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A Rapid and Simple Cannulation Technique for Repeated Sampling of Cerebrospinal Fluid in Freely Moving Rats[1]

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BOUMAN, H. J. AND T. B. VAN WIMERSMA GREIDANUS. *A rapid and simple cannulation technique for repeated sampling of cerebrospinal fluid in freely moving rats.* BRAIN RES. BULL. 4(4) 575–577, 1979.—A cannulation technique for frequent sampling of cerebrospinal fluid (CSF) in unanaesthetized freely moving rats is described. A permanent stainless steel cannula, constructed in such a way that no loss of CSF occurs, is placed into the rat's cisterna magna and fixed to the skull by anchoring screws and dental cement. A special CSF outflow opening of the cannula is connected to polyethylene tubing for CSF sampling. Amounts of 50–150 μl CSF can be collected repeatedly without any sign of disturbing the animal. The technique lends itself not only to pilot studies in which within a short period of time a large amount of CSF is wanted, but also to experiments in which physiological conditions are required.

Cerebrospinal fluid (CSF) Cisterna magna

SEVERAL techniques have been described for collecting cerebrospinal fluid (CSF) from various animals species which vary from a simple lumbar puncture to complex perfusion systems.

Only few techniques meet the requirements needed for studying neuroendocrine roles of the CSF. Since the activity of the hypothalamo-pituitary axis is affected by a variety of conditions such as anaesthesia [2, 4, 6], stress [1,5], withdrawal of blood and/or CSF [4] and other manipulations of the animal, generally a compromise is made between the required physiological conditions and personal research interests. In order to avoid some of the manipulations which may induce changes in blood and/or pituitary hormone level, we developed a new CSF sampling technique, for studying the role of the CSF in brain-endocrine interactions under more physiological conditions.

For obvious reasons the technique has to fulfill the following requirements:

A simple and quick operation (a one man task).
No loss of CSF during its collection.
Controllable quantity and sampling rate of CSF.
No anaesthesia during collection of CSF.
The CSF collecting system must be operational for at least a week, because the animals need to recover from the operation and to be faimiliar with handling.
Repeated collection of CSF.
Simultaneous collection of CSF and systemic blood.
No contamination of CSF samples by blood.

METHOD

Construction of Cannula

The stainless steel cannula (Fig. 1) is constructed in such a way that before removal of the plug (Fig. 2) the CSF outway (Fig. 1-5) can be connected to polyethylene tubing (I.D. 0.34'', O.D. 0.50''). For this reason a silicone rubber disc (thickness 1.5 mm) is implanted in the cannula-construction (Fig. 1-3) so that a CSF one-way outflow will be ensured (Fig. 2). By means of a pressure screw (Fig. 1-2) leakages from the system can be avoided. Spontaneous CSF flow can be controlled by the plug (Fig. 1-1) which is guided by a stainless steel ring (Fig. 1-4).

After finishing experiments the cannula can be used again after removing the acrylic dental cement (which fixes the cannula to the skull) by acetone or chloroform. The silicone rubber disc can be renewed by removing the pressure screw of the cannula, thus gaining access to the exchangeable disc.

Surgery and Implantation

Male rats (weighing 250–300 g) are maintained under ether-anaesthesia for the duration of the operation. The animal's head is mounted with the skull in a horizontal position on a stereotaxic apparatus. A 3 cm incision is made in the skin from the back of the head and the overlying connective tissue is removed to expose the skull. A small hole is drilled in the skull using a dental burr (009) on the sagittal midline

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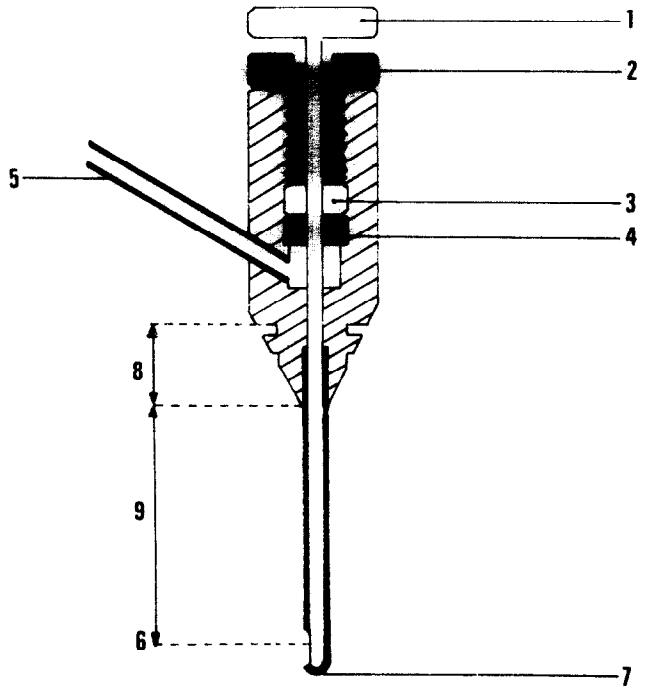

FIG. 1. Diagram illustrating the cannula system and its dimensions. The plug (1) is guided by a stainless steel ring (4). The silicone rubber disc (3) directs the CSF outflow through the CSF-outway (5) when the plug is removed from the cannula. The pressure screw (2) type m2 prevents the system from leakages of CSF. The longitudinal window (6) of 1.5 mm serves for CSF inflow. The tip of the cannula (7) is fashioned in such a way that the risk of obstruction of the cannula by tissue is minimized. The length of the stainless steel cannula from inflow opening to cannula anchoring construction (8) is 6.5 mm (see 9). The inner diameter of the cannula is 0.5 mm. The outer diameter is 0.8 mm.

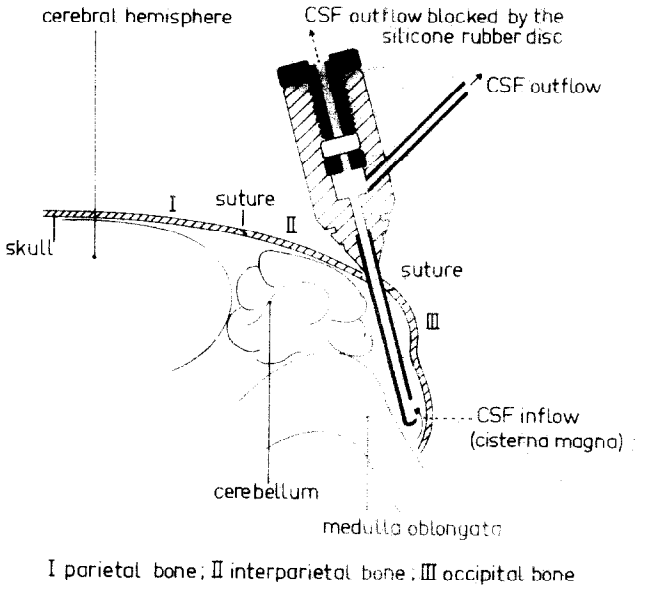

FIG. 2. Diagram of the location of the cannula showing the CSF flow through unplugged cannula.

immediately rostral to the interparietal-occipital bone suture. The hole is drilled in such a way that the occipital bone can be used as a guide while inserting the cannula (see Fig. 2). Additionally two or three holes are made in the (inter)parietal bone using a larger dental burr (010) for placement of stainless steel screws (m 1,2) to anchor the cannula. The cannula is then slowly placed into the cisterna magna without damaging the cerebellum. Correctness of placement of the CSF-inflow window (Fig. 1, Fig. 2) is checked by removal of the plug and clearing the CSF-outway, at which time a spontaneous flow of CSF should occur. For optimal effectiveness of the cannula system the spontaneous CSF flow must be without blood contamination. When successful implantation occurs the operation area on the skull is cleaned and dried and a small amount of dental acrylic cement (Simplex Repair Material) is applied to cover part of the inserted cannula (Fig. 1-8) and the anchoring screws. The whole operation takes approximately 10 min. Rats are housed singly without special care and are allowed to recover from the operation. Food intake returns to normal levels after one or two days.

CSF-Sampling Procedure

In order to avoid disturbance of the animal during collection of CSF whilst staying in its cage, the CSF outway of the cannula is connected to polyethylene tubing before replacing the animal in his cage. During the handling period to prevent the tube from hampering the movements of the animal it is counterbalanced by a light weight. There is no need for a rotating adapter as described by Steffens [7] to keep the tube in place at all times, because after a perfect handling period the animals are generally sleeping during CSF sampling. To measure the collected quantity of CSF the polyethylene tube is marked with 25 μl units.

DISCUSSION

For several reasons the cisterna magna has been chosen for cannulation. This enables us to use the high CSF pressure in this compartment to obtain CSF without a forced withdrawal. In case larger volumes of CSF are needed the sample tube can be connected to a syringe and CSF withdrawal can be performed by hand (to 10 μl/sec) or by a perfusion pump (from 0.1 μl/min).

The surgical procedure necessary for implanting the cannula requires a minimum of time and skill and in addition no sophisticated or costly equipment is needed.

Because of a separate CSF outway which can be closed, the cannula system prevents CSF losses which may occur when the cannula is connected to the sampling tube, as a result of the high CSF pressure. The plug cuts off the CSF outway, prevents the cannula from being clogged, and decreases the chance of infection. The possible leakage of CSF is prevented by the silicone rubber disc which allows only the plug to pass. The top of the cannula (Fig. 1-7) is fashioned in such a way that the risk of obstruction of the cannula by tissue is minimized.

In order to be sure that the CSF inflow-window of the cannula is always in contact with the CSF in the cisterna magna, a longitudinal window in the cannula is used instead of a round one because of the biological variation in the exact location of the cisterna magna.

The technique presently described is recommended for experiments in which sequential CSF samples are required from an animal without anaesthesia.

The rat does not seem to be disturbed while sampling the CSF, even when relatively large quantities of CSF are collected successively. Knowing that the CSF formation-rate in rats is about 2.2 μl/min [3], the technique lends itself not only to pilot studies in which a large amount of CSF is required within a short period of time, but also to experiments in which physiological conditions are necessary.

Preliminary experiments on hormone levels in CSF after pharmacological manipulation collected from freely moving rats result in a significantly more reliable effect than in anaesthetized animals.

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