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A Convenient and Inexpensive Method for Chronically Implanting Multiple Intracranial Cannulae

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SCHULZE, H. G. AND B. B. GORZALKA. *A convenient and inexpensive method for chronically implanting multiple intracranial cannulae*. PHARMACOL BIOCHEM BEHAV **37**(3) 411–416, 1990.—A simple and inexpensive method is described for the chronic implantation of multiple cannulae in small animals. This method employs a simple die cast from dental acrylic cement which is then repeatedly used to retain the cannulae during implantation. No special workshop tools are required and assembly is rapid.

Multiple cannulae Microinjection Chronic cannulae Chemical stimulation Microinfusion Intracranial cannula

INVESTIGATIONS of brain-behavior relationships sometimes require the simultaneous stimulation of different brain sites through the microinjection of psychoactive agents. This may require the use of several chronically implanted cannulae in the same animal. Many of the presently available techniques for the implantation of cannulae only allow the construction of single [e.g., (2,3)] or bilateral cannula assemblies [e.g., (4,6)]. There are also a few methods that describe the construction of multiple cannulae headpieces [e.g., (1, 5, 7–11)] and although some of these are ingenious, they frequently suffer from disadvantages such as being cumbersome, time-consuming, limited in scope to the implantation of cannulae in close proximity, complicated to execute and requiring special workshop tools or equipment.

In order to surmount these problems, we recently developed a method for the implantation of multiple cannulae that is easy to use, inexpensive, durable and reliable. Our approach consists of casting a dental acrylic cement die which is then used to retain several cannulae at the appropriate positions relative to each other. The die with cannulae is then manipulated as a single unit. This method, apart from the construction of the die, is almost as simple to use as the implantation of a single, durable, chronic cannula using the method of Gray and Gorzalka (7).

METHOD

The multiple cannulae system requires several easily made components. Except for the clay, all the materials should be readily available in the laboratory. The following sections describe these components: 1) the die that is needed to retain the guide cannula holders; 2) the die holders that allow for insertion of the die itself in the stereotaxic apparatus; 3) the place holders that are used to retain the appropriate positions for guide cannula holders during the construction of the die, these place holders are eventually replaced with the guide cannula holders; and 4) the guide cannula holders that are needed to retain the guide cannulae which are then implanted intracerebroventricularly to allow the microinfusion of psychoactive substances. In summary, the die holders retain the die, the die retains the guide cannula holders which in turn hold the guide cannulae during implantation while the place holders are exclusively used for construction of the die. The reader may wish to refer to Figs. 3 and 5 for identification of the components.

These components are then assembled via the following basic steps, illustrated as a sequence in Fig. 1: (a) the place holders, the die holders, the guide cannula holders and guide cannulae are prepared; (b) the place holders are implanted into the clay; (c) the mold is constructed and used to cast the die; (d) the die holders are inserted into the die; (e) the place holders are removed from the die, replaced with the guide cannula holders and guide cannulae are inserted into guide cannula holders; and (f) the die is then used to retain the guide cannulae during surgery.

Die Holders

The bevelled ends of two denuded 23-gauge stainless steel hypodermic needles are filed away on a rotary grinder and the outer surface of one end is serrated lightly to improve adhesion to the dental acrylic cement during implantation. Disposable 23-

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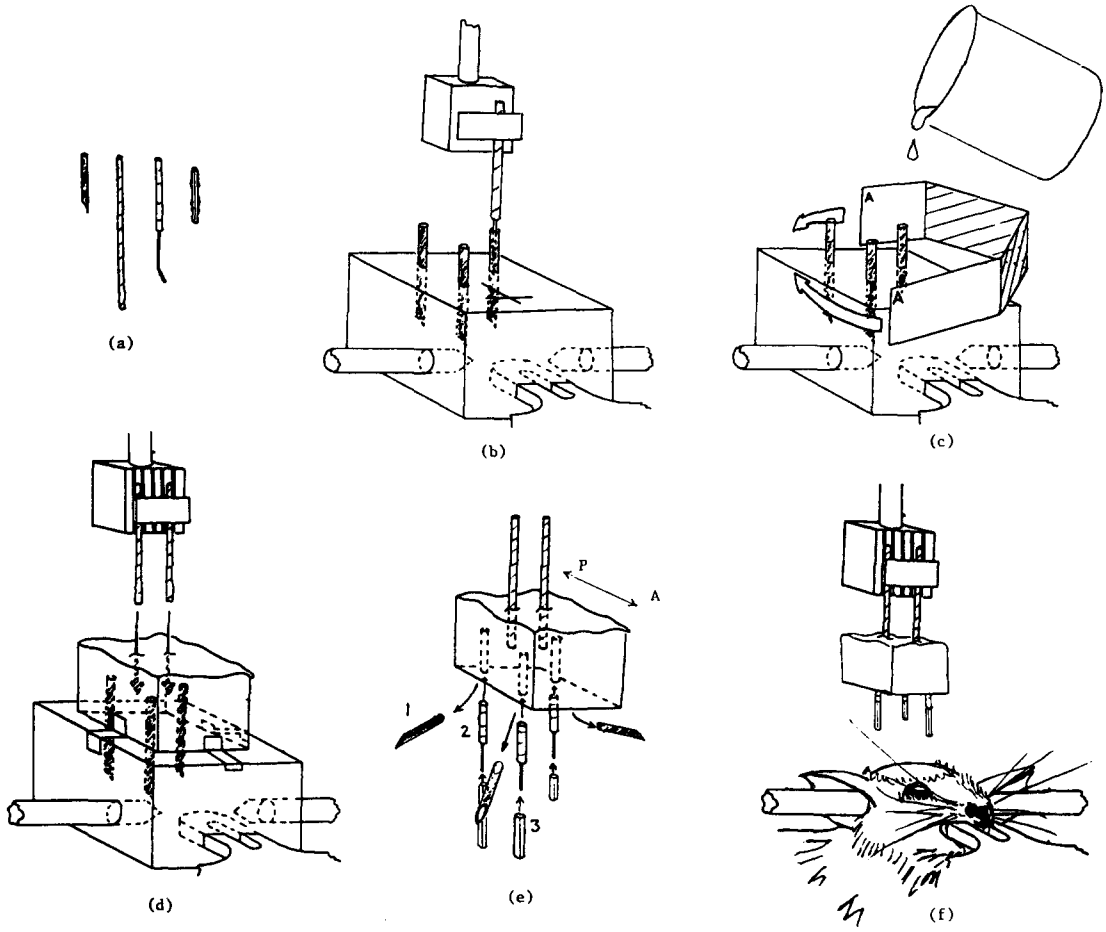

FIG. 1. Illustrated in this diagram is the sequence of basic steps for construction of a multiple intracranial guide cannula assembly: (a) from left to right (showing one of each): the place holders with bevelled ends, the die holders, the guide cannula holders and guide cannulae are prepared; (b) the place holders are implanted into the clay; (c) the mold is constructed and used to cast the die; (d) the die holders are inserted into the die; (e) the place holders are removed from the die 1), replaced with the guide cannula holders 2) and guide cannulae are inserted into guide cannula holders 3); and (f) the die is then used to retain the guide cannulae during surgery.

gauge hypodermic needles are denuded by separating the needle portion from the plastic hub.

Guide Cannula Holders

Denuded 23-gauge stainless steel needles are cut into equal sections of approximately 10 mm each. A short length of 30-gauge stainless steel tubing or needle is then inserted into the larger gauge section and soldered or cemented into place. The protruding section of smaller tubing is then manually slightly bent to hold the guide cannula more firmly during implantation. An additional holder is required for the implantation of place holders. This is also made from a denuded 23-gauge needle with a denuded 30-gauge needle inserted approximately 7 mm into the larger diameter part and fixed in position. The protruding portion of the smaller gauge needle is then manually given a slight bend.

Guide Cannulae

These are made from disposable stainless steel 23-gauge needles cut to the required lengths and lightly serrated on the outer surfaces.

Die Construction

A piece of synthetic modeling clay is fashioned into a cube with sides of approximately 50 mm each and inserted into a stereotaxic instrument as if it were the head of a small animal. On

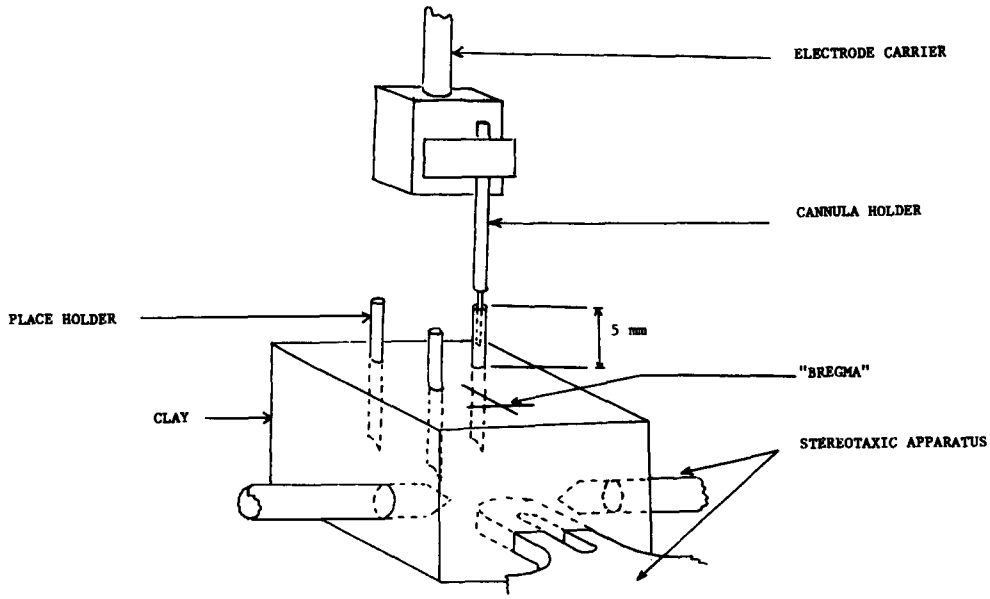

FIG. 2. Diagram shows the positioning of the clay in the stereotaxic instrument, the cross that serves as a reference point in the manner that bregma does and three implanted place holders. Also indicated is the use of a cannula holder to implant a place holder.

the "dorsal" surface of the clay, approximately in the middle, a light cross is made with a scalpel or other sharp instrument. This cross then serves as a reference point in the same manner that bregma does on an animal's skull. Using the stereotaxic instrument, place holders (denuded 23-gauge stainless steel needles) are implanted at the appropriate positions relative to the cross (bregma) as if one were implanting guide cannulae into an animal's brain at these locations. The place holders are implanted into the clay in a manner which allows about 5 mm to protrude above the surface. One place holder is implanted in this manner for every guide cannula required as illustrated in Fig. 2.

The next item to be constructed is a mold; this can be made from a sturdy type of paper such as that from a milk carton. A strip of paper about 7 mm high and of sufficient length so that a rectangle can be folded to enclose all the place holders is fashioned as indicated in Fig. 3. There should be at least 2 mm between the wall of the mold and any one place holder. Adhesive tape can be used to form the rectangle and to secure it to the clay surface. Once the mold is firmly in place, the place holders are lightly painted with a vegetable oil to prevent the acrylic cement from adhering to them. Dental acrylic cement is then poured into the mold until it is flush with the top of the mold. The two die holders are then fixed to the electrode carrier and lowered about 5 mm into the still viscous acrylic cement and left at this position until the cement has hardened fully. Details are given in Fig. 4.

When the dental acrylic cement has fully cured, the die holders are released from the electrode carrier and the die is removed from the clay. The place holders are removed from the die and the holes cleaned with a tiny bit of cotton wool. A small amount of glue or very diluted dental acrylic cement is applied to each hole and then

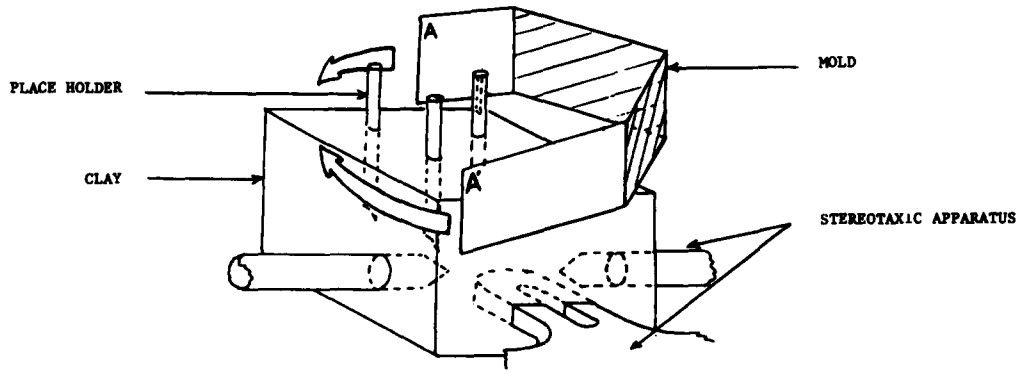

FIG. 3. A sturdy section of paper about 7 mm high is cut and folded so that the ends A' and A meet to form a rectangle encompassing all the cannulae in such a way that no cannula is closer than about 2 mm from any wall. Once positioned, this rectangular mold is secured in place using adhesive tape. Dental acrylic cement is now poured into the mold until flush with the upper edge of the mold.

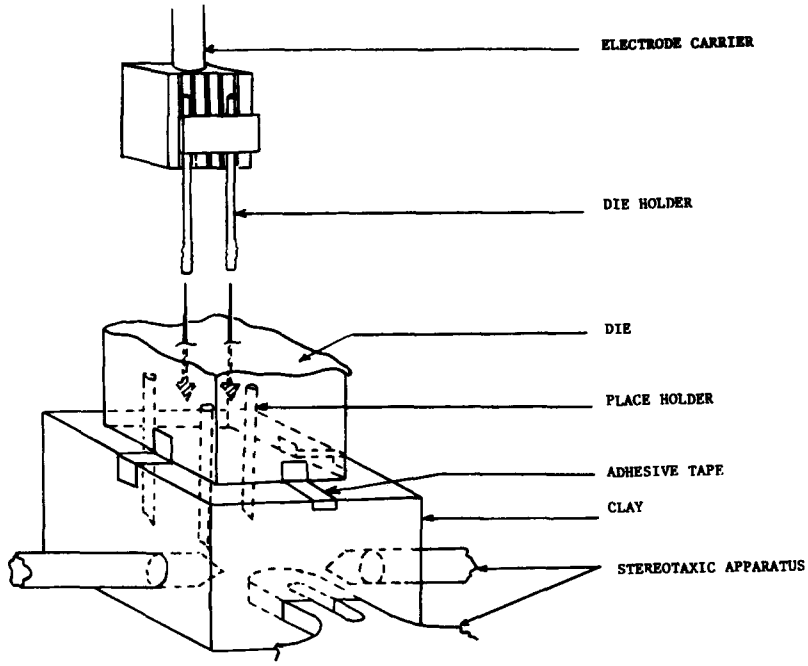

FIG. 4. The die holders are implanted to a depth of about 5 mm in the still viscous dental acrylic cement and left in position until the acrylic cement has fully cured. The die is then removed from the clay and the stereotaxic instrument.

a cannula holder is firmly pressed into each hole as indicated in Fig. 5. All cannula holders should be of the same length, so that when properly inserted, they all protrude to the same extent from the die.

Cannulae of the appropriate lengths can now be inserted into their respective holders. The lengths of the cannulae can be determined by using a stereotaxic atlas and making a correction for the more posterior cannulae relative to the most anterior cannula as explained in Fig. 6. It is advisable to mark or otherwise label the anterior and posterior sides of the die to ensure correct insertion into the stereotaxic apparatus.

Surgery

The surgical procedure involves obtaining coordinates from the skull or dura with the tip of the cannula closest to bregma, and will be briefly outlined. After the animal has been securely positioned in the stereotaxic instrument and the skull exposed, three or four mounting screws are implanted into the skull to anchor the dental acrylic cement used for embedding the cannulae. The dental acrylic cement die is now positioned in the electrode carrier of the stereotaxic instrument. Since two die holders are used in the construction of the die, incorrect positioning of the die will only

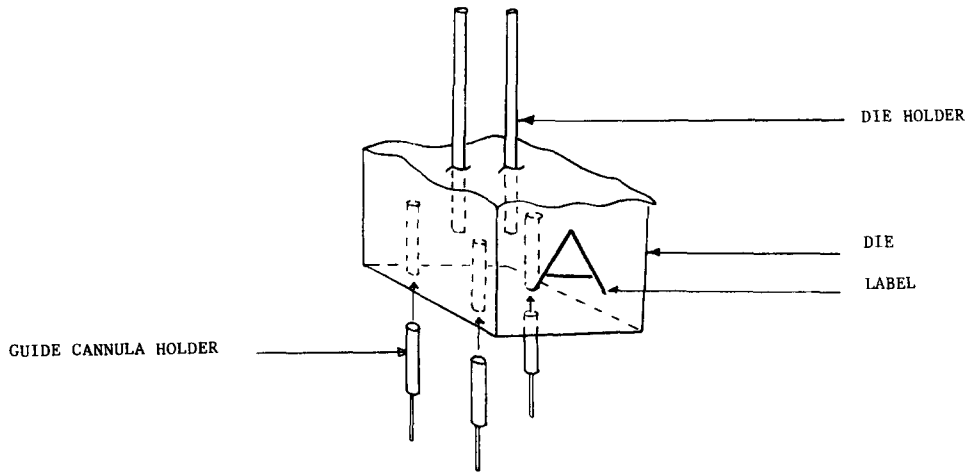

FIG. 5. The place holders are removed from the die and guide cannula holders are cemented into their positions as indicated. The anterior side of the die is labelled as shown with an "A" (posterior side with a "P") using a felt tip pen.

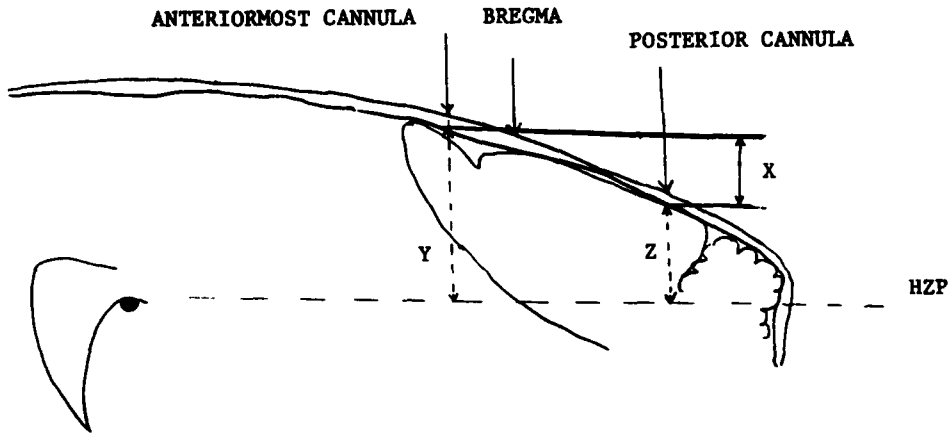

FIG. 6. Guide cannula length adjustment for posterior cannulae. The distance "Z" between the dura and the horizontal zero plane (HZP) for the relevant cannula is subtracted from the distance "Y" between the dura and HZP for the most anterior cannula to give the length "X" which should be added to the length of the relevant cannula. Measurements are taken from the stereotaxic atlas of Pellegrino *et al.* (12).

result in a reversal of the anterior and posterior orientation and should not occur if the anterior and posterior sides of the die had been marked as suggested in the section on die construction. At this point, the die holds only the anterior-most guide cannula which is used to obtain the three coordinates for its correct implantation. Since the die fixes the positions of all the cannulae relative to one another, they should all hit their targets upon correct implantation of the anterior-most cannula. Once these coordinates have been taken, the remaining cannulae are carefully inserted into their respective holders and implantation proceeds as normal. Care should be taken to drill the appropriate number of holes into the skull to accommodate all the cannulae. When the die is positioned over the skull of the subject and has been lowered to the correct depth, it may be slightly difficult to apply dental acrylic cement to embed all the cannulae. Our advice would be to use a rather diluted mixture of dental acrylic cement and to apply it in such a fashion that its distribution around the cannulae can be accomplished with the aid of gravity. This is readily accomplished during surgery, especially if all the cannulae are made to be slightly (1–2 mm) longer than needed for individual implantation. When the dental acrylic cement has hardened thoroughly, the die with cannula holders can be carefully retracted before the protective collar is fashioned around the embedded guide cannulae (7).

DISCUSSION

This method allows the construction of a multiple chronic cannulae system that is easy to assemble, remarkably inexpensive and durable. Animals with chronically implanted multiple cannulae can then be used in studies employing drug infusions into multiple sites and on multiple occasions. We believe that this method has several advantages: it allows for the independent and/or simultaneous manipulation of several brain sites, minimizes the number of measurements to be taken and consequently reduces variance, and increases statistical power and reduces the number of animals required by permitting multiple within-subject comparisons. This procedure has been used in about 50 animals in our laboratory. The results to date indicate excellent durability and quite acceptable placements. It should be noted, however, that the more cannulae that are required, the more difficult it becomes to ensure accurate placements of all cannulae. Placement accuracy is also dependent on the types and proximity of sites selected. A judicious selection of placement coordinates to maximize the amount of error tolerance for each site is recommended. We also suggest that several trial surgeries be performed and, depending on the results of the histological analyses, adjustments be made to the die and/or placement coordinates to ensure proper implantation of all cannulae.

This convenient and inexpensive technique provides, to laboratories with limited resources, a new method of chronically implanting multiple intracranial cannulae.

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REFERENCES

1. Altaffer, F. B.; de Balbain-Vester, F.; Hall, S.; Long, C. J.; D'Encarnacao, P. A simple and inexpensive cannula technique for chemical stimulation of the brain. Physiol. Behav. 5:119–121; 1970.
2. Brakkee, J. H.; Wiesant, V. M.; Gispen, W. H. A simple technique for rapid implantation of a permanent cannula into the rat brain ventricular system. Lab. Anim. Sci. 29:78–81; 1979.
3. Caulfield, M. P.; Clover, K. F.; Powers, D. A.; Savage, T. A rapid and convenient freehand method for the implantation of cerebroventricular cannulae in rats. J. Pharmacol. Methods 9:231–236; 1983.
4. Czech, D. A.; Stein, E. A. Bilateral cannula system for intracranial chemical microinjection in small animals. Pharmacol. Biochem. Behav. 20:811–813; 1984.
5. Dougherty, G. G.; Ellenwood, E. H. A simple, multiple-cannulae headpiece for the rat. Physiol. Behav. 26:897–900; 1981.
6. Elliott, P. J. A reliable, rapid and inexpensive method for producing and implanting chronic cannulae into brains of small animals. Pharmacol. Biochem. Behav. 24:1809–1811; 1986.
7. Gray, D. S.; Gorzalka, B. B. An easily constructed durable chronic intracerebral cannula system. Pharmacol. Biochem. Behav. 11:463–466; 1979.
8. Hepler, J. R.; Myers, R. D. New multicannula pedestal device for microinjection of drug into brain tissue or cerebral ventricle. Pharmacol. Biochem. Behav. 18:791–795; 1983.
9. Hoebel, B. G. Electrode-cannulas for electrical or chemical treatment of multiple brain sites. Electroencephalogr. Clin. Neurophysiol. 16:399–402; 1964.

10. Malkinson, T. J.; Jackson-Middelkoop, L. M.; Veale, W. L. A simple multipurpose cannula system for access to the brain and/or systemic vascular system of unanesthetized animals. Brain Res. Bull. 2:57–59; 1977.
11. Myers, R. D. Handbook of drug and chemical stimulation of the brain. New York: Van Nostrand; 1974.
12. Pellegrino, L. J.; Pellegrino, A. S.; Cushman, A. J. A stereotaxic atlas of the rat brain. New York: Plenum Press; 1979.