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Construction and implantation of a cannula system for repeated injections into localized regions of the rat brain

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The construction and method of implantion of a brain cannula for chronic use in rats is described. All the components of the cannula system are easily made from stainless steel tubing and no machined parts are required. The use of a metal key eliminates the need for tapped holes during implantation. Although little time is required to prepare each animal the system remains firmly fixed to the skull and allows injections to be made accurately into selected brain areas.

Various cannulae systems which allow injections to be made into specific areas of the rat brain have been described (Grossman, 1962; Hayden, Johnson & Maickel, 1966; Myers, Casaday & Holman, 1967). Some of these require special machined parts and most rely on small screws to key the implant to the skull. The following is a description of a simple cannula system for use in rats which may be rapidly and easily constructed using standard laboratory materials and tools and which eliminates the need for screws or tapped holes during implantation.

Construction

The cannula system is made entirely from stainless steel and polyethylene tubing and consists of the six parts depicted in Fig. 1. The length of cannula described is suitable for injections into the hypothalamus or other areas near the base of the brain. The stainless steel tubing is cut and the ends levelled with a needle file. Internal burrs are removed by rotating a syringe needle tip in the cut ends. Soldered joints are made using phosphoric acid as a flux.

(a) The cannula guide is constructed from a 7-8·5 mm length of 23 S.W.G. stainless steel tubing to which a second length (approximately 4 mm) is soldered at right angles; as near as possible to one end.

(b) A 2 mm length of 23 S.W.G. tubing is threaded onto and soldered to one end of an 8 mm length of 30 S.W.G. tubing to form the stylet. One end of the wider tubing is left partially occluded after filing to hold it in place during soldering.

(c) The injection cannula is made by threading a 5-10 mm length of 23 S.W.G. tubing onto a 30 mm length of 30 S.W.G. tubing and soldering it in place to form a collar 11 mm from one end.

(d) The key is made by bending a length of 28 S.W.G. tubing into a U-shape so that the parallel arms are 2 mm apart. The length of the U is adjusted to 8 mm and a 45° bend is made at points one-third and two-thirds along the length to give the side elevation depicted in Fig. 1 (inset).

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(e) The crown consists of a 6 mm length of 4 mm diameter heat-shrinkable polyethylene tubing in which 9-12 cuts, each 4 mm long, are made from one end along the length of the tubing. The flaps so formed are bent outwards to give the configuration shown in the figure.

(f) The cannula guide holder consists of a length of 30 S.W.G. tubing soldered inside a length of 23 S.W.G. tubing (approximately 30 mm) and arranged so that 7 mm of the narrower tubing protrude from inside the wider one.

All components with soldered parts are boiled in distilled water to remove remaining traces of flux. The cannula guide is measured to the nearest 0·25 mm using a microscope and all guides, keys and stylets are stored in ethanol before use.

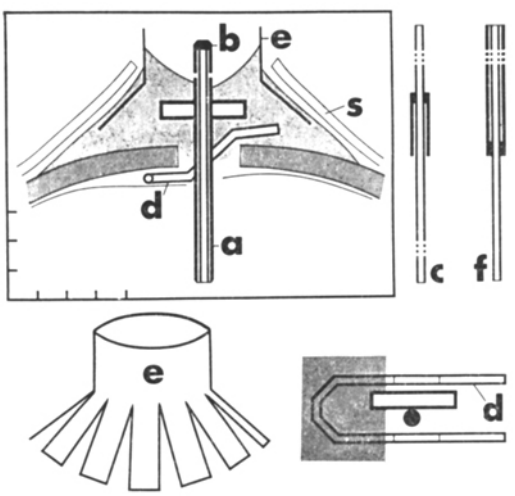

FIG. 1. Diagram of apparatus drawn to scale (each division of the scale in the inset represents 1 mm). (a) cannula guide, (b) stylet, (c) cannula, (d) key, (e) crown and (f) cannula guide holder. The inset shows a diagrammatic view of a section through the implanted apparatus, the cross-hatched area represents the skull, the stippled area dental cement and (s) the skin. The plan of the implanted key (lower right) shows the proportion lying below the skull and the relative positions of the key and cannula guide *in situ*.

Implantation and use

A rat, 180–200 g, is anaesthetized using an ether face mask 10 min after an injection of atropine (1 mg kg^{-1} s.c.). The head is placed in a stereotaxic instrument and the dorsal surface of the skull is exposed and dried. The cannula guide is attached to the guide holder and mounted in an electrode carrier. An oval hole (2 x 3 mm) is drilled in the skull in the required position at co-ordinates selected from König & Klippel (1963). The key is inserted so that approximately one-third lies between the skull and dura mater, then the dura is pierced to allow the guide to be gently lowered between the arms of the key until the top of the guide is 3·5 mm above the brain surface (Fig. 1 inset and plan). The skull surface is carefully dried and a little dental cement applied to fix the key and guide in place. When this is dry the carrier and guide holder are removed and the crown is positioned centrally around the top of the guide and cemented in place. After the acrylic cement has hardened the stylet is inserted and the animal is removed from the instrument. The wound is sutured, leaving the crown protruding between the skin edges. The rat is housed individually

and allowed to recover for about a week before use. In use, the cannula is attached to a 10 μl Hamilton syringe via a length of silicone rubber tubing and the whole system is filled with the solution to be injected. The stylet is removed from the guide and replaced by the cannula, inserted as far as the collar. The depth of the injection site below the brain surface is determined by the length of the cannula minus 3·5 mm (the distance between the top of the guide and the brain surface). Normally 1·0 μl of solution is injected and the cannula is left in position for 30 s before it is removed and replaced by the stylet.

The accuracy of the placement may be verified at the end of a series of experiments by injecting Indian ink into the brain via the cannula and subjecting the brain to histological examination.

DISCUSSION

This cannula system has been in use for more than a year during which time 230 rats have been prepared. The main advantages of this cannula over more conventional ones are (i) that the manufacture of the component parts requires little in the way of technical skill, time, materials or tools and (ii) that the use of the key to anchor the guide to the skull ensures that the implantation procedure is simple and much less time consuming than those requiring holes to be tapped or screws to be fixed to the skull. With part of the key beneath the skull and the remainder embedded in a block of solid acrylic cement extending over the outer surface of the bone the whole structure is essentially clamped to the cranium. Provided that the skull is thoroughly dry when the dental cement is applied the structure remains firmly fixed for at least two months. The polythene crown prevents the skin growing over the implant and the rat removing the stylet whilst grooming.

The placement of the top of the guide at a predetermined height above the brain surface ensures that injections to all similarly prepared animals via a cannula of a particular length, are made to a constant depth regardless of minor variations in the length of the cannula guides. Thus although the implantation procedure is simple, a high degree of accuracy is achieved. Histological evidence indicates that in more than 90% of the preparations the position of the final placement was within 0·5 mm of that selected. Also, with the exception of the guide path there was no evidence of tissue damage even in rats that had received multiple injections into the hypothalamus.

Since the guide tip is positioned 3–4 mm above the target area the sensitivity to drugs of regions above and below this may be explored by using cannulae of different lengths. Although the cannula described is used to make injections into the area of the hypothalamus the system may be readily modified to allow the administration of drugs into other brain areas or the ventricles merely by altering the length of the injection cannula and guide.

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