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BRIEF COMMUNICATION

A Reliable, Rapid and Inexpensive Method for Producing and Implanting Chronic Cannulae Into Brains of Small Animals

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ELLIOTT, P. J. *A reliable, rapid and inexpensive method for producing and implanting chronic cannulae into brains of small animals.* PHARMACOL BIOCHEM BEHAV **24**(6) 1809–1811, 1986.—A new method for constructing and implanting cannulae into brains of small animals is described. This system allows drug microinjections to be performed on multiple occassions with little tissue damage, at a very low cost.

Cannula Microinjection Chemical-stimulation

PERIPHERAL routes of administration are often used to assess effects of drugs on the central nervous system (CNS). However, many agents do not or only weakly circumvent the blood-brain barrier, or are rapidly metabolized after oral or intravenous adminstration. To overcome these problems, direct intracerebral microinfusion procedures have been developed to study the biochemical and behavioural effects of drugs on various discrete brain areas [19]. Such methods typically involve the implantation of commercially available cannulae into the CNS. However, these pre-made headpieces are usually unsuitable for bilateral implantation due to their relatively large diameter mounting devices [20]. Custom designs have been previously reported [1, 3–18, 21–23] but these generally involve complicated construction and often require a substantial initial cost to cover specialized machine-workshop costs. The present report describes a quick, inexpensive and reproducible surgical procedure to stereotaxically implant bilateral cannulae for subsequent CNS microinfusion studies.

MATERIALS AND CONSTRUCTION

The multiple microinfusion procedure requires the production of four easily made parts. All the material used for this cannulae system are commonly found in the laboratory, are inexpensive and require no special workshop equipment.

Guide Cannulae Holders

These are used to maintain a fixed distance between the guide cannulae when they are implanted. Use of a standardized cannulae holder minimizes deviations from the injection site and thus provides a uniform group of cannulated animals.

Guide Cannulae

Guide cannulae can be made from 1/2 in., 23 gauge, hypodermic needles clipped below the plastic syringe adapter. The lumen should be cleaned with dental broaches (Healthco Ltd., Boston, MA) and their outer surface serrated lightly to allow maximum adhesion to the dental acrylic cement during implantation. An alternative, cheaper method for the production of the guide cannulae would be to prepare them from lengths of 23 gauge stainless steel tubing (Small Parts Inc., Miami, FL). However, the bevelled ends are more difficult to standardize.

Injection Cannulae

These injection needles, made from 23 and 30 gauge tubing, are "L" shaped, rather than straight, to allow easier insertion into the guide cannulae. Like the guide cannulae and their holders the ends of the 23 gauge tubing should be as

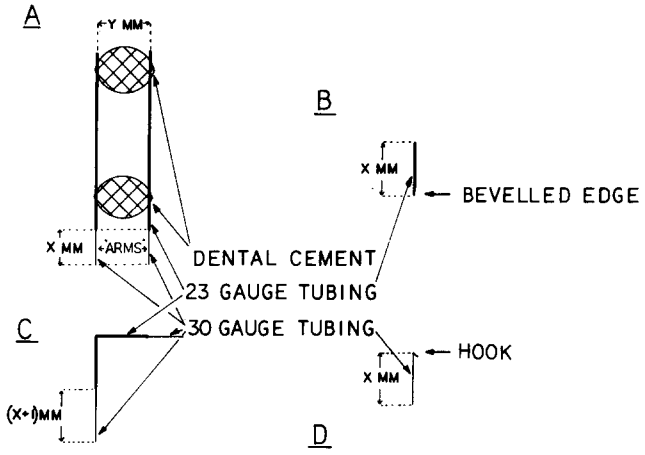

FIG. 1. Diagrammatic representation of the complete cannula system used for microinjection of drugs into the brains of small animals. (A) cannula-holding device, (B) cannula, (C) injection cannula, (D) stylet.

near perpendicular to the length of the tubing as possible, to ensure that all three parts fit together well.

Stylets

These plugs (made from 30 gauge tubing) are used to reduce infection and to keep the lumen of the guide cannulae free. Animals can remove their stylets during play and grooming and it is therefore better to house the subjects separately. However, this is not completely necessary if small hooks (approximately 0.5–1.0 mm), formed by bending the 30 gauge tubing at a 30° angle are used, as this decreases the chance of the stylets being removed.

SURGICAL PROCEDURE

Once the subject is securely positioned in the stereotaxic apparatus and the fur on top of the head has been shaved, a midline incision extending from a point approximately between the eyes to the back of the skull is made. Parting of the ectoderm reveals the periosteum which can be scraped from the midline towards the sides of the calvarium. After removing any blood present, futher bleeding may be prevented by topical application of an adrenaline solution (sparingly), thus allowing better visualization of the skull sutures. Four mounting screws (Plastic Products Co., Roanoke, VA) are then implanted approximately 0.3 mm into the skull—one in each bone-plate of the calvarium. This process may be quickened greatly when using a screw-holding screwdriver available from most electrical supply stores.

Bregma can be located using one of the 30 gauge "arms" of the guide cannulae holder. Once located, guide cannulae can be affixed to the apparatus by the addition of water onto the 30 gauge "arms." The bevelled sides of the guide cannulae are positioned to face each other to minimize problems arising from tissue distortion. These bevelled ends of the guide cannulae serve two purposes: (i) to puncture dura; and (ii) to allow easier penetration of the brain tissue. Note: Coordinates taken from skull or dura should be measured from the tip of the guide cannulae.

The guide cannulae tips should be implanted 1 mm above the desired injection site to ensure that no cellular damage occurs to the tissue under investigation. Once the guide cannulae are in position, dental acrylic cement is layered around their outer surfaces, on the calvarium and also the ectoderm surrounding the wound. To reduce the size of the bore holes required for both the screws and the guide cannulae it is recommended that a 2 mm drill bit be used.

Stylets are inserted after the guide cannulae holder has been raised. The total time for the whole operation takes between 15–25 min, depending upon the experience of the surgeon.

Animals cannulated in this way can be kept for several weeks and upon histological examination show no signs of tissue necrosis around the wound edges. Furthermore, although sterile surgical equipment and operating areas are desirable, they are not essential. In the above operation no antibiotic medications are needed, reducing the total cost of the procedure and speeding up the implantation. Thereby, drug-antibiotic interactions are avoided.

INFUSION PROCEDURE

To perform bilateral injections the following apparatus is required: two Hamilton syringes mounted on an infusion pump connected to the injection cannulae via lengths of polypropylene tubing (Cole Palmer Inst. Co., Chicago, IL). To facilitate insertion into the cannulae and syringes the tubing can be "bulbed" using heat from a soldering iron. The complete system is then filled with distilled water. At the tips of the injection cannulae an air bubble (approximately 0.1 μl) is introduced into the system to act as a barrier between the water and the drug and also to provide a means of verifying that the drug is infused correctly.

Conscious animals are hand-held while the stylets are removed and the injection cannulae are inserted into the guide cannulae. Subjects are allowed to move freely throughout the infusion procedure.

Injection cannulae are left in place for a 1–2 min period after the drug administration to ensure that the drug has diffused away from the cannulae tip.

DISCUSSION

Using the procedures and methods illustrated above, a cannulae system can be rapidly and inexpensively produced. Animals with chronically implanted cannulae can be then used for drug infusions on multiple occasions. This is in contrast to other studies where acute drug administration require surgical manipulations on the same day as the behavioural or biochemical testing. The procedure described here minimizes the problems of drug-anaesthetic interactions and furthermore allows multiple CNS drug infusions in the same animal. Thus, this method dramatically reduces animal costs as well as allowing subjects to be used as their own controls. Furthermore, multiple drug infusions permit the researcher to study the effects of chronic CNS drug administration. Finally, because the cannulae are very small multiple implantations into the same animal can be performed simultaneously.

With the chronic intracranial cannulation technique described behavioural changes elicited by the drug can be studied immediately after the infusion, rather than waiting for the effects of the anaesthetic to fade. This is a valuable attribute over other methods since the behavioral effects of many drugs and some neuropeptides are short lasting due to their rapid degradation or distribution [2,7]. Moreover, this

technique could be used in conjunction with osmotic minipumps, where the connecting microtube between the pump and the cannulae would be covered by the acrylic cement.

The relative low cost and ease of implantation of the above cannulae system permits therefore the use of conscious animals in studying the neurochemical and behavioural effects of centrally administered drugs where a supply of a large number of expendable subjects is needed. This technique is thus a useful tool for the biochemist, pharmacologist and behaviourist alike.

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