An Improved Method for Chronic Catheterization of the Rat Spinal Subarachnoid Space

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Spinal cord Subarachnoid space Catheterization

IN 1976, Yaksh and Rudy described a method for chronically catheterizing the spinal subarachnoid space of the rat. In this technic, the free (exteriorized) end of the catheter was cemented into a 20 ga needle hub which was in turn affixed to the skull with screws and dental acrylic. This method has been used successfully to study the effect of intrathecally injected agents on nociception [3, 7, 9, 10, 11], sensorimotor reactivity [1], cardiovascular tone [2] and thermoregulation [6]. However, there are several technical problems associated with the catheterization method of Yaksh and Rudy: (1) fabrication of the catheter and fixation of the needle hub to the skull are time consuming, (2) cements of any type do not bind well to the polyethylene catheter; thus, during handling of the free end of the catheter, the tubing sometimes pulls free of the cement, resulting in an upward displacement of the catheter tip within the subarachnoid space, (3) occasionally, the free end of the tubing breaks off due to repeated flexion at the tubing-cement junction or due to friction between the tubing and the edge of the needle hub, and (4) the projecting needle hub can become caught in caging or laboratory apparatus, which can dislodge the hub from the skull. The present paper describes a simple method for catheterization of the rat spinal subarachnoid space which circumvents these problems.

METHOD

Preparation of Spinal Catheters

A portion of a completed spinal catheter is illustrated in Fig. 1. To make the catheters, we cut polyethylene tubing (PE 10) into segments 20 cm long. A length of stainless steel wire (0.005 in. diameter) is inserted into a segment and a small section of the tubing near its midpoint is briefly exposed to a jet of heated air. After the polyethylene has softened, heating is terminated and the sections of tubing on either side of the softened region are gently pushed toward one another. This produces a small bead in the tubing. The wire, which prevents the lumen of the tubing from collapsing during formation of the bead, is then withdrawn. A hot air jet can be fashioned with a 10 cm length of 18 ga stainless steel tubing which is connected by flexible plastic or rubber tubing to a source of pressurized air. The metal tube is attached to a ring stand and is heated by a Bunsen burner placed beneath it. With only a brief period of practice, the proper skills for forming a bead can be acquired. The beaded catheters can then be mass produced at the rate of about one per minute.

Yaksh and Rudy [8] reported that insertion of the catheter into the spinal subarachnoid space of the rat could be facilitated by stretching the portion of the tubing to be inserted. This can be accomplished by grasping the bead tightly between the thumb and index finger of one hand, the tip of the catheter in the other hand and pulling with a strong, steady motion. Stretching of the catheter is made easier by submerging the tubing in a water bath maintained at 60°C and pulling while the catheter is submerged. Ideally, the tubing should be stretched to just short of the breaking point. This degree of stretching produces a catheter approximately one-third again its original length. The stretched portion of the
catheter is then cut to the desired length. For a catheter designed to end at the lumbar enlargement in a 350-400 g rat, the bead to tip distance should be 8.5 cm.

The beads which can be formed conveniently by the hot air jet method have a diameter slightly less than that required for the catheterization method described below. We have found that the easiest way to increase their size is to apply a thin coating of dental acrylic. The acrylic should completely encase the bead and also about 1 mm of the tubing on each side of the beaded region. The optimal size of the finished enlargement is indicated in Fig. 1. We suggest that the acrylic be applied after rather than before the stretching step because the thin acrylic shell surrounding the bead can be crushed during the stretching process. After the acrylic coating has completely dried (about 10 minutes), the completed catheters can be sterilized by immersion in 70% alcohol. Prolonged storage in alcohol has no adverse effect on the catheters. Yaksh and Rudy [8] suggested that, before insertion, the spinal catheters should be treated with a silicone preparation to reduce the incidence of gliotic/fibrotic scar formation around the catheter tip. However, subsequent experience indicates that this procedure is of little value; the incidence of scar formation around the tips of treated and untreated catheters seems to be similar.

Surgical Procedure

To implant a catheter, a rat is anesthetized and mounted in a stereotaxic instrument or any other device which will hold the animal’s head firmly. An incision beginning at a line joining the ears and extending about 3 cm in a caudad direction is made in the midline: the fascia is retracted from the skull. The initial incision of the skin exposes a superficial layer of neck muscles which are then separated by a midline incision beginning at the occipital crest and extending caudally about 2 cm. Separation of the superficial musculature exposes an underlying layer of muscles which can be easily separated along the midline by blunt dissection. Next, a scraping tool is used to free the muscles from their point of origin on the occipital bone for about 0.5 cm on either side of the midline. The back of the skull can now be seen, and gentle retraction of the neck musculature will expose the atlanto-occipital membrane. This fascial membrane connects the base of the skull with the first vertebra (atlas).

The remaining steps in the implantation procedure can best be appreciated by referring to Fig. 2. A small (1 mm diameter) hole is drilled through the interparietal bone in the midline about 1 mm rostral to the occipital crest. A second hole of the same size is drilled at the midline of the occipital bone about 1 mm below the occipital crest. These holes are large enough to allow passage of the unstretched end of the polyethylene catheter but small enough to inhibit passage of the bead. Once the holes are made, the atlanto-occipital membrane can be incised. To obtain a better exposure of this membrane, the rat’s head is freed from the incisor bar and rotated downward to about a 45° angle. A small slit (1–2 mm long), beginning at the base of the skull, is made along the midline of the membrane. A 26 ga disposable needle can be used as a cutting edge. If the incision has been performed correctly, clear cerebrospinal fluid should immediately well up through the slit. The stretched end of the catheter can now be inserted in the subarachnoid space. Yaksh and Rudy [8] suggested inserting a stainless steel wire into the catheter to assist in passing the catheter through the subarachnoid space. We have found that the resulting lack of flexibility due to the inserted wire increases the risk of placing the catheter tip within the cord rather than within the subarachnoid space. Therefore, we have dispensed with this procedure, and to insure that the catheter does not penetrate the dorsal surface of the cord, the animal’s body is placed on a platform such that the body and head are in the same horizontal plane. The rat’s head, which has been freed from the incisor bar (see above), is rotated nose downward so that the head is at a 90° angle to the body. This position facilitates insertion of the catheter parallel to the dorsal aspect of the cord. A catheter is flushed thoroughly with sterile saline, and the stretched end is then carefully advanced in a caudal direction while rotating it between the thumb and forefinger.

Once the stretched portion of the catheter has been inserted into the subarachnoid space, the free end of the catheter is threaded through the occipital hole and out the top of the skull via the interparietal hole. To aid in the threading process, the tip of a 2 cm length of 30 ga stainless steel wire bent into a semicircle is inserted into the free end of the catheter. The wire and attached catheter are then passed through the occipital and interparietal bone holes. When the wire and catheter exit the interparietal bone hole, the wire can be removed and the free end of the catheter grasped. The tube should be pulled forward until the bead butts against the hole in the occipital bone. Next, the skin incision is closed and the exposed portion of the catheter is cut to the desired length. In our studies, we leave 4–5 cm of tubing exposed. Shorter external catheter lengths can be used but longer lengths are impractical because most rats will eventually bite the end off of longer catheters. When the external part of the catheter has been cut to the appropriate length, the catheter...
is cleared by injecting a few microliters of physiological saline, and the open end is occluded with a short length of 30 ga stainless steel wire.

**DISCUSSION**

The technic described for spinal catheterization of rat has been used in several long-term studies of thermoregulation involving multiple intrathecal injections of drugs [4,5]. We have found that this procedure circumvents all of the problems mentioned in the introduction. The catheters are easier to make and to install than those described by Yaksh and Rudy [8]. The bead prevents any appreciable shifting of the catheter tip within the subarachnoid space. Rostrad movement is prevented by the inability of the bead to pass through the hole in the occipital bone. In theory, the catheter could slip backwards, but in practice this does not occur because the bead is larger than the slit in the atlanto-occipital membrane and because the overlying musculature of the neck inhibits movement of the bead. Furthermore, “decatheterization” is virtually impossible, and breakage or tearing of the catheter due to flexion has never been observed in rats catheterized using this method. Although it might appear from Fig. 2 that cerebellar damage would be a common consequence of application of the present technic, it in fact is not.

When the skull holes are drilled correctly and the bent wire used to pull the tubing through is of the correct shape, the cerebellum is not injured during the catheterization procedure, and the intercranial portion of the inserted catheter rests in the liquor space between the cerebellum and the back of the skull. Furthermore, in spite of the fact that we make no effort to plug the skull bone holes through which the catheter passes, postoperative infections within the cranial or spinal subarachnoid space or in the soft tissues surrounding the bone holes are extremely rare. Finally, it should be mentioned that we have encountered no difficulty in regard to maintaining the patency of the implanted catheters as long as they remain filled with saline; periodic flushing of the catheters is not necessary. However, the catheter tip and, indeed, the entire catheter will eventually become encased in scar tissue. The presence of this tissue does not detectably impede the inflow of injectate, but it does significantly alter the pattern of distribution of injected drug within the subarachnoid space. Formation of scar tissue seems to depend upon the length of time the catheter has been in place and upon the number of injections made through the catheter. We have found that daily flushing of the catheter does not inhibit the deposition of scar tissue and may actually enhance it.

**REFERENCES**