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Techniques for the Chronic Cannulation of the Jugular Vein in Mice

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BARR, J. E., D. B. HOLMES, L. J. RYAN AND S. K. SHARPLESS. *Techniques for the chronic cannulation of the jugular vein in mice.* PHARMAC. BIOCHEM. BEHAV. 11(1) 115–118, 1979.—The use of chronic intravenous cannulae implanted in the jugular vein of mice utilizing techniques previously developed for larger rodents is discussed. Two cannula designs and a chronic infusion chamber are illustrated. Cannula insertion depths for mice of three strains and various body weights, and estimates of operative mortality and cannula durability are given.

Mouse Intravenous cannula Chronic drug administration

THE use of indwelling venous cannulae in freely moving rats and cats has become a standard technique in the behavioral analysis of drug effects. Since the method's introduction [4, 5, 6, 9] numerous refinements in cannula design and support equipment have been made: several recent chapters have explained the procedure in quite adequate detail [7,10]. To date, however, the technique has not been applied to mice: yet the mouse is the standard test subject for the preliminary analysis of drug effects. Its use in these animals would make available the large number of readily obtainable inbred strains facilitating the genetic analysis of chronic drug effects without the trauma, absorption of loading problems of intraperitoneal or subcutaneous injections or the taste aversion and gastric degradation problems associated with oral administration. Repeated intravenous injections via the tail vein have been employed but are as traumatic as the other methods. Recently, one method for the chronic cannulation of the tail vein of DBA/2J mice was described [3]: however, the attachment device is awkward and cumbersome, requiring the animal to remain continually attached to the injection apparatus. The ideal procedure would be flexible enough to allow both chronic and one-time testing and be rapid and reliable enough to be applied to large groups of subjects. We describe the design of two cannulae for implantation into the jugular vein and describe the techniques and equipment we have developed for chronic intravenous drug administration into mice. Estimates of operative mortality and cannula endurance are also given.

METHOD

Cannula

Figure 1 diagrams both cannulae and explains their construction. Three features of the cannula are important to the success of the implant. First, the cannula must be constructed of silicone rubber tubing: polyethylene tubing, often used in rats, is unsuitable because it is not flexible enough to bend without putting undue, and possibly damaging, tension on the inserted portion of the cannula. Secondly, the junction between the two different diameter tubings must be made some distance above the point of insertion. Clotting is apparently induced in cannula 1 when blood flows above this junction: the resulting clots cannot be forced through the smaller diameter tubing when clearing the cannula. The possibility of such clotting is substantially reduced by increasing the length of the smaller diameter segment. One piece cannulae using only the smaller diameter tubing were tested but the portion exiting from the animal was too fragile for sustained usage: a single piece larger diameter cannula has been successfully implanted, but the insertion is less deep, usually only to the junction of the jugular and vena cava, and is more difficult. Cannula 2, which we have used for longer term implantations, avoids this problem: rather than employing an open lumen, the end is plugged with silicone medical grade adhesive: the outflow is through two small pinholes placed in the tubing above the plug [1]. Thirdly, due to the relatively large change, with body size, of the length of the jugular-vena cava above the atrium, we have determined, from autopsies of 63 mice, the length of cannula to be inserted into mice of different body sizes for three inbred strains. The length is measured between the point of insertion, which is 3 mm above where the jugular vein courses under the pectoral muscles, to 1 mm above the atrial valve. These lengths are indicated in Table 1.

Implantation Procedure

Adult mice are anesthetized with 280 mg/kg chloral hydrate and 140 mg/kg ketamine in saline vehicle [2]. The incision, location of the vein, insertion of the cannula and clos-

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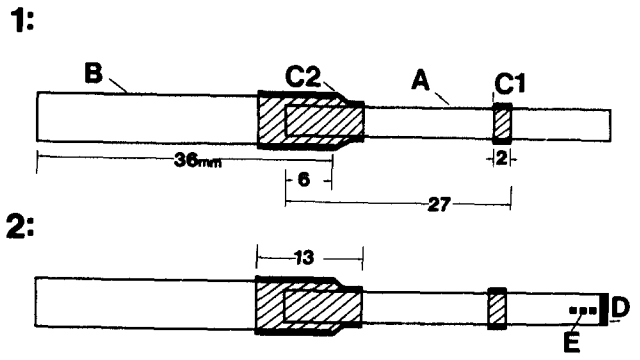

FIG. 1. Cannula 1: A—Silicone rubber tubing, id 0.30 mm, od 0.64 mm, length between C1 and end determined from Table 1. B—Silicone rubber tubing, id 0.51 mm, od 0.94 mm, insertion of A into B accomplished by allowing B to expand in either chloroform or trichloroethane and inserting A 4 mm into B. C1—1.2 mm heat shrinkable polyolefin tubing, marks length of insertion and acts as a tie-down structure. C2—1.2 mm heat shrinkable tubing used to strengthen junction. Total fluid volume=8.8 μl. Cannula 2: Same as cannula 1, but D—1 mm plug of medical grade silicone adhesive, injected into the cannula tip from a 1-ml disposable syringe with a 27 ga needle, E—two opposed pin holes, made with the beveled tip of a 27 ga needle 2 mm above plug. Both cannulae may be cut at a bevel to facilitate implantation, but bevels of more than 30° off of the transverse plane are not recommended as they tend to puncture the vein.

ing of the wound follow the operative procedure developed for rats [7,10]; we suggest that a 27 ga needle or fine microdissection scissors be used to make the insertion hole in the vein. The use of a dissection microscope is extremely helpful in visualizing the finer operative details. For most applications, mice are allowed a four day recovery period prior to testing. It is especially important for the cannula to be flushed with saline one day after implantation, and every other day thereafter to maintain the patency of the cannula.

Chronic Administration

The chronic setup currently in use in our laboratory is shown diagrammatically in Fig. 2. Using this system we have left mice attached for two weeks with no discernible problems with the mice or the patency of the cannula. Two points of the arrangement are worth emphasizing. The use of a saddle or subcutaneous implant as typically used in rats to prevent the tension of the attachment tubing from acting directly on the cannula is eliminated by modifying an electronic mini-hook connector to hook onto a stainless steel catch cemented to the mouse's skull in order to absorb the strain. A 4–5 cm dia. circle of skin is cut off the mouse's head, the thin layer of fascia is scraped off with a scalpel blade, and the skull is scratched in a cross-hatch pattern to facilitate the dental cement binding. A thin layer of dental cement is applied, and, as it hardens, the steel catch is positioned. Dental cement is built up around the catch to hold it in place. Secondly, a coiled attachment tubing allows free movement of the mouse without requiring a flowthrough liquid swivel. The cannula, though, must be unattached and the tubing allowed to unwind every 24 hr. The use of this coiled attachment is contraindicated with activating drugs that can greatly increase the animal's locomotor activity; use of a more restrictive and elaborate attachment is required. Details on the control equipment can be found [7].

TABLE 1

INTRA-JUGULAR CANNULAE INSERTION LENGTH

Strain	Age (Days)	Sex	Weight (g)	Length of Insertion (mm)
C57BL	65	M	18–20	12
			21–24	13
			25–30	14
C3H	65	M	18–20	11
			21–24	12
			25–30	13
DBA/2	65	M	18–20	10
			21–24	11
			25–30	12

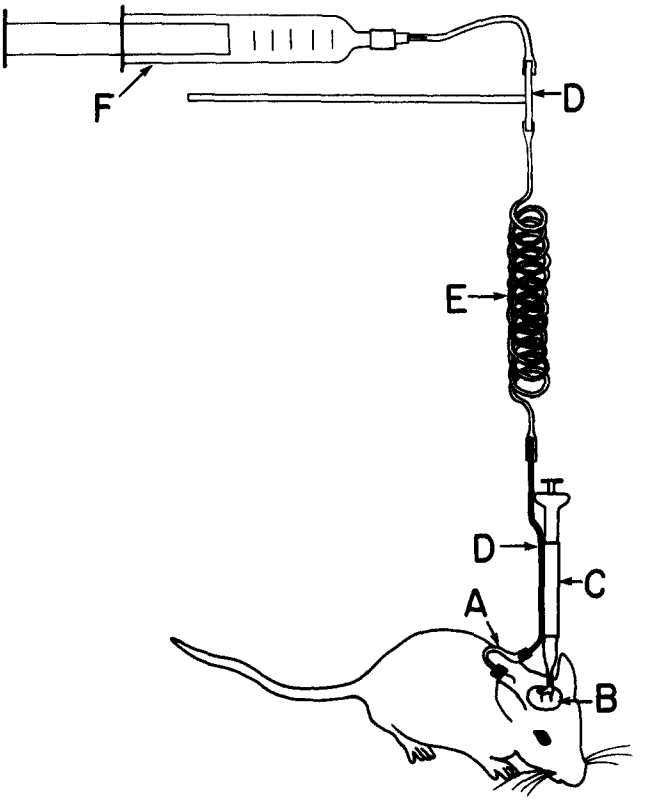

FIG. 2. A method for attachment to an infusion apparatus for use on freely moving mice. The cannula (A) exiting from the mouse's back attaches to a 35 mm length of shaped 23 ga stainless steel tubing (D), attached to the side of a miniature electrical test clip, the top of which was removed to reduce weight, (D), with a 25 mm section of 6.4 mm dia. heat shrinkable tubing. The attachment loop of 0.61 mm stainless steel wire is anchored to the skull with dental cement (B). Polyethylene tubing (E, PE 50), coiled by wrapping around a piece of 8 mm od glass tubing and dipping several times in boiling water, is attached above to a short section of 23 ga tubing positioned above the center of the cage to eventually connect to a syringe or infusion apparatus (F). Typically, 30 coils are used: however, this may be varied to reduce the tubing volume, elevate connector D, or increase the amount of twisting the tubing may accommodate.

Operative Mortality

In a recent study, implants were attempted on 244 mice. Of these, 11 died during or following the operation. Of the survivors, only three of the Type 1 cannulae were inoperable when tested 24–48 hr later. The average duration of each operation, after the experimenter had become skillful, was less than 12 min when operations were conducted on mice in groups of 6–12.

DISCUSSION

The implantation of cannulae of either design into the jugular vein of mice opens many previously inaccessible lines of both chronic and acute experimentation in mice. With experience, the implantation can be both rapid and effective, and the cannulae maintained for periods of at least two weeks.

SOURCES OF MATERIALS

Electronic mini-hook connector: No. X100W, Tek-test Inc., E-Z Hook Division, 114 St. Joseph St., Arcadia, CA 91006.

Heat-shrinkable tubing: No. 37N1165 (1.2 mm) and No. 37N1173 (6.4 mm) Type FPS-Voltrex flexible polyolefin tubing, white, Newark Electronics, 500 N. Pulaski Rd., Chicago, IL 60624.

Silicone rubber tubing and adhesive: Silastic No. 602-105 (0.30 mm id × 0.64 mm od) and No. 602-135 (0.51 mm id × 0.94 mm od), No. 891 Medical grade silicone adhesive, Dow Corning Corporation, Medical Products, Midland, MI 48640.

Stainless steel tubing and wire: HTX-23 (23 ga hypodermic tubing), and SWX-024 (0.61 mm wire), Small Parts, Inc., 6901 N.E. Third Ave., Miami, FL 33138.

Dental cement: No. 58063, Durelon carboxylate cement, fast set, Premier Dental Products Co., 1710 Romano Drive, P.O. Box 111, Norristown, PA 19701, or from ESPE GmbH, Seefeld/Oberbay, West Germany.

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