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A MICRODIALYSIS PROBE FOR RECOVERY OF NEURO-TRANSMITTERS FROM THE SPINAL CORD

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Brain microdialysis was first utilized to collect putative neurotransmitters in the late '60s when Bito et.al. (1966) used blunt dissection to prepare a pocket in cerebral cortex and inserted into it a dialysis sac approximately 10 mm in length. Microdialysis techniques have been substantially refined since then. The probes are smaller and minimizing tissue damage has become a priority. The aim, however, remains the same, to collect neurotransmitters or other small molecules from the extracellular fluid of neural tissue and to correlate changes in the levels of these substances with some experimental manipulation. Quantita-tion usually is performed with either chromatog-raphy or radioimmuno assays.

SPINAL CORD MICRODIALYSIS

Dialysis within the spinal cord is of more recent vintage. As in brain dialysis, the probes have a semipermeable membrane that allows exchange of substances between the dialysate and the extracellular fluid as well as plumbing that conducts the perfusate to the exchange point (dialysis site) and then on to a collection point. Perfusate consists of either an artificial cerebrospinal fluid or Ringers solution.

There are two basic probe designs; 1) vertical probes with inflow and outflow arranged concentrically or in a U-shape can be lowered into the spinal cord or 2) transverse probes with one way flow of the dialysate can be inserted through it (Ungerstadt, 1984). Advantages of dialysis over other collection techniques include exclusion of most enzymes from the dialysate (depending on the pore size in the dialysis material), ability to administer pharmacological agents as well as to retrieve neurotransmitters, relatively small sampling area and the capability to pair it with recording electric activity of single cells (Sorkin, et.al., 1988).

Our group has chosen to use a transverse approach (Figure 1). This is an adaptation of Un-gerstedt's technique. Since the transverse probe requires only one way flow of the perfusion solution, it can be constructed with a smaller diameter than a vertical probe. The dialysis probes that we use are modified 30 cm lengths of Cuprophan hollow fibers (150 (J.m inner diameter, 9 |J,m thick wall) with a 9 kDa molecular weight cutoff

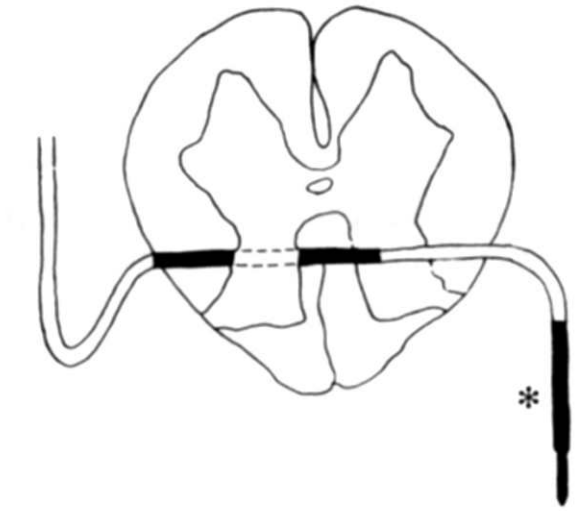

Figure 1. Schematic of a transverse section of the spinal cord dorsal horn and a dialysis probe. The leading end of the fiber includes a dissecting pin (*). The pin is cut off and the probe connected to the syringe pump. The uncoated area, the dialysis site, is indicated by the dotted lines and the ink spots used for placement are also shown. Lengths of the fiber and pin are not to scale

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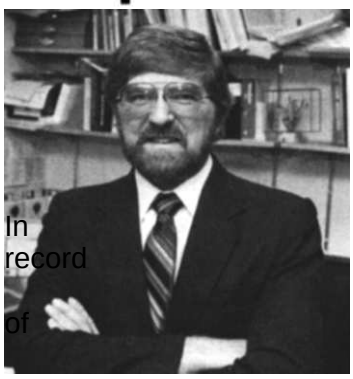

Editor's Column

In record of What wonderful winter weather we are having here in Ohio this month. December, we had breaking low temperatures, with lots snow, when usually it is fairly mild here. Now it is mild, in the 50s and 60s (F) and not a trace of snow when it is usually fairly cold and snowy. Maybe the world is not getting warmer, it just has its seasons confused.

The recent break-in and vandalism by the Animal Liberation Front at Adrian Morrison's laboratories at the University of Pennsylvania is a real attack on all science. Dr. Morrison is chair of the Committee on Animals in Research of the Society for Neuroscience and the avowed intent of the break-in was to intimidate him. Please consider this new tactic and write to your congressperson about the seriousness of these groups and the threat to science and human progress they represent.

Please notice the back page of this issue of the *Carrier.* It contains two important announcements. The lower one concerns an issue which is becoming increasingly important and which has been addressed before here. The importance of cleaning your equipment before returning it to Kopf Instruments for calibration or repair cannot be overemphasized. The company has received equipment which was covered with blood and other matter. It is a real risk for the technicians and staff who must handle that equipment without knowing what the material is or what pathogens might be carried on it. Kopf will send back equipment received in dirty condition, so please clean it up before sending it in.

The article in this issue of the *Carrier* is another example of a unique use of dialysis. The application described is innovative and useful to many readers. I hope that some of you who read it will be moved to write up your techniques for the *Carrier.* Just call or write to me at this address:

Happy New Year!

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(Spectrum Industries). Although this is our choice for collection of monoamines and amino acids because small diameter fibers are available, it is unsuitable for collection of peptides; other materials for this purpose are presently available (Kendrick, 1989).

DIALYSIS PROBE CONSTRUCTION

To seal the pores in the dialysis fiber, a thin layer of silicon rubber (Dow Chemical 3140 RTN) is applied along its length except for a segment intended for dialysis. The length of the dialysis site varies; we generally use 1 mm in cats and monkeys when the intention is to confine sampling to the dorsal horn and 2 mm in rats when we sample from the entire dorsal half of the spinal cord. To accomplish this the fiber is first marked by two 1 mm long indelible ink spots separated by the length of the intended dialysis site. It is then suspended between a pair of mi-cromanipulators with two short sleeves of polyethylene (PE 10) tubing placed around the fiber, one on either side of the ink spots. A few drops of silicon rubber are applied on each end of the fiber and the PE tubing is moved back and forth several times to produce a seal of uniform diameter. Irregularities which are especially prone to be formed at the edges of the bare site are smoothed down with a pair of jewelers forceps. The uncoated dialysis site is flanked by two 1 mm long marks. The coating encompasses both ink spots, but not the space between them. All manipulations are performed under a dissecting microscope. Fiber length is measured before and after the sealing procedure; any fiber that stretches more than 2 mm is discarded. Stretching changes the molecular weight cutoff of the dialysis material. The length of the uncoated dialysis site and the maximum diameter from the leading end to several cm past the marks are measured. Any fiber with a maximum diameter in excess of 250 nm is also discarded. Average maximum outer diameter is 210-230 (im. After the silicon rubber dries, the dull end of a mini-dissection pin (0.1 mm diameter cut to 10 mm length) is inserted into the lumen of the leading end of the fiber and glued into place with cyanoacrylate. The dull end of the pin is electrolytically etched to reduce its diameter previous to insertion.

DIALYSIS PROBE INSERTION

After the dura is cut the spinal cord is raised slightly and stabilized using a glass hook. The dissection pin is grasped in jewelers forceps, inserted transversely through the spinal cord and the fiber is pulled through the positioned. Medi-olateral placement is accomplished by pulling the fiber through the spinal cord and using the second ink spot for reference.

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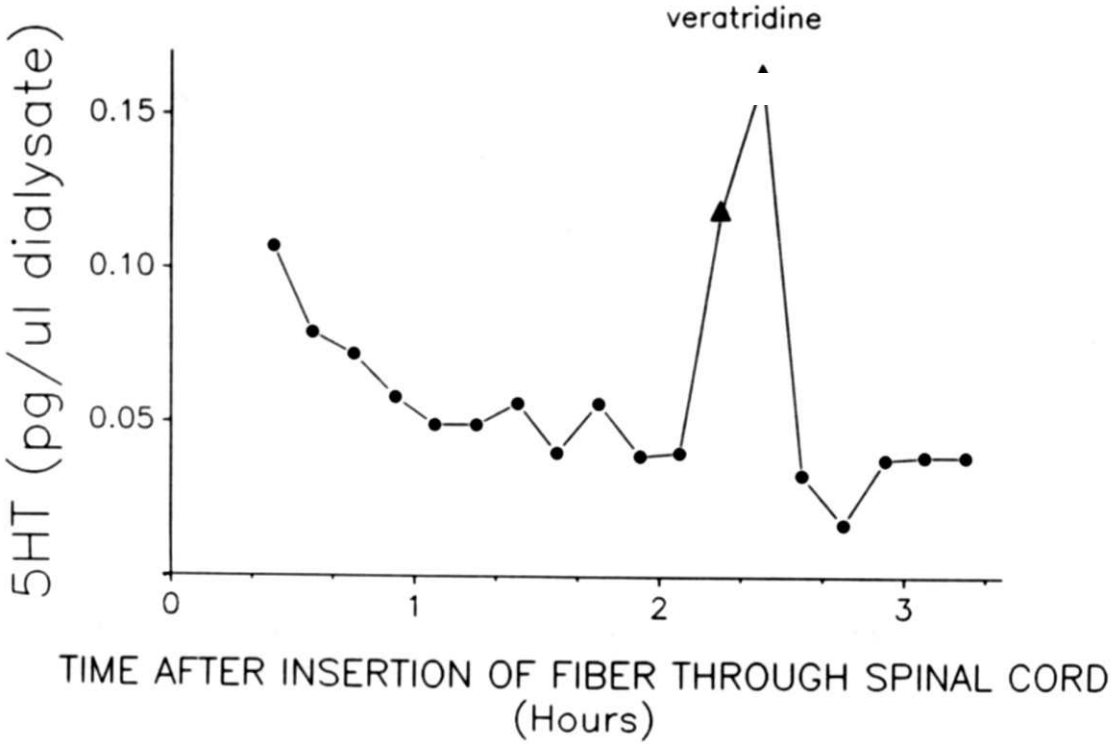

Figure 2. Simultaneous administration of a pharmacological agent and collection of a neurotransmitter within a localized area. Time course of a single experiment in which serotonin (5HT) was collected and measured using high pressure liquid chromatography. The probe was inserted through the spinal cord at time = 0. Samples were assayed in 10 min aliquots. Levels of 5HT were initially high. After a baseline was established, veratridine was administered through the fiber of two sampling periods (filled triangles). The increase in extracellular 5HT caused by the depolarizing agent is clearly seen.

Dorso-ventral placement is accomplished using the dorsal root entry zone and dentate ligaments for landmarks. The glass hook is removed, the pin cut off and the leading end of fiber connected to a syringe pump via PE 20 tubing. Flow rates of 4-5 jil/min maximize collection efficiency and do not lead to obvious problems with transmitter depletion. During all manipulations precautions must be taken to ensure that the fiber and the silastic coating are not damaged.

IN VIVO EXPERIMENTS

Extracellular levels of many substances including neurotransmitters are often elevated after fiber insertion, and a 'washout period' lasting for a few hours is necessary before stable background levels of these substances can be ascertained. Figure 2 illustrates washout, control levels of collected serotonin, and the response of serotonin levels to veratridine, a depolarizing agent, introduced through the dialysis fiber. At the end of each experiment dye (we use a filtered crcsyl violet solution) is run through the fiber for 15 minutes and then washed through with the perfusion solution. Histological examination of the spinal cord and dye mark determined the location of the dialysis site.

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