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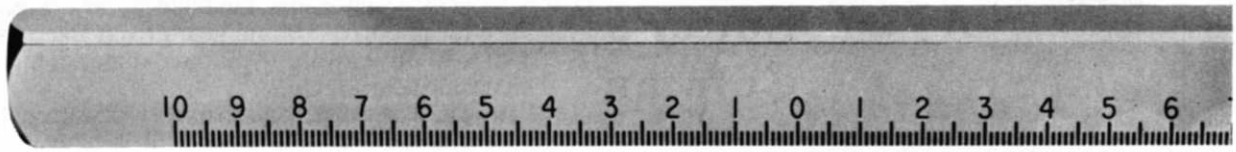

A NEW APPROACH TO REPEATED SAMPLING OF CSF FROM THE ANESTHETIZED RAT

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Determination of the CSF concentration of a neuro-transmitter or drug following experimental injection into the central nervous system often can be used to determine the actual effective concentration of the injected substance (Myers, 1977). In the rat, CSF is most commonly sampled from the cistema magna, which is located between the base of the cerebellum and the top of the medulla. The ultimate chemical makeup of the CSF is a consequence of filtration, diffusion and active transport. The normal flow of CSF is from the lateral ventricles through the intraventricular foramen to the third ventricle, then on to the fourth ventricle via the cerebral aqueduct and then into the cisterna magna. Thus, the CSF that reaches the cistema magna may be thought of as representative of the sum effects of production of CSF and the addition and removal of substances by the ependyml tissue of the ventricles.

The simplest way of sampling CSF from the rat involves removal of the musculature overlying the atlan-to-occipital membrane to expose the cisterna magna. The pool of CSF is then sampled by needle and syringe aspiration. Using this procedure, the rat is anesthetized and it is generally not possible to obtain repeated samples from the same rat. Surgical implantation of a cannula allows the investigator the luxury of sampling CSF from the awake, unrestrained rat. In most procedures, the cannula is introduced through a hole in the occipital crest, travels ventrally through the space between the cerebellum and the skull and terminates in the cisterna magna (Kiser, 1982; Lai, 1983). These cannulae are also used when repeated sampling of CSFis desired. However, the cannulae are characterized by a limited duration of patency and furnish variable, often small, volumes of CSF (Kiser, 1982). Some other disadvantages associated with permanently implanted cannula include tissue damage, temporary breakdown of the blood-brain barrier, and potential for infection and catheter obstruction (Wood, 1980; Myers, 1977; Kiser, 1982; Lai, 1983).

When anesthesia is not an impediment to the purpose of the experiment, the investigator may benefit from the ease of the technique described here, which facilitates repeated collection of CSF from the rat (Frankmannn, 1986). This technique does not necessitate implantation of a cannula. It is fast, minimally invasive and consistently yields large volumes of blood-free CSF. Repeated sampling, which allows the rat to serve as its own control, is possible with no apparent ill effects.

By taking advantage of the differences in resistance to negative pressure between the muscle tissue and CSF fluid, removal of the musculature overlying the cisterna magna is avoided, and the CSF is sampled without direct visual confirmation of the location of the cisterna magna. The collection apparatus is a 30 g needle attached by a length of polyethylene tubing to a syringe filled with water except for a small air bubble at the tip of the needle. The sampling needle is held in a modified Kopf electrode carrier which allows the needle to travel in parallel with the arms of the "U" frame (Fig. 1). The needle is guided stereo taxi cally into the musculature overlying the cisterna magna at which point a small vacuum is created by withdrawing gently on the syringe. As the needle advances further, the atlanto-occipital membrane is punctured and the needle enters the cistema magna. At this time, the CSF flows into the collection apparatus as observed by the movement of the air bubble in the tubing.

The modified carrier is constructed of a 16.5 cm length of aluminum rod. The original 8/32" diameter is reduced to 7/32" at either end to facilitate a good fit with the electrode carrier. At approximately 3 cm from the distal end a 90° bend is made. A cast acrylic block which fits the standard electrode clamp is then cemented to the distal end. When the modified electrode carrier is mounted in the stereotaxic instrument, the 30 g collection needle will travel in parallel with the "U" frame (see Fig. 1). The following paragraphs detail the CSF collection technique.

A Kopf model #900 small animal stereotaxic instrument is adapted with a Kopf #925 swivel mount that allows the "U" frame and the ear bars to be held approximately 15 cm above the table surface

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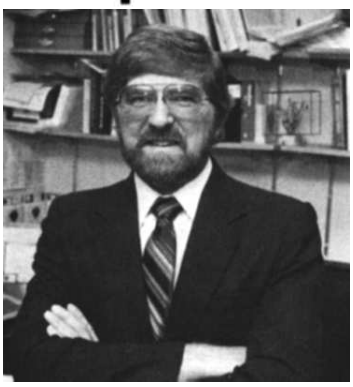

Editor's Column

We are back. After a short respite from publishing, the Carrier is resuming its quarterly appearance. It will be published as usual in the early summer, just before the Society for Neurosci-ence Convention in the fall, in the winter, and just before the FASEB meetings in the spring. In addition, the Kopf Instrument Company is looking into the possibility of sponsoring a newsletter for the Chapters of the Society for Neuroscience. Such a newsletter would help the more than 90 Society Chapters communicate with each other, trade ideas and hints about chapter organization, and other items of interest. Such support from companies such as Kopf, Grass, and others are vital to the ongoing health of the neuroscience community.

I want to remind readers that the Carrier has published a large number of interesting and helpful articles in the past. We will be providing an index of past articles in a coming issue in case there are any which you want to see. Copies would be available from the Editor or directly from the Kopf Instrument Company. In addition, the company has an annotated listing of current stereotaxic atlases available free of charge, which is also available from the editor or the company. If you have any suggestions for things which might be included in the Carrier, please let me know at the address below.

It is a beautiful evening in Ohio as I write this, the sort of spring evening that almost makes winter seem worthwhile as a contrast. It means that the academic year is drawing to a close and we can look forward to more time for research, travel or relaxing. It is a good time to look at your Kopf equipment and make sure it is ready for the next round of studies. As with any very precise equipment, the stereotaxic and positioning equipment and drives must be handled carefully for best results. As mentioned in an earlier column, the electrode carriers should not be heat sterilized due to the precision bushings and lubricants which could be damaged. If you have any questions about the operation of the equipment or how to care for it, please let me know and I will find the answer and publish the question and answer in this colunia

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(Fig. 1). It is critical to have a worm gear-driven anterior-posterior (A-P) drive (Kopf #960 three-dimensional electrode manipulator).

The CSF-collection apparatus is constructed of a 1 ml syringe; PE-10, PE-50, and PE-90 polyethylene tubing; 30 g disposable needles; distilled water, and a 100 ul collection pipette. At one end of a 60 mm length of PE-10 tubing, a heat flange is made and the blunt end of a 30 g needle (removed from its hub) is inserted approximately 2 mm into the lumen of the PE-10 tubing. To facilitate transfer of the CSF-collection line from the syringe to the 100 ul collection pipette, a collar of 5 mm PE-50 within 5 mm of PE-90 is slipped over the free end of the PE-10 tubing. The sharp end of a second 30 g needle, with its hub still attached, is inserted into the lumen of this end of the PE-10 tubing. The 1 ml syringe, filled with distilled water is fitted to the 30 g needle. The completed collection apparatus is then flushed with distilled water to insure patency and to check for leaks.

The free end of the CSF-collection apparatus is mounted in the electrode carrier with the lumen of the needle facing up. The heat flange is ahead of the collar, and the portion of the PE-10 tubing containing the 30 g needle is firmly clamped. A small air bubble (extending 1 cm behind the needle) is created by withdrawing very gently on the syringe plunger. This provides a barrier of

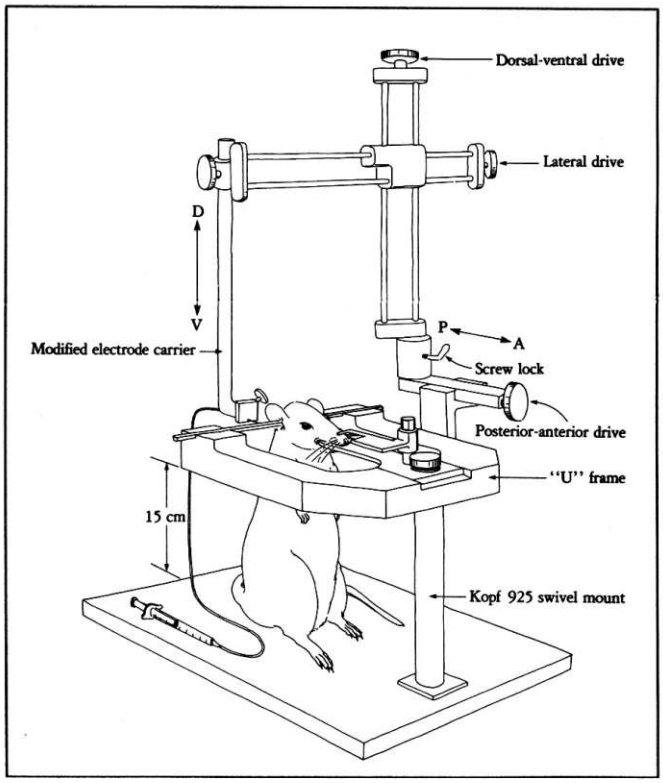

Figure 1. Schematic illustration of the CSF collection apparatus with the rat mounted in the stereotaxic instrument (Frankman, 1986).

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air between the CSF and the distilled water.

Using the three worm gear drives, the needle is aligned in the midsagittal plane at ear bar zero (see Fig. 1). All subsequent movements of the electrode carrier are confined to the A-P and dorsal-ventral (D-V) worm gear drives. The A-P worm gear drive is then used to move the needle posteriorally and the electrode carrier is temporarily rotated away from the "U" frame. The anesthetized rat is centered in the stereotaxic instrument, placing the tooth bar at its lowest position to maximize dorsoflexion and thus expose maximally the surface area of the atlanto-occipital membrane.

A small (less than 5 mm is sufficient) midsagittal incision is made through the skin, paralleling the midline at about 7 mm below the occipital crest. The electrode carrier is then rotated back into its original position, The A-P and D-V drives are used to position the CSF-collection needle so that it is just resting on the skull at the level of the occipital crest and the D-V position is noted. The needle is then moved posteriorly a distance sufficient to clear the head and using the D-V drive, the needle is lowered to a position 6.8 mm ventral. This distance closely approximates the midpoint of the atlanto-occipital membrane and thus the cisterna magna for rats weighing between 225 and 350 g.

The needle is advanced towards the cisterna magna using the A-P worm gear drive. If the skin incision does not coincide with the point of entry, it is gently moved until it does. When the needle has been sufficiently advanced such that the lumen is entirely within the muscle, the syringe plunger is pulled back. More pressure than was initially used to create the air bubble at the tip is necessary to see the air bubble begin to travel toward the collection syringe. The air bubble does not travel far, even with greater withdrawal pressure, because of the resistance of the muscle. The needle is advanced slowly, while gentle negative pressure is maintained with the syringe. The distance that the needle must be advanced before the cisterna magna is encountered varies widely from rat to rat 5-9 mm). When the air bubble begins to move toward the syringe, needle advancement is stopped. The CSF will follow the air bubble into the PE-10 tubing. Gentle negative pressure may be used to continue the flow of CSF. Once the air bubble has entered the collection syringe, the collection line is removed from the syringe and inserted into a 100 ul pipette. By positioning the pipette below the rat, the continued flow of CSF will be aided by gravity. When sufficient CSF has been collected (the collection line contains a significant volume of CSF) the needle is withdrawn from the head by using the A-P worm gear drive. The skin incision is then sutured, or if small enough, the edges simply pressed together.

The CSF samples are normally free of contamination by blood or muscle tissue. Occasionally, in attempting to sample CSF, the collection needle will encounter bone or blood instead of the cisterna magna. In this event the needle is withdrawn, flushed, the D-V position adjusted to a point slightly above or below the initial entry point, and the process is repeated.

This technique is unique in that all other reported methods of obtaining repeated samples of CSF from the rat involve surgical implantation of a cannula. Sampling of the CSF by the method described is fast, reliable and yields large volumes of blood-free CSF. Collection of 100 ul of CSF generally takes from 3 to 12 minutes. This procedure has been used successfully to obtain 100 ul samples of CSF as often as once every 3 days over a 2 week period. The rats continued to gain body weight and had normal food and fluid intake.

This technique has been used successfully to document differences in CSF immunoreactive insulin levels between obese and lean Zucker rats and in Wistar rats under freefeeding and fasting conditions (Stein, et al, 1983). Similarly, changes in the concentration of sodium in the CSF following extracellular fluid depletions have also been documented using this technique (Frankmann, Sakai and Simpson, 1987), which provides for repeated fluid sampling over long periods of time.

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