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Intrathecal Chronic Catheterization in the Rat

BASSAM DIB

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DIB, B. *Intrathecal chronic catheterization in the rat.* PHARMACOL BIOCHEM BEHAV **20**(1) 45–48, 1984.—This article describes the fixing of intrathecal cannula to the processus transversus T1. This method of fixing prevents rejection and allows the use of chronic cannula for experiments on animals over long periods up to one year. The intrathecal implantation may be made caudally from T1 using cannula from 1 to 7.5 cm. The technique for repeated perfusion in the freely behaving rat is described.

Surgical procedure Cannula fixation Intrathecal Long term

HISTORICALLY, the method of injecting a drug or other compound directly into an animal's nervous system has been a powerful tool, in order to understand the central nervous system (CNS) mechanisms of the control of body temperature, hormone release, autonomic functions, drinking, nociceptive transmission, etc. Another method was developed in the early 1960s. A procedure was devised whereby a chemical could be injected repeatedly into the cerebrospinal fluid [4,6] of the unanesthetized and unrestrained animal. In species with a large brain, the implantation of a chronic cisternal cannula appears to be more feasible [1,5]. The cannula or thermode is attached to the perfusion system in the freely moving animals. However, chronic maintenance of the intrathecal cannula or thermode is actually very difficult. The intrathecal device is always rejected. To circumvent these difficulties, we have developed a simple technique which permits repeated injection of drug or infusion of a thermode into any selected level of the spinal subarachnoid space in any medium-sized animal.

Yaksh and Rudy [7] developed a method for chronic catheter implantation in the spinal subarachnoid space. This cannula was used for drug administration. The small polyethylene catheter (PE-10) was inserted through a puncture in the atlanto-occipital membrane and secured to the skull. We tried this technique for chronic spinal thermode implantation for cooling and/or heating the spinal cord in the rat. By pressing a lever, a rat could modify the temperature of the water circulating in the thermode. This method was not entirely satisfactory for our purpose.

(1) The small tube does not allow for continuous perfusion under pressure because water circulation in a polyethylene U-shaped is very difficult.

(2) The cannula fixation at the occipital skull bone induces a traction when the rat turns its head left and right or up and down. The thermode or cannula moves in the spinal canal.

(3) The thermode implantation through the attlanto-occipital membrane is very difficult and the percentage of surviving rats is very small.

METHOD

Semi-sterile instruments, thermodes and cannula were used.

Cannula Preparation

Polyethylene tubing 0.70 mm external diameter and 0.30 mm internal diameter, and 11.5 cm long was used. A polyethylene tube 3.5 long 1.57 mm external diameter and 0.86 mm internal diameter was fixed to the former tube. Around this, 5 or 6 turns of surgical steel wire were wound and fixed by cyanoacrylic glue. The cannula is capped by a silicone tube 2.5 cm long (Fig. 1).

Thermode Preparation

The same polyethylene tubing was used. The thermode was U-shaped using a soldering iron. The same steel wire was around 5 or 6 turns and fixed with cyano-acrylic glue.

The cannula or thermode length varies according to the spinal cord locus under study. The rat was anesthetized IP Nembutal 35 mg/kg. No stereotaxic instrument was necessary for cannula or thermode implantation. A small cushion was inserted between the rat's neck and the floor to facilitate dissection and insertion of the cannula into the subarachnoid space. A 3 cm midline incision was made in the neck. The superficial and deep neck muscles were delicately separated and then drawn apart by a retractor. When the processus transversus T1 was bared, a small scraper was used to free the tissues from their point of insertion on either side of the midline. Using a forceps, the intervertebral disc between

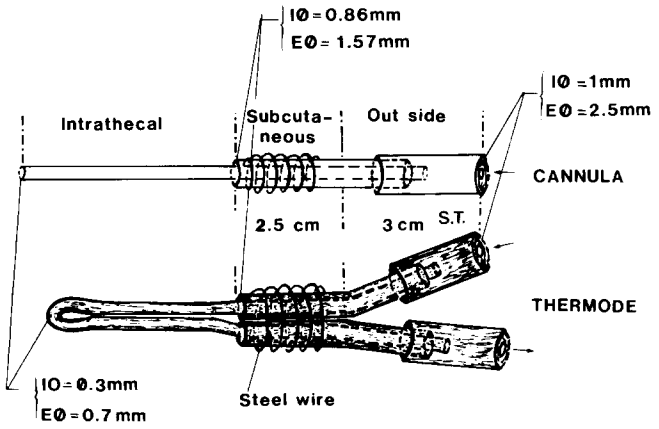

FIG. 1. Cannula and thermode perfusion. IØ: Internal diameter; EØ: External diameter; ST: Silicone tube.

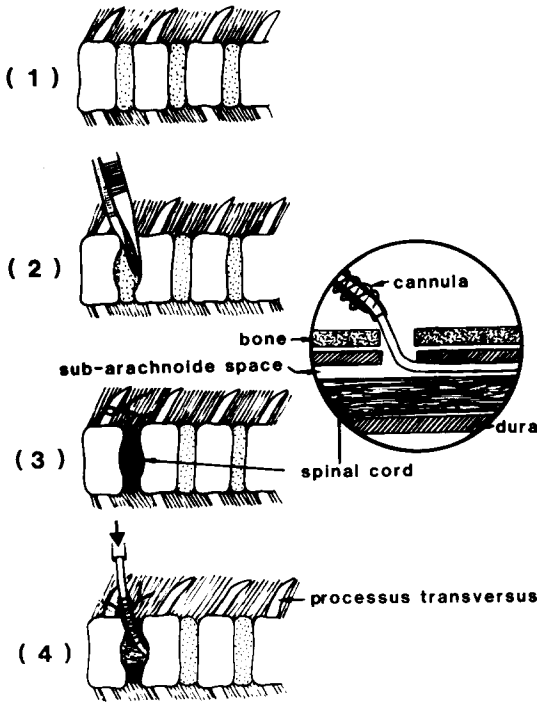

FIG. 2. Successive steps of implantation and fixing of cannula. No. 4: later steps.

C_8-T_1 and the dura was delicately removed without harming the spinal cord. When the vessels of the spinal cord are seen, and the cephalorachidian liquid came out, we could be sure that the cannula was inserted intrathecaly. The processus transversus T1 was denuded only on one side in order to loop and fix a steel wire around the processus transversus (steps No. 1, 2, 3, Fig. 2). This wire was finally used to fix the cannula or thermode after implantation. The thermode or cannula should be inserted slowly, during 5 min into the subarachnoid space in order to avoid apnea or hemorrhage. The thermode was attached to the processus transversus T1 by a previously prepared steel wire (later steps, Fig. 2). The incision was sutured. The implantation of cannula or thermode lasted from 15 to 20 min. Then the animal received 36,000 units of deposit penicillin subcutaneously. One week after surgery and recovery, the rats were apparently in good health; their motility and behavior were normal.

The following details should be noted: (1) Before insertion in the subarachnoid space, the cannula is filled by isotonic saline (NaCl) and the silicone tube is plugged. (2) If a cannula sticks during insertion we can do little but withdraw it, change the position of the animal's body and reinsert the cannula. The external part of the thermode or cannula is left free in the rat's neck. This is an advantage, since the flexibility of the cervical vertebra is not effected by the cannula. Furthermore, when the rat is grooming, the cannula does not budge, and is not displaced in the subarachnoid space since it is solidly attached to the processus transversus.

Figure 3 shows the radiographs of 3 rats implanted in the caudal direction with 3 types of intrathecal cannula 2 cm from T1 up to T4, 4 cm from T1 up T9, and 7.5 cm long from T1 up to L3. The insertion of cannula of 2 cm or 4 cm were well tolerated. The survival exceeded one year. When cannula 7.5 cm reach L3 from T1, the survival varied from 7 to 10 months, and sometimes exceeded one year. Long cannula sometimes cause apparent nervous trouble.

Twenty days after intrathecal cannula 7.5 cm long implantation from T1, histological control showed that there was a compression of spinal cord at T1 level (a). This compression is very small on section 1 cm from T1 (b) (Fig. 4).

Drugs are injected easily in 5 or 20 μl. This injection is delivered by a Hamilton syringe.

Water was continuously circulated through a U-shaped catheter under pressure by means of a pump. The flow of water through the thermode was 8–12 $ml \cdot mm^{-1}$ [3].

Our present study on the effect of capsaicin or substance P using cannula of 7.5 cm on the nociceptive threshold and on thermoregulatory behavior showed that 82 out of 92 rats tolerated the cannula implantation [2]. Of the 10 rats which did not tolerate the cannula implantation, 4 rats were discarded 2 or 3 days after surgery due to motor deficit in the hind legs. The 6 other rats showed motor deficit 3 or 4 weeks after intrathecal drug administration.

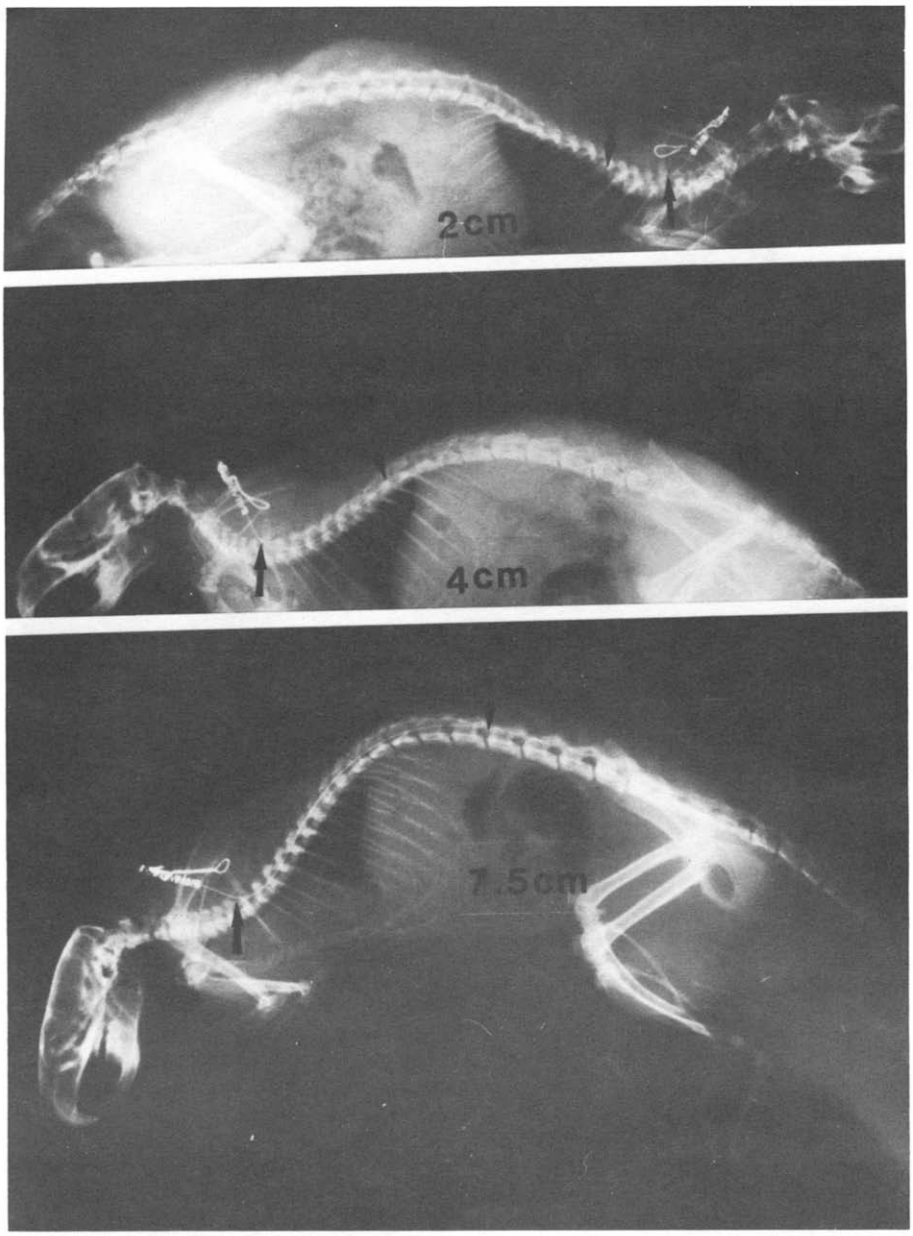

FIG. 3. Radiograph of three rats implanted with three types of intrathecal cannula: 2 cm from T1 up T4, 4 cm, from T1 to T9, and 7.5 cm long from T1 up to L3. Black arrow indicates the zone cannula insertion.

Eight rats were implanted with a thermode of 4.5 cm long. Only 1 rat was discarded. Five rats were implanted with a cannula towards the cisterna. They tolerated the cannula implantation attached to T1. Implantation cannula through the attlanto-occipital membrane which was fixed on the occipital skull bone, was not satisfactory [7]. This limits the implantation to one type of cannula (PE-10). Furthermore, in our hands the insertion of thermode by this technique was never successful. On the reverse our technique allow implantation cannula or thermode in 15–20 min. There is no need to open the occipital skull bone, because cannula was attached solidly at processus transversus T1. The use of this method can be extended to any experiment for any medium-sized animal over a long period.

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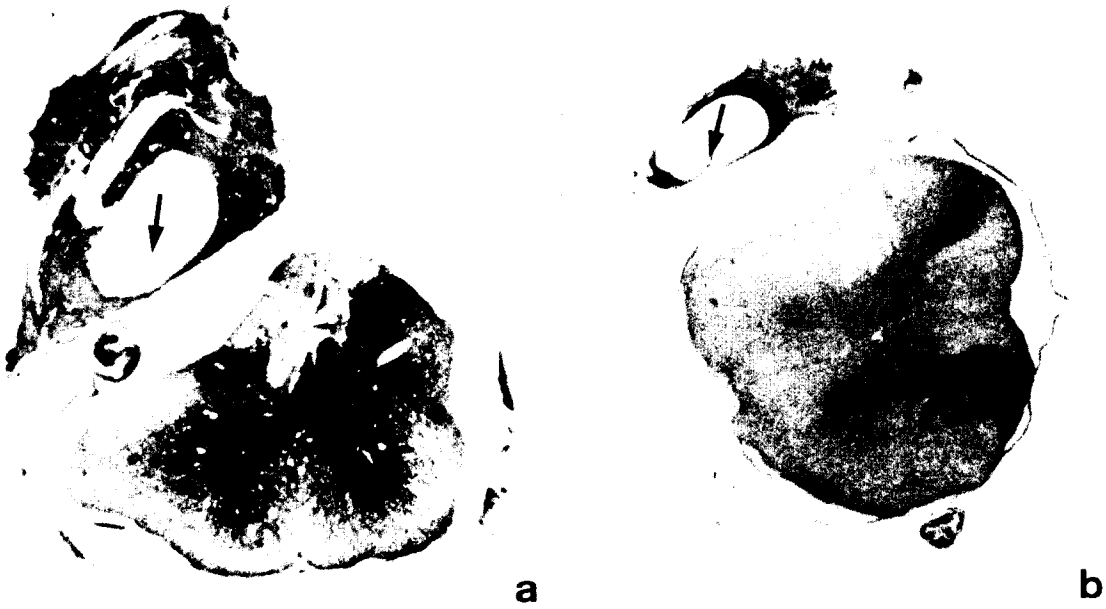

FIG. 4. Histological section of spinal cord at the T1 (a), and at 1 cm from T1 (b) (×50). Paraffin section (hematoxylin-eosin). Black arrow indicates the cannula implantation.

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