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BRIEF COMMUNICATION

A Miniaturized Push-Pull Cannula for Use in Conscious, Unrestrained Animals

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DLUZEN, D. E. AND V. D. RAMIREZ. *A miniaturized push-pull cannula for use in conscious, unrestrained animals.* PHARMACOL BIOCHEM BEHAV **24**(1) 147–150, 1986.—In this report we present a detailed description for construction of a miniaturized push-pull cannula. This device can be used for *in vivo* perfusion of discrete brain loci in a variety of conscious, unrestrained animals for evaluation of brain neurochemistry/behavior interactions.

Push-pull cannula Construction

THE push-pull cannula as a technique for *in vivo* determinations of neurochemical release within discrete brain loci was described almost 25 years ago by Gaddum [4]. Subsequently, Myers [9] reviewed the technical aspects of push-pull perfusion and related a number of important considerations in the use of this technique. The use of push-pull perfusion has been limited, primarily due to the lack of sensitive assays which are required to detect the low levels of release in push-pull perfusate samples. However, recent advances in the development of sensitive assays (e.g., radioimmunoassays and high pressure liquid chromatography with electrochemical detection) have made push-pull perfusion a more viable and attractive procedure for determination of *in vivo* neuroregulator release in the awake freely moving animal. While push-pull cannulae are commercially available (Plastic Product, VA), we feel the increased adaptability, ease of construction and relative inexpense of the cannula described in this report better fulfills the needs of research laboratories examining the relationship between brain neurochemistry and behavior. In our laboratory we have adapted and miniaturized the push-pull cannula and have extensively used this technique [11,12] to successfully measure *in vivo* release of the neuropeptide hormone, luteinizing hormone-releasing hormone from the medial basal hypothalamus of rats [2, 5, 7, 8], rabbits [13], and sheep [6], as well as the release of dopamine and its metabolites from rat corpus striata [1]. In the present report we provide a detailed description for the construction of the push-pull cannula we have used in these experiments.

MATERIALS

The following materials are required for construction of the push-pull cannula: (1) Stainless steel tubing, hypodermic—Type 304, two gauges—(a) 33 g (HTX 33) o.d.=0.008″, Wall=0.002″ and (b) 29 g (HTX 29) o.d.=0.013″, Wall=0.003″ (Distributor: Small Parts Inc., 6801 NE Third Avenue, Miami, FL 33138); (2) Syringe needles, 24 g 1 Regular bevel B-D Yale Hypodermic needle, Luer-LOK hub—Stainless Steel (Distributor: Becton Dickinson, Division of Becton, Dickinson and Company, Rutherford, NJ 07070); (3) Polyethylene tubing, PE 20 i.d.=0.38 mm (0.15″) o.d.=1.09 mm (0.43″) (Distributor: Clay Adams, Division of Becton, Dickinson and Company, Parsippany, NJ 07054); (4) Pump tubing, 0.25 mm i.d. (Distributor: Gilson Medical Electronics, Inc., P.O. Box 27, Middleton, WI 53562); (5) Plastic syringes, 1 cc tuberculin (Distributor: Becton Dickinson) and (6) Glue—(a) quick set epoxy resin and (b) quick set epoxy hardener (Distributor: 3M or Scotch).

CONSTRUCTION

Outer Cannula

The 24 g (0.56 mm o.d.) syringe needle serves as the outer cannula assembly which is permanently implanted in the animal. Mark off the appropriate legnth of the syringe needle for the specific site in the brain to which the cannula will be directed. In our experiments we use 11.0 mm for cannulae directed at the medial basal hypothalamus and 5.0 mm for the corpus striatum in rats. Score the circumference of the syringe needle at this length and with needle nose pliers snap off the end. If necessary, file down to the exact length required and remove any burrs.

Stylette

The 33 g (0.20 mm o.d.) stainless steel tubing is used in constructing the stylette as well as for the push cannula. Cut off approximately 5.0 cm of 33 g tubing. Remove the plunger from the 1 cc plastic syringe and cut off the bottom of the syringe at the 0.05 ml level. Insert this bottom portion of the

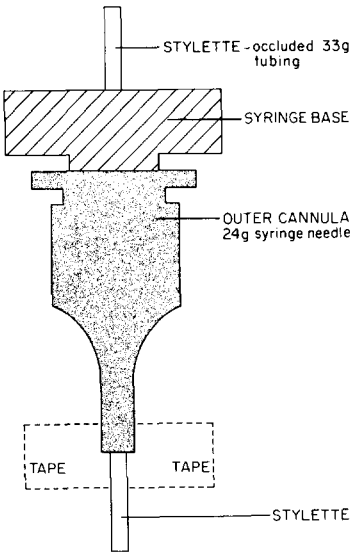

FIG. 1. Outer cannula and stylette assembly. Plastic syringe base is inserted into outer cannula and stylette is inserted through entire unit. Folded over tape secures stylette to unit while glue is sealing stylette to plastic syringe base. After curing, stylette protruding from end of needle comprising outer cannula is filed until length extends only 1 mm and excess stylette protruding from top of syringe base is also removed. This entire unit (syringe base with attached stylette/outer cannula) is stereotaxically implanted into the animal and permanently secured with dental cement.

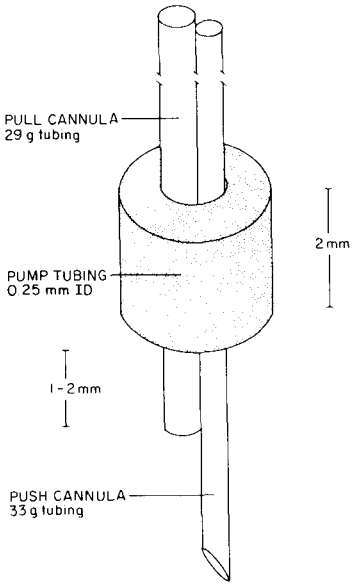

FIG. 2. Pump tubing with secured push and pull cannulae. After cutting and filing push (33 g) and pull (29 g) tubing to required lengths, they are inserted into center hole of pump tubing where they can be adjusted and secured to correct lengths. This entire unit is gently forced into the bottom of a plastic syringe base, permanently secured with glue and forms the inner cannula assembly.

syringe into the 24 g syringe needle (outer cannula) and insert the 33 g tubing (stylette) through this syringe base/needle combination. Place tape across the stylette where it extends beyond the needle of the outer cannula and fold tape over itself to secure stylette to outer cannula (Fig. 1). Mix quick set epoxy resin and hardener (in approximately equal amounts) with a toothpick until the mixture is viscous (approximately 0.5–1.0 minute). While holding the outer cannula in a vertical position apply the glue into the plastic syringe base allowing glue to fill the base and secure the stylette. By rotating the outer cannula in a vertical position, the glue will fill the base of the syringe without sticking to the outer cannula. Once the glue has sealed (typically about 2–4 minutes) remove the tape, file the stylette until it extends only 1 mm beyond the outer cannula, making certain that this end of the stylette is occluded. Remove any excess stylette extending beyond the top of the glue.

Inner Cannula Perfusion Assembly

For construction of the inner cannula assembly first score and cut off approximately 3 cm of 33 g tubing (push cannula). File one end at an angle (approximately 45°) and flush tubing with distilled water to make certain that tubing is patent. Score and cut off approximately 2 cm of 29 g tubing (pull cannula), again flushing with distilled water to insure patency. Cut off approximately 2.0 mm of pump tubing. This cylindrical piece of pump tubing will be used to secure the push and pull cannulae. Insert the push and pull cannulae through the center hole of this pump tubing. The pull cannula (29 g) should extend approximately 1–2 mm beyond the cylindrical piece of pump tubing and the extension of the push cannula (33 g) will vary depending upon the outer cannula length (Fig. 2). Cut off the bottom of another 1 cc plastic syringe as previously described and gently insert the cylindrical piece of pump tubing containing the push and pull cannulae into the bottom of this syringe base until the pull cannula is just slightly above the bottom of the plastic syringe base. This unit will now be referred to as the inner cannula assembly. Place this inner cannula assembly into the previously prepared outer cannula and adjust the length of the push cannula until it extends 0.5 mm beyond the tip of the outer cannula. Prepare the glue as previously described, apply into the syringe base of the inner cannula assembly and allow the glue to flow through the base until it reaches the top of the cylindrical piece of pump tubing. Once glue has sealed, re-check both push and pull cannulae lengths and make certain both are patent. Place PE 20 tubing over the push and pull cannulae extending beyond this first gluing and with a second gluing of the inner cannula assembly, glue these together while building up the glue around the tubing (Fig. 3). This PE 20 tubing will then be connected to the pump tubing during perfusion.

IMPLANT AND PERFUSION

The outer cannula with stylette are implanted and permanently secured with dental cement using classical stereotaxic techniques. On the day of perfusion, the stylette is removed and replaced with the inner cannula assembly. It is imperative to equilibrate the flow rates of the push and pull cannulae prior to perfusion by maintaining a constant sized drop on the tip of the outer cannula/inner cannula assembly as described by Myers [9]. We have found that this can best be

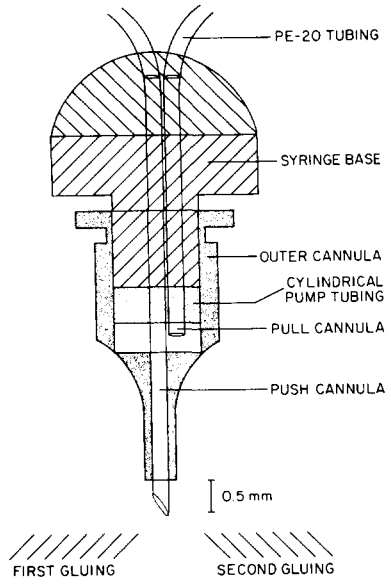

FIG. 3. Cutaway view of outer cannula housing the completed inner cannula assembly. After a first gluing which secures the push and pull cannulae to the plastic syringe base, PE 20 tubing is inserted over cannulae extending from the top of the syringe base and a second gluing will secure the PE tubing to the cannulae. Each PE-20 tube is connected to individual peristaltic pumps with equilibrated flow rates to maintain equal pressure between push and pull cannulae during perfusion.

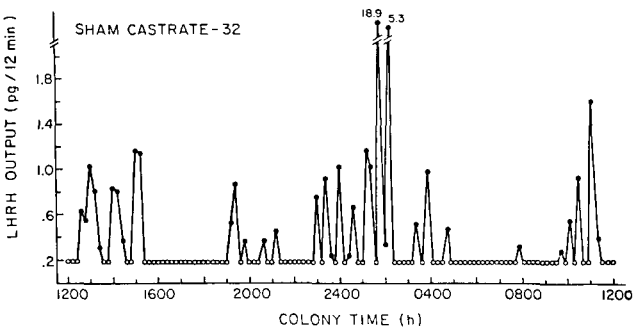

FIG. 4. Data from a continuous 24 hr push-pull perfusion of a male rat [2]. The adult male rat was implanted with a push-pull cannula directed at the medial basal hypothalamus. Following a 7 day recovery period, the male was perfused for a continuous 24 hr period. Samples were collected at 12 minute intervals and assayed for luteinizing hormone-releasing hormone (LHRH). This *in vivo* pulsatile release rate profile of LHRH from the medial basal hypothalamus represents a physiological determination of spontaneous release and corresponds well with the pulsatile active and silent periods of LH levels in the blood of intact male rats as previously reported by others [3]. Throughout the perfusion period, the behavior of the animal appeared relatively normal displaying periods of activity and rest as well as eating and drinking. This data illustrates the power of this technique for extended use in the conscious, unrestrained animal.

accomplished with the use of two separate pumps (Rabbit-Miniature Peristaltic Pump, Rainin Instrument Company, Inc., 94 Lincoln Street, Brighton, MA 02135) which permits a greater degree of flexibility in equilibrating the flow rates of the push and pull cannulae.

The major disadvantage of the push-pull perfusion technique is the lesion produced. The size of the lesion is a function of the size of the cannula implanted and the flow rate used during perfusion. With the miniaturized push-pull cannula described in this report this lesion is greatly attenuated. In addition, the slower flow rate (<20 μl/minute) permitted with this reduced size cannula further alleviates tissue damage. This represents a substantial improvement over commercially available cannulae as well as those described by others which are considerably larger and use excessive (150 μl/minute) flow rates [10]. While clogging always remains a potential problem, with vigorous aseptic conditions, careful insertion of the inner cannula assembly, exact equilibration of push and pull flow rates and a slow flow rate, minimal technical problems are encountered. With this procedure, as illustrated in Fig. 4, we have been able to effectively perfuse discrete brain loci with minimal tissue damage for a continuous 24 hr period [2] as well as perform repetitive perfusions within the same animal [12].

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