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BRIEF COMMUNICATION

Bilateral Cannula System for Intracranial Chemical Microinjection in Small Animals

DONALD A. CZECH*[1] AND ELLIOT A. STEIN†

**Department of Psychology and †Biology, Marquette University, Milwaukee, WI 53233*

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CZECH, D. A. AND E. A. STEIN. *Bilateral cannula system for intracranial chemical microinjection in small animals.* PHARMACOL BIOCHEM BEHAV **20**(5) 811–813, 1984.—An easily constructed and inexpensive bilateral cannula assembly for microinjection of chemicals into neural tissue in small animals is described. It reduces problems sometimes encountered with commercially available units, making it useful in both research and teaching laboratory settings. Suggestions for implant procedures and modifications for use in unique applications are suggested.

Intracranial cannula Bilateral cannula assembly Chemical microinjection
Behavioral psychopharmacology Chronic implant

MICROINJECTION of small amounts of neurobiologically active agents has become a powerful and widely used tool in neuroscience for studying the biochemical substrate of specific brain nuclei. Both endogenous ligands and pharmacologic agents may be applied to localized brain sites in an awake preparation with the examination of subsequent behavioral or physiologic responses. There are a number of excellent reviews of the application of this technique (e.g., [8,9]).

With the use of substances that activate or enhance activity in neural systems, unilateral injections are often adequate. However, application of blocking agents is best made bilaterally to provide effective blockade. Implantation of symmetric bilateral cannulae, however, can present several potential complications. For example, even after trephine holes are correctly placed and anchoring screws set, the sequential positioning and anchoring of individual cannulae at precise depths can be time-consuming, and the error factor potential is increased. Typically, measurements corresponding to selected coordinates are individually checked as each unit is positioned. In addition, most commercially available single guide cannulae (e.g., Plastic Products Co., Roanoke, VA) have relatively large diameter plastic mounting bodies which prohibit close skull placements required to reach many of the more medial target nuclei in small animals. In such cases, more difficult angular approaches are required. Further, commercially available units can be quite expensive. Custom fabrication designs reported in the literature for multiple cannula assemblies are often quite complicated, requiring considerable technical skill and/or construction time [6, 7, 13]. Dougherty and Ellinwood recently described a design in which the cannula assembly is molded, providing for more efficient construction [5].

We report below a new technique, also using a molding principle, to rapidly and inexpensively manufacture symmetric bilateral cannulae for intracranial injections. Suggestions for surgical implantation and chronic drug injections are also described. The advantages of this system facilitate its use not only in research settings, but also in experimental laboratory courses such as neurobiology, psychopharmacology and physiological psychology.

MATERIALS AND CONSTRUCTION

Figure 1A shows the unit used for fabricating the assemblies. It is made from a piece of 1/2 in (~13 mm) thick Teflon into which several wells are machined. Each of the wells is 3 mm deep and 3 mm in diameter along its minor axis. Diameter along the major axis is determined by the inter-cannula distance of the assemblies to be fabricated; overall diameter is set at 2 mm wider than this distance. Pairs of guide holes are drilled completely through the block, centered in each of the wells, with distance between pairs of holes determined also by the inter-cannula distance required for a given guide cannula assembly. For example, if the lateral coordinate for the targeted brain tissue is determined to be ±1.5 mm from the saggital skull suture, the holes would be centered 3 mm apart. Since it is difficult to keep the small diameter drill bit (No. 77, 0.018 in.) straight when drilling through a very thick piece of Teflon, a large well, 6 mm deep,

[1]Requests for reprints should be addressed to Donald A. Czech, Department of Psychology, Marquette University, Schroeder Complex—Rm. 461, Milwaukee, WI 53233.

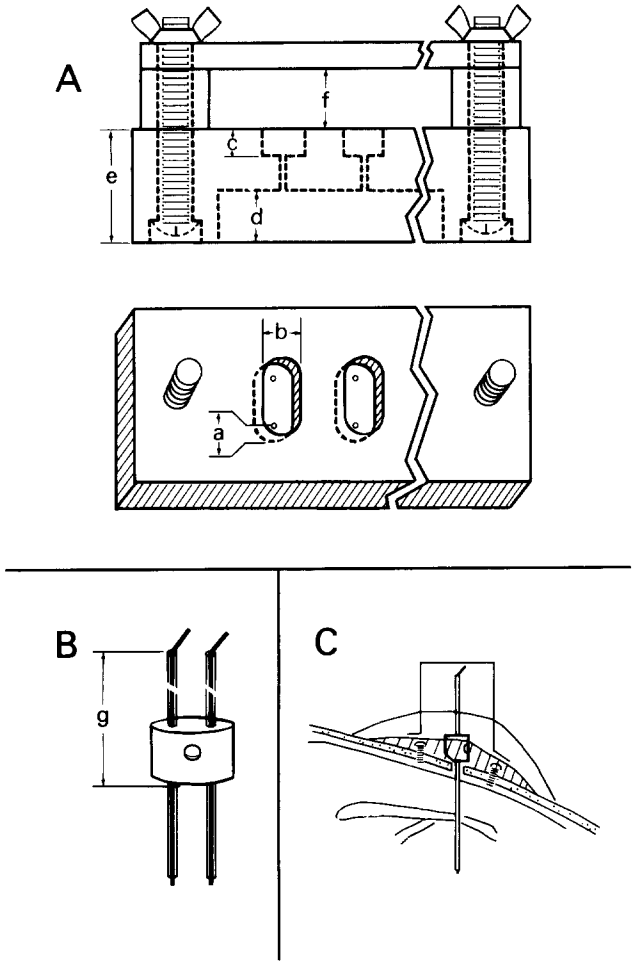

FIG. 1. (A) Side and top views of Teflon form used in fabricating cannula assemblies. Measurements are: a=1 mm, b and c=3 mm, d=6 mm, e=13 mm and f=7 mm. (B) Completed bilateral cannula assembly with stylets, and shallow anchoring hole. Length g=10 mm (constant). (C) Implanted cannula assembly showing bevel for inclined skull and protective collar. Hatched area is initial acrylic layer.

is machined into the bottom of the unit to facilitate drilling accurate parallel holes that are perpendicular to the surface of the well floor. With this under-well, the drilled section is now only 4 mm thick, which is more than adequate to produce a sturdy assembly.

Preparatory to constructing an assembly, 26 gauge stainless steel hypodermic tubing (Small Parts Inc., Miami, FL) is rough cut to approximately the desired length. The ends are then cleanly cut and polished smooth electrolytically, by immersion of the end into a saturated NaCl solution and passing current, using the tubing as the anode in the circuit. Overall length can be determined by the formula: Length (mm) = 10 (constant) + stereotaxic depth measurement required + 2 (excess for final cutting). For example, if the cannula tip is to end 5.5 mm below the skull surface (or bottom surface of assembly), the guide tubes should be at least 17.5 mm long. To facilitate removal of the completed assembly (see below), tubing length should never be less than 15 mm. Sections of tubing are scored with a fine file approximately 8 mm from one end. This will provide an anchoring point for later assembly.

To construct the assemblies, appropriate lengths of 26 gauge tubing are inserted into the guide holes, such that the scored area is positioned just into its block well. A Plexiglas cover plate, with a 7 mm spacer at each end, is then positioned over the screws at each end of the block, and tightened down evenly with wing nuts, thereby pushing the guide cannula tubes slightly deeper through the holes and resulting in a constant 10 mm length of tubing between the lower surface of the cover plate and the floor of the well for all guide tubes; the etched area will end up positioned approximately 1–2 mm above the well floor. The wells are then filled with dental acrylic and left to cure completely.

Gentle pressure, evenly applied to the two guides of the assembly from below, is used to dislodge the cured assembly slightly from its well. The acrylic body can then be grasped and the assembly carefully pulled out. Care should be taken to pull the assembly out straight, so as not to bend the guide cannulae. The acrylic body can be slightly notched, e.g., with a small file or by drilling a shallow hole, to provide secure anchoring when implanting the assembly. A second electrolytic cut is now made to produce the final overall length desired. The cannulae are then cleaned and tested for patency by flushing with ethyl alcohol, using a syringe and short length of PE20 polyethylene tubing. To maintain patency, a stylet, made from 33 gauge stainless steel tubing or wire and bent at 45 degrees for 1–2 mm is inserted into each cannula. The stylet is cut so that it extends approximately 0.5 mm out of the end of the cannula when fully inserted up to the 45 degree bend. Slightly bowing the stylet will guarantee a snug fit. The completed unit (see Fig. 1B) can now be stored in 10% ethanol or distilled water until ready for use.

IMPLANT PROCEDURE

The animal is surgically prepared for implantation using standard stereotaxic procedures. For additional detail, step-by-step instructions can be found in a number of laboratory publications (e.g., [1, 12, 14]). Care should be taken that anchoring skull screws are positioned far enough anterior and posterior to the guide cannula trephine holes so that the assembly will clear them when lowered into position. Solid anchoring is routinely achieved with 3–4 screws (2 posterior and 1 or 2 anterior). It is critical that the trephine holes are accurately placed, such that they are directly over the targeted brain sites and match the fixed relative positions of the guide cannulae in the assembly. Careful measurements will permit one to drill holes with diameters only slightly larger than the guide cannulae. We accomplished this by first inserting a blunted needle in the vertical drive of the stereotaxic unit. Using bregma as a reference point, the drive is then manipulated to a selected anterior-posterior/lateral position, a small amount of black India ink is placed on the needle tip, and the needle is lowered until it just touches the skull, thereby leaving a tiny ink mark on the skull. All positions are thus marked. The point of a No. 11 scalpel blade is then lightly pressed into the ink mark and rotated back and forth several times, making a small indentation in the skull. This provides an accurately positioned and stable starting point, much like a center punch, for the drill bit. The No. 70 drill bit used produces a hole (o.d.=0.028) just slightly larger than the 26 gauge cannulae (o.d.=0.018). Held in the vertical drive of a stereotaxic frame by the upper section of one of

the guide cannulae, the implant is now positioned so that the guide cannulae are approximately centered directly over the small, accurately placed trephine holes, and carefully lowered until the body contacts the skull surface. If guide cannulae lengths have been properly selected, the tips of the guide cannulae should be positioned accurately in the brain tissue. It is typically recommended that guide cannulae end at least 1 mm dorsal to the targeted injection site to prevent tissue damage. It is important to determine the vertical distance between the skull surface and the targeted brain site at a given anterior-posterior location. It should be noted that even when using a flat skull preparation (e.g., [10]) the skull surface is slightly higher between bregma and lambda. If an inclined skull (e.g., [11]) is used, it is helpful to file the underside of the assembly at a bevel so that skull contact is made where the guide cannulae protrude from the bottom of the assembly (see Fig. 1C).

With the assembly held in position, a layer of acrylic (~2 mm thick) is applied to secure the assembly to the 3–4 anchoring skull screws. This thin layer will harden quickly, after which the stereotaxic drive unit can be uncoupled. To prevent the rats from dislodging the stylets, a protective collar is formed from a piece of heat shrinkable tubing, approximately 10 mm long and with an o.d. 2–3 mm wider than the intercannulae distance. Collars of different diameters can easily be made by shrinking standard diameter tubing. Small flaps may be cut at the base, or the base slightly stretched, to form anchor points (see Fig. 1C). The collar is centered around the guide cannulae, resting on the anchoring layer of dental acrylic, and a pedestal of acrylic is formed around the base of the collar. The center is then partially filled with a thinner slurry of acrylic. In this way, a protective collar is sealed into what would otherwise be a standard acrylic pedestal. Several sutures are used in a standard manner to secure the skin around the base of the pedestal.

Injection cannulae are made from 33 gauge stainless steel tubing. Because of the small diameter of this tubing, a 5 mm sheath of 26 gauge tubing is fitted over one end of the injector and epoxied in place, with approximately 3 mm of the 33 gauge tubing protruding. This sheath will accommodate standard PE20 tubing used to connect the injector to a microliter syringe and infusion pump delivery system. To insure good bonding of the sheath, hand scoring at the junction of the tubes is recommended. Finally, the injector is electrolytically cut to the desired length with the epoxy bead acting as a stop in the guide cannula. The injection system is modified from a design reported by Crane and Glick [2].

DISCUSSION

Using these procedures, sturdy bilateral guide cannula assemblies can be produced in quantity very quickly and inexpensively. We use them routinely for bilateral injection of opioid peptides and antagonists into cortical and subcortical brain tissue in rats [3,4]. Our university machinist has made a series of blocks, drilled to accommodate the various lateral coordinates that we use, each with multiple wells for constructing 10–12 assemblies at once. If only unilateral placements are required, one can simply cut an assembly into two parts and use the guide cannulae singly. Alternatively, a thin cardboard partition can be inserted into the well between the two cannulae before or immediately after pouring the dental acrylic; the assembly can then easily be broken in half after removal. An attractive feature of this procedure is that assemblies can be easily and rapidly produced in sufficient quantities to meet the needs in student teaching laboratories that employ stereotaxic techniques. In addition to low cost and ease of construction, the time required for bilateral implantation is substantially reduced. If desired, variations involving more than two placements, differing depths, and/or non-bilaterality of placement can easily be achieved, some of these requiring alternative unique block configurations. With the protective collar, no special construction techniques are required to prevent dislodging of stylets or bending of cannulae between test trials. The bent ends of the stylets, as well as the guide cannulae themselves, are below collar height and thus protected from disturbance by grooming-like behaviors, sleeping postures where the head is tucked under the body and/or getting caught on cage parts. Since the exposed part of the collar is somewhat flexible, it also cushions the implant from some of the force which would be exerted on it by such activities or events, and thereby the chances of its being dislodged are greatly reduced. We have experienced no difficulty, even though our experimental protocols sometimes require that animals be maintained over several months.

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REFERENCES

1. Cooley, R. K. and C. H. Vanderwolf. *Stereotaxic Surgery in the Rat: A Photographic Series*, 2nd edition. Ontario: A. J. Kirby Company, 1978.
2. Crane, L. A. and S. D. Glick. Simple cannula for repeated intracerebral drug administration in rats. *Pharmacol Biochem Behav* **10:** 799–800, 1979.
3. Czech, D. A., M. J. Blake and E. A. Stein. Drinking behavior is modulated by CNS administration of opioids in the rat. *Appetite: J Intake Res*, in press.
4. Czech, D. A., E. A. Stein and M. J. Blake. Naloxone-induced hypodipsia: A CNS mapping study. *Life Sci* **33:** 797–803, 1983.
5. Dougherty, G. G., Jr. and E. H. Ellinwood, Jr. A simple multiple-cannula headpiece for the rat. *Physiol Behav* **26:** 897–900, 1981.
6. Gray, D. S. and B. B. Gorzalka. An easily constructed durable chronic intracerebral cannula system. *Pharmacol Biochem Behav* **11:** 463–466, 1979.
7. 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:** 791–795, 1983.
8. Myers, R. D. *Handbook of Drug and Chemical Stimulation of the Brain*. New York: Van Nostrand Reinhold Company, 1974.
9. Myers, R. D. Chronic Methods: Intraventricular Infusion, Cerebrospinal Fluid Sampling, and Push-Pull Perfusion. In: *Methods in Psychobiology*, vol 3, *Advanced Laboratory Techniques in Neuropsychology and Neurobiology*, edited by R. D. Myers. New York: Academic Press, 1977, pp. 281–315.
10. Paxinos, G. and C. Watson. *The Rat Brain in Stereotaxic Coordinates*. New York: Academic Press, 1982.
11. Pellegrino, L. J., A. S. Pellegrino and A. J. Cushman. *A Stereotaxic Atlas of the Rat Brain*, 2nd edition. New York: Plenum Press, 1979.
12. Skinner, J. E. *Neuroscience: A Laboratory Manual*. Philadelphia: W. B. Saunders Company, 1971.
13. 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.
14. Webster, W. G. *Principles of Research Methodology in Physiological Psychology*. New York: Harper and Row, 1975.