomplished by the use of a hot and finely-tipped soldering iron in conjunction with non-flux solder. When the cannulae-nut assembly is positioned within the plastic base (see Fig. 1, step 6), a weak solder joint could result in a bending or loosening of the cannula from the nut so that the tubes would not be stereotaxically parallel. A distinct advantage of Epoxy Steel is that this difficulty is eliminated.

In the final adjustment of the cannula cap, it is important to note that the bolt is extended 1.0–2.0 mm below the bottom portion of the plastic cap itself. This in turn will prevent too tight a friction fit of the cap to the pedestal base, which could result in the separation of the bolt from the plastic base.

If mass production procedures are used for the assembly of the cannula system, as many as 10 or 12 cannula devices can be constructed by a skilled individual in a single afternoon. Although the prototype example utilizes a disposable syringe and needle as its main components, other readily available materials can be utilized according to a specific application and/or a required stereotaxic distance between cannulae. If the separation of the cannulae is to be 5.0 mm, for example, the dust cap from a Unopette capillary pipette (Becton-Dickinson) is used as the pedestal base, which is simply fitted with a larger receiving nut (#2-56). Similarly, the pedestal cap is cut from a disposable plastic Micro-Test Tube (Eppendorf) with a corresponding #2-56 cap bolt inserted into its top. Thus, whatever materials are selected, the principles of construction illustrated by Figs. 1 and 2 are identical.

DISCUSSION

The same surgical procedures and scientific principles for micro-injection of a drug, chemical or other substance into the brain should be followed [10] when this cannula device is

used. These include: the use of anchor screws and cranioplastic cement during implantation of the pedestal; a calibrated infusion pump; a microliter syringe for ejecting minute volumes; the monitoring of the miniscus of a bubble of air inserted into the PE tubing line; and the aseptic precautions required for maintaining a viable ventricular or tissue preparation [11]. Figure 4 illustrates diagrammatically the position of an infusion needle inserted into a guide tube affixed within the pedestal. After the infusion is completed and the needle removed, the cap is replaced as described earlier.

Once initial practice is gained in the construction procedure, the cannula system will provide a flawless experimental technique. For example, it is virtually impossible for the cannula cap and the underlying stylets to be dislodged if the cap is screwed on by two or three full turns just to the point of friction fit. In over 75 rats that have been prepared with this cannula system no evidence of infection, trauma, pathology or occlusion of a guide tube has arisen. Thus, an aseptic, contaminant- and dust-free preparation can be maintained for an indefinite period during which time the animal may be tested repeatedly. In this connection, it is important to note that ethanol and other hydrocarbons used for sterilization can soften and structurally weaken epoxy cement; thus, 70% ethanol or other sterilizing solution should not saturate the epoxy cement bond in either the cap or pedestal base.

A distinct advantage of this cannula device over previous designs [9,12] is that the pedestal base and its opposing cap do not require threading by tap and die. Other than the use of a hacksaw for notching the receiving nut in the pedestal base, no special hand tools are required. Another advantage of the system is that when properly constructed, the caps are readily interchangeable. In addition, the cannula base can be re-used without difficulty. Once it is removed from the skull of the animal, wire side-cutters are used to remove the cranioplastic cement with care taken not to bend the guide tubes.

Finally, with the usage of a specific stereotaxic configuration, a number of guide tubes can be implanted simultaneously so that different compounds can be readily infused into discrete structures. To illustrate, a four-cannula array can be utilized for micro-injection of a solution in the anterior and posterior hypothalamus as well as CA1 and CA4 fields of the hippocampus. This strategy is advantageous since different structures in the brain can be studied concurrently, with the animal serving as its own anatomical control.

ACKNOWLEDGEMENTS

This research was supported in part by grants BNS-78-24491 from the National Science Foundation, AA-04200-03 from the National Institute of Alcohol and Alcohol Abuse and by USAF Contract F33615-81-K-0604. The authors are grateful to Dr. E. C. Critcher and Dr. T. F. Lee for tests of the cannula device in their animals and to A. R. Talbot for excellent technical assistance.

REFERENCES

1. Brakkee, J. H., V. M. Wiegant and W. H. Gispen. A simple technique for rapid implantation of a permanent cannula into the rat brain ventricular system. *Lab Anim Sci* **29**: 78–81, 1979.
2. Cervós-Navarro, J., E. Betz, G. Ebhardt, R. Ferszt and R. Wüllenweber. *Advances in Neurology. Pathology of Cerebrospinal Microcirculation*, vol 20. New York: Raven Press, 1978.
3. Crane, L. A. and S. D. Glick. Simple cannula for repeated intracerebral drug administration in rats. *Pharmacol Biochem Behav* **10**: 799–800, 1979.
4. Criswell, H. E. A simple chronic microinjection system for use with chemitrodes. *Pharmacol Biochem Behav* **6**: 237–238, 1977.
5. Gray, D. S. and B. B. Gorzalka. An easily constructed durable chronic intracerebral cannula system. *Pharmacol Biochem Behav* **11**: 463–466, 1979.
6. Harvey, E. A. and R. J. Stephens. Construction and implantation of a cannula system for repeated injections into localized regions of the rat brain. *J Pharm Pharmacol* **27**: 43–45, 1975.
7. Lavenhar, S. R. and A. L. Palanker. Cannula system for local stimulation of the rat brain. *Pharmacol Biochem Behav* **4**: 351–352, 1976.
8. Malkinson, T. J., L. M. Jackson-Middelkoop and W. L. Veale. A simple multi-purpose cannula system for access to the brain and/or systemic vascular system of unanesthetized animals. *Brain Res Bull* **2**: 57–59, 1977.
9. Myers, R. D. Methods for chemical stimulation of the brain. In: *Methods in Psychobiology*, vol 1, edited by R. D. Myers. London: Academic Press, 1971, pp. 247–280.
10. Myers, R. D. *Handbook of Drug and Chemical Stimulation of the Brain*. New York: Van Nostrand Reinhold Company, 1974.
11. Myers, R. D. Chronic methods: Intraventricular infusion, cerebrospinal fluid sampling, and push-pull perfusion. In: *Methods in Psychobiology*, vol 3, edited by R. D. Myers. New York: Academic Press, 1977, pp. 281–315.
12. Myers, R. D., G. Casaday and R. B. Holman. A simplified intracranial cannula for chemical stimulation or long-term infusion of the brain. *Physiol Behav* **2**: 87–88, 1967.
13. Rezek, M. and V. Havlicek. A chronic intracerebral cannula. *Physiol Psychol* **3**: 263–264, 1975.
14. Staton, D. M. and P. R. Solomon. An easily mass produced cannula system for chemical stimulation of the brain. *Pharmacol Biochem Behav* **11**: 363–365, 1979.
15. Stein, E. A. and D. Rodd. A new injection technique for intracerebral drug administration in behaving animals. *Pharmacol Biochem Behav* **12**: 815–817, 1980.
16. Yamamoto, B. K. and C. L. Kutscher. The use of fluoride for the prevention of chronic intracranial implant dislodgment. *Pharmacol Biochem Behav* **15**: 663–664, 1981.