Permanent Cannula-Injection System for Intracerebral Injections in Small Animals

LEE R. GRUNDEN* and GEOFFREY E. LINBURN†

Abstract ☐ The procedure for constructing a simplified cannula-injection system for making repeated intracerebral injections in conscious, free-moving animals is described. The system is made from standard hypodermic needles and polyethylene tubing. The absence of screw joints and the small size make this system particularly useful for chronic injections in small animals. Additional advantages are discussed.

Keyphrases ☐ Cannula-injection system—cerebral injections ☐ Intracerebral injections—small animals ☐ Brain—localized injection ☐ Intraventricular injections—rats, chickens ☐ Diagram—cannula-injection system

Much interest has developed in recent years in the chemistry and pharmacology of the brain. A useful tool for studies in the neurological sciences has been the implanted cannula (1). Implanted with a stereotaxic apparatus for precise localization in a specific area of the brain, the cannula permits the introduction of a given amount of compound directly into the brain of an unanesthetized animal. The *in vivo* effects of the compound may then be studied with a verifiable knowledge of the specific area treated.

Few detailed descriptions (2–5) of a chronic cannula-injection system suitable for small animals are available. The cannula-injection system which has been developed and described in detail here has several simplifying features which make it compact and easy to construct and use. Moreover, the cannula is easily reclaimed for repeated implants.

METHODS

The system consists of a double-barreled unit. The outer barrel is the guide cannula which is implanted stereotaxically and is secured to the skull with a quick-drying acrylic dental cement.[1] An injection cannula is inserted into this guide cannula at the time of injection. Following injection, the injection cannula is replaced by a stylet. A tight friction fit is sufficient to hold both the stylet and injection cannula in place. The guide cannula, stylet, and injection cannula are machined from hypodermic needles[2] on a small metal lathe.[3] The needles are easily mounted in the chuck of the lathe by first securing them to an adapter.[4]

The guide cannula is made from a 23-gauge Yale hypodermic needle (Fig. 1). The taper of the needle is machined to a cylinder with a diameter of approximately 2 mm. The taper should not be machined to a distance more than 4 mm. from its tip, since a decreasing wall thickness may cause the machined cylinder to break off prematurely if machined further. For better binding by the cement, one or two grooves are machined into the cylinder of the 23-gauge needle with a fine pointed cutting tool. The cylinder is then cut from the body of the syringe sleeve with a small cut-off tool. The cannula is finished with a fine file or grinding wheel.[5] The opening at the top of the cylinder is flared with a small burr[6] for easier insertion of the stylet and injection cannula. The shaft of the cannula is cut with wire cutters to a length slightly longer than the final length desired; it is then ground to the precise length with the grinding wheel. This process reopens the pinched end. The finished product is represented diagrammatically in Fig. 2A. The overall length of the guide cannula is determined by the depth of the desired implant site. For example, an overall length of 9 mm. is adequate when placing implants into the lateral cerebral ventricles of 250-g. rats.

The stylet is made from a 30-gauge Yale hypodermic needle. To provide a better fit between the stylet and the guide cannula, the end of the syringe sleeve taper of the 30-gauge needle is faced to a flat surface. The taper is then machined to a cylinder as described for the cannula. The machined cylinder of the stylet is cut from the body of the syringe sleeve with a hacksaw and is thereby sealed. The needle is cut to length with wire cutters, thereby sealing the lumen, and both ends are smoothed with the grinding wheel. The tip of the stylet, when fully inserted into the guide cannula, should be flush with the tip of the cannula (see Fig. 2). For the stylet to fit tightly inside the guide cannula, slight crimping of the cannula shaft near the cylinder with wire cutters may be necessary.

The injection cannula is also made from a 30-gauge hypodermic needle and is machined, initially, like the stylet. Starting approximately 1 mm. from the faced surface, the cylinder is then further

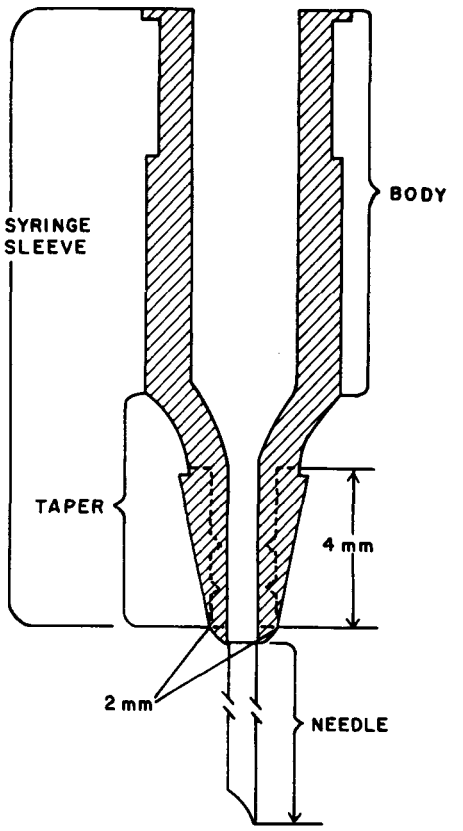

Figure 1—*Diagram of a standard hypodermic needle with the various parts labeled. The dotted lines represent the outline of the machined cylinder.*

[1] NuWeld, L. D. Caulk Co., Milford, Del.
[2] B-D Yale type, Becton, Dickinson & Co., Rutherford, N. J.
[3] *e.g.*, Unimat lathe, American Edelstaal Inc., New York, N. Y.
[4] One-Way Valve, female Luer-lok to male Luer-lok; Becton, Dickinson & Co., Rutherford, N. J.
[5] *e.g.*, Hypodermic needle sharpener, Van Waters & Rogers Inc., Brisbane, Calif.
[6] Unimat accessory.

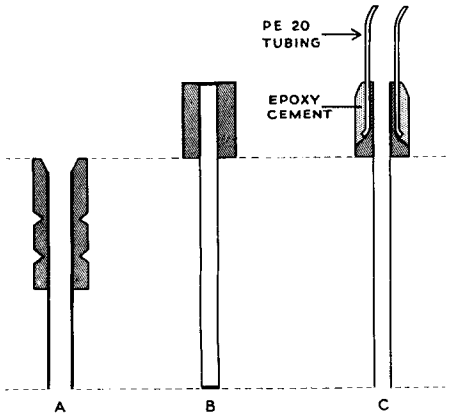

Figure 2—*Diagrammatic representation of the finished components. Key:* A, *guide cannula, 23G;* B, *stylet, 30G;* C, *injection cannula, 30G, and tubing.*

machined to as small a diameter as possible. The machined needle will break from the body of the syringe sleeve when the diameter becomes sufficiently small. The resulting cylinder can be fitted into polyethylene tubing (PE 20). After roughing the surface of both the cylinder and tubing with a small file, the two are cemented together with epoxy cement. The other end of the tubing is fitted to a blunted 25-gauge hypodermic needle which may then be attached to a syringe. The injection cannula is cut to length and reopened with the method already described for the guide cannula. The tip of the injection cannula, like the stylet, should be flush with the tip of the guide cannula when fully inserted (Fig. 2). The injection cannula can be used with all guide cannulas of a given length.

A simple adapter for implanting the guide cannulas with any standard stereotaxic instrument can be made from 23-gauge and 30-gauge stainless steel hypodermic needle tubing.[7] The 30-gauge tubing is inserted inside an 8-cm. length of 23-gauge tubing and is secured by crimping at one end. The 30-gauge tubing should extend a sufficient length beyond the other end of the 23-gauge tubing so that when fully inserted into a guide cannula, the two tips are flush.

The adapter with the tightly fitting cannula is mounted like an electrode in a stereotaxic instrument. A permanent implant is made without any elaborate devices for securing the cannula to the skull. A strong bonding of the guide cannula to the skull is achieved by tightly threading several small stainless steel machine screws[8] to the skull and thoroughly drying the surface before applying the cement (Fig. 3). After positioning the guide cannula, the exposed portion of the cannula is surrounded by acrylic dental cement which is applied with a small brush. When the cement has hardened, the adapter is removed from the cannula and a stylet is inserted.

COMMENT

This cannula-injection system is designed for fluid injections but could be readily adapted for the introduction of solids. For fluids, microliter volumes are easily injected with a microliter syringe.[9]

The authors have used this cannula-injection system successfully for repeated intracerebral and intraventricular injections in conscious, unrestrained rats (6, 7) and chickens (Grunden, unpublished). The system has proven to have several advantages over previously used systems. The small size of the guide cannula makes it particularly useful for rats and other small animals. Since the cannula is completely surrounded by cement resulting in a low-profile implant, the animal is unable to exert leverage which might lead to loss of the cannula. In over 120 animals implanted to date, the cannulas have remained useable for periods of up to 11 months. The system has proven to be virtually leakproof without the use of screw joints. Thus injections are made simply by pulling out the stylet and inserting the injection cannula.

[7] 304 F-hard, seamless; Tubesales, Los Angeles and New York.
[8] 0–80 × 0.32 cm. (1/8 in.).
[9] Hamilton microliter syringes, available in sizes as small as 1 μl. total volume; Hamilton Co. Inc., Whittier, Calif.

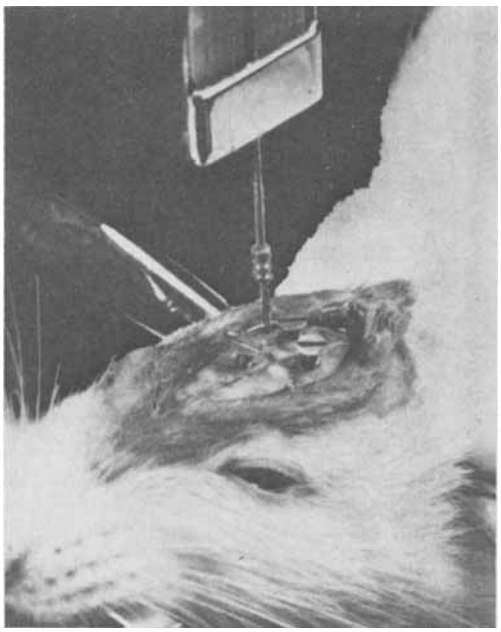

Figure 3—*Photograph showing a guide cannula ready to be implanted in a rat. The cannula is fitted on the adapter which is mounted in the electrode holder of a stereotaxic instrument. The tip of the cannula is lowered to a predetermined depth through a hole in the skull, and the cannula is then secured in place with dental cement. Three stainless steel screws are used to anchor the cement to the skull.*

An added advantage is the ease with which the guide cannula may be reclaimed after sacrificing the animal. By immersing the embedded cannula in chloroform for a few hours, the acrylic dental cement is dissolved away, thereby freeing the metal cannula for use in subsequent implants.

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* Present address: College of Pharmacy, The University of Arizona, Tucson, AZ 85721

† Present address: Stanford University Medical School, Stanford, CA 94305