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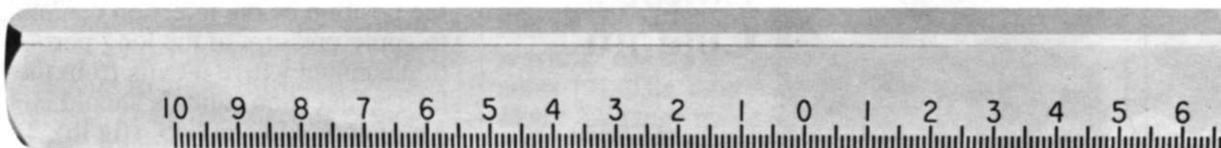

DRUG MICROINJECTION IN DISCRETE BRAIN REGIONS

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INTRODUCTION

Microinjection is the mechanical administration of chemicals to specific brain regions to localize the site of drug action. The technique was refined during the 1960's and has since been used to determine the site of drug action in studies of pain, sleep, neuroendocrine function and epilepsy to name a few.

Component Construction

The microinjection technique as used in my laboratory is intended to administer small volumes of drug solution directly into specific brain regions. Although commercial products are available, it is more cost effective to construct our own components. In addition, because our system uses smaller diameter fluid lines without liquid swivels we have found that our technique produces the most reliable and consistent microinjection volumes.

There are 4 components involved in our microinjection system: the guide cannula; the injection cannula; the stylet (also known as the obu-trator or dummy cannula); and the reamer or guide cannula cleaning device. The guide cannula is stereotaxically placed 1 mm above the microinjection site a number of days prior to the actual experiment. The injection cannula is inserted into the guide cannula at the time of the experiment. The fully inserted injection cannula protrudes 1 mm beyond the end of the guide cannula. This means that the guide and injection cannulas must be constructed meticulously so that all components are of a consistent, predetermined length. The third component is the stylet, which is placed into the guide cannula when the animal is not in use to reduce the incidence of occlusion. The fourth component is the reamer, which we use after the stylet is removed to insure that the guide cannula is completely clean.

The guide cannulas are constructed from 26-gauge stainless steel tubing (Small Parts, Inc.). Stainless steel will not leach contaminants into the tissue and does not interact with the injected chemicals [1]. The length of the tubing will be discussed below but it is suggested that the sections be cut slightly longer than required as the refinement of the ends of the guide cannulas usually consumes the extra length. The ends of the guide cannula should be finished so that they are smooth, rounded, and open (fig la). The rounded edges insure a smooth insertion and placement of the injection cannulas. The smooth edges also reduce the tissue damage induced when the guide cannulas are inserted into the brain. The outside edges of the cannulu can be refined using a fine grade file or emery cloth. The lumen edge can be reamed with the tip of a new 28 gauge (or smaller) syringe needle. A dissecting scope greatly facilitates this process.

The injection cannulas are constructed from 32 or 33 gauge stainless steel tubing (Small Parts, Inc.). The tubing for the injection cannulas is cut 3-4 times longer than the guide cannulas. The extra length provides sufficient tubing for attachment of the PE-10 tubing that is used as the fluid line as described below. The ends of the injection cannula should be smoothed and rounded as described for the guide cannulas. This allows for easier insertion into the guide cannula, reduces tissue damage at the site of injection and facilitates the attachment of the PE-10 tubing.

An injection cannula is prepared by insertion into a guide cannula until the end of the injection cannula protrudes 1 mm beyond the end of the guide cannula (fig Ib).

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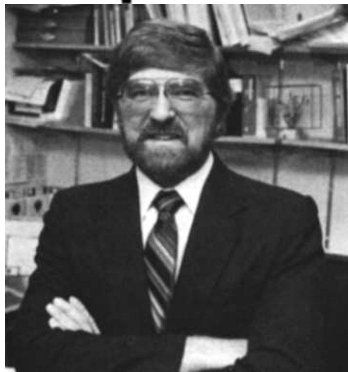

Editor's Column

It is a beautiful day in Kansas City as I write this column. I think that Labor Day completely delineated summer from fall here; sweltering one day, crisp and cool the next. It looks like a great fall coming up.

The *Carrier* is coming to you again in anticipation of the Society for Neuroscience Meetings. Please stop by the David Kopf Instruments booth to see the new instruments that will be there this year and say hello to us. Take a look at the great new Neuro-Hyperdrive - a device for using multiple electrodes simultaneously, and the new MRI Stereotaxic Instrument for those of you doing MRI imaging. It will be a great show. See the back page of this issue for the booth numbers.

This issue of the *Carrier* was written by Ste-ven Peterson. In it he discusses various issues pertaining to cannula construction and use. This information is valuable to those thinking about using these devices who wish to construct their own cannulas or purchase ready made ones such as those sold by David Kopf Instruments. The use of cannulas has produced much information, and is certainly a very useful technique.

Those of you who regularly receive the *Carrier* remember that the past two issues were written by Jan Galik and his colleagues. Jan is now back in Kospice, Slovak Republic. I hear from him from time to time by e-mail. He seems to be doing well. The use of e-mail certainly is a wonderful convenience, and even more than that at times. I am sure I would not be able to communicate with Jan nearly as much were it not for the computer and net. I have heard from scientists in Russia via e-mail and look forward to hearing from more colleagues around the world with their comments on the *Carrier*.

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Then, without disturbing the position of the injection cannula, use a finger to apply pressure to the long portion of the injection cannula where it exits from the guide cannula. The injection cannula should be bent to an angle of approximately 45° (fig Ib). The bend in the injection cannula will serve as an automatic stop to position the cannula exactly 1 mm beyond the end of the guide cannula. In this way the guide cannula never actually reaches the target tissue thereby reducing gliosis and chronic tissue damage in the site of interest. At the time of injection the smaller injection cannula induces less damage in the previously undisturbed target tissue than the larger guide cannula would have caused.

Reamers are also constructed from 32 or 33 gauge stainless steel tubing. They are made by inserting the tubing into a guide cannula until it reaches the end of the cannula (stand the cannula on a flat surface and put the reamer in). The reamer is then bent to a 45° angle and cut about 4 cm from the bend to serve as a handle. The difference between reamer and the injection cannula shown in Ib is that the reamer does not project beyond the guide cannula.

The fluid lines are PE-10 polyethylene tubing (Harvard Apparatus, Inc.) that connect the injection cannula to the 10 uL syringe which controls the volume administered. We use 30-40 cm fluid lines which are long enough for convenient use but not so long as to create a large dead space that affects the accuracy of the infusion volume. The fluid line is attached to the injection cannula with Super Glue®. The 32 or 33 gauge stainless steel tubing used for the injection cannulas slides easily into the PE-10 tubing. With the tubing inserted into the PE-10 tubing about 4-5 mm, the conjunction of the tubing is placed into a suspended drop of Super Glue® (fig Ic). Keeping the end of the PE-10 tubing in the drop of glue, the stainless steel tubing is quickly slid into the PE-10 tubing. The PE-10 should be stopped about 1 cm from the 45° bend in the injection cannula. This procedure forms a tight bond between the stainless steel tubing and the PE-10 fluid line.

We perform bilateral microinjections which requires bilateral injection guide cannulas. To fabricate the bilateral guide cannulas, two independent guide cannulas must be positioned exactly in parallel, separated by the required distance. Our experiments require bilateral micro-injections into brainstem nuclei that are 1 mm from the midline. Thus our cannulas are positioned 2 mm apart. We have found that the standard electrode holder with the corner clamp

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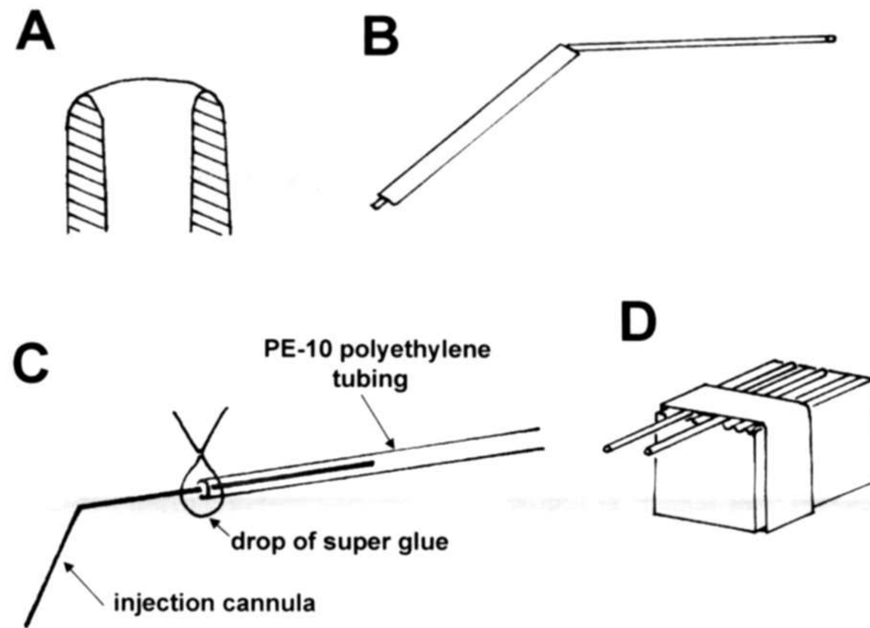

Figure 1. A) Cross section view of the end of an injection guide cannula after the inside and outside edges have been rounded and refined. B) Injection cannula fully inserted into the guide cannula. Note that the injection cannula protrudes 1 mm from the end of the guide can cannula. The end of the injection cannula that will be attached to the fluid line is bent to a 45° angle. The bend will serve as the stop mechanism for positioning the injection cannula 1 mm beyond the end of the guide cannula. C) The conjunction of the 32 ga. stainless steel tubing and the PE-10 polyethylene tubing is introduced into a drop of Super Glue®. The end of the PE-10 tubing is held in the drop while the stainless steel tubing is pushed into the PE-10. D) Positioning of independent guide cannulas in a Kopf electrode carrier to form a bilateral injection cannula. A solder bead may be formed across distances between cannulas of at least 4 mm.

(Kopf model 1770) works very well for positioning our guide cannulas as the grooves of the acrylic block on the electrode holder are exactly 1 mm apart. Positioning guide cannulas that are not exactly 1,2, 3, 4, or 5 mm apart requires extra preparation. Typically a machine shop is needed to create parallel grooves of the required separation in either a block of stainless steel or a ceramic material (Ed. note: Contact David Kopf Instruments for custom spacing on a corner clamp). Once fastened in the grooves, the two independent guide cannulas are soldered together using stainless steel solder. We have found that a solder joint or bead will form easily across distances up to 4 mm between the guide cannulas.

Several issues must be considered when determining the length of the guide cannulas. The length of the ventral or bottom end of the guide cannula (fig 2a) must allow for the depth of the nucleus in the brain with the consideration that the end of the guide cannula will be 1 mm above the actual site of microinjection. The ventral portion also must allow for the thickness of the skull, which varies depending on the age of the rat. The solder joint typically requires 2-3 mm. The dorsal or top part of the guide cannula (fig 2a) must be of sufficient length to allow accurate positioning of the cannula in the electrode or cannula holder. In our experience, this requires 7-8 mm of cannula. A final consideration is that the overall length of the guide cannula should be kept as short as possible to prevent bending once positioned in the awake, freely moving animal.

A stylet should be placed in the guide can cannula when the animal is not in use to avoid occlusion. The 33-gauge tubing is inserted into the full length of the guide cannula but not beyond. The tubing protruding from the insertion end of the guide cannula is then bent to approximately a 150° angle and cut leaving a 2-3 mm section (fig 2b). The stylet is kept in place by sliding a section of 22-gauge polyethylene tubing (Small Parts, Inc.) over the stylet and guide cannula (fig 2b). This is best done at least 1 hour after application of the dental acrylic that anchors the guide cannula to the skull.

Guide cannulas can be used multiple times. After the implant is removed from the skull, it is soaked in chloroform for several days. This solubilizes the dental acrylic and allows separation from the guide cannulas. The inside of the

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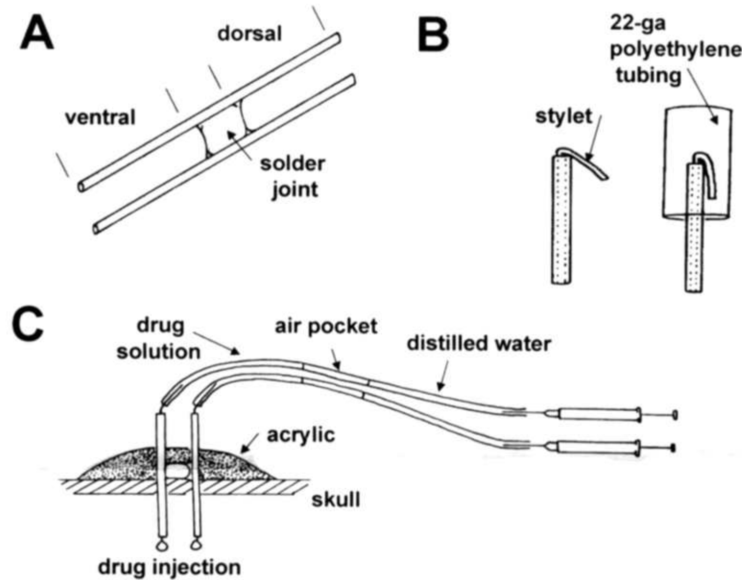

Figure 2. A) A depiction of the bilateral microinjection cannulas used in our laboratory. Indicated are the ventral and dorsal portions as well as the relative position of the solder joint. B) Placement of the stylet (orbutrator or dummy cannula) in the guide cannula. A section of 22-gauge polyethylene tubing is positioned over the stylet to prevent stylet loss. The style and 22-gauge tubing are positioned after the implantation of the guide cannula when the dental acrylic is well dried. C) Schematic of the final microinjection procedure. Note the positioning of an air pocket between the distilled water in the syringe and the drug solution in the injection cannula. (Components not to scale.)

guide cannulas should be cleaned carefully to remove any residual acrylic. The stylets tend to break easily at the point where they are bent, so they should be used only once.

Microinjection Procedure

A 10 µL syringe driven by a syringe pump is used for the microin-jections. The syringe is filled with distilled water and should never retain any drug solution. The PE-10 fluid line and injection cannula are initially filled with distilled water and then fitted over the permanent needle of the 10 µL syringe. Once attached, the contents of the syringe should be expelled through the injection cannula to insure that there are no leaks in the system. The plunger of the syringe is then withdrawn such that a 2-3 cm pocket of air is pulled into the fluid line (fig 2c). The tip of the injection cannula is then inserted into the drug solution to be microinjected and the solution drawn into the PE-10 fluid line (fig 2c). Thus, the drug solution is separated from the distilled water by the air pocket, which also allows visual determination of how much drug solution remains in the fluid line and whether or not the drug solution is moving.
The syringe pump drives the 10 µL syringe at the previously determined injection speed. The pump should be allowed to run at the infusion speed until a drop of drug solution is observed at the tip of each infusion cannula. The injection cannulas are placed into the guide cannulas without stopping the syringe pump. The injection cannulas are again observed for drops of drug solution once they are removed from the animal to confirm that the cannulas did not become occluded during the injection. In short, we do everything possible to assure ourselves that the micro-injection system is delivering the drug solution before, during and after the injection.

The rats are restrained by swathing during the microinjection. That is, the rat is wrapped in a towel or sheet such that only the head protrudes from the wrapping. The animals tolerate this very well, especially if they are conditioned to such restraint for a 5 minute period the previous day. The stylets and the 22-gauge polyethylene tubing that hold the stylets in place are removed using thumb forceps. The guide cannulas are cleared using the reamers. This may require several passes of the reamer if the guide cannulas have accumulated some organic matter. The injection cannulas that have been verified to be freely flowing are then inserted. We administer 0.5 µL of drug solution over 5 minutes at a rate of 0.1 µL/min. At the end of

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tthe injection period, the syringe pump is turned off and the injection cannula left in place for one minute to allow diffusion of the solution into the tissue. Once the injection cannu-las are removed the stylets are replaced, either with or without the 22-gauge polyethylene tubing, depending on the needs of the experiment. The syringe pump is restarted to insure the injection cannulas are still flowing freely.

It is critical that the location of the drug infusion be determined. To do this we anesthetize the rat and microinject a saturated solution of Fast Green dye using the same 0.5 µL volume and injection rate. The animal is then sacrificed and the location of the tip of the cannula determined by standard histological procedures. Technical Considerations The spread of the microinjected drug solution in the brain is dependent both on the volume and the rate of administration. The larger the injection volume, the larger the spread of the drug solution. A 0.5 µL volume spreads to approximately a 1.0 mm diameter sphere whereas 2.0 µL spreads to 2.4 mm [2]. The volume of the spread is consistent for up to an hour after infusion [2, 3]. It has been recommended that no more than a 0.5 µL volume be administered to rats if the drug is to be retained within the desired structure [4]. However, in larger structures, a 1.0 µL volume that spreads approximately 2.0 mm [2, 5] might prove useful. No tissue damage occurs with volumes of 2.0 µL [6] unless rapidly administered in a 1 minute period [7]. Volumes of 10 µL induce significant tissue damage [6] and are not recommended [1].

The rate of microinjection also affects the spread of the drug solution. The faster the injection, the greater the spread. A 0.1 µL volume administered over 5 minutes gave an estimated volume of 0.04 mm3 while the same volume administered over 1 minute gave a 0.12 mm3 volume and resulted in the drug solution leaking back up the cannula tract [7]. Rapid injections tend to distend and disrupt the target tissue and injection rates over 1 µL/min. are not recommended [1]. Spread of injection also has been shown to affect the physiological response [7]. We use a rate of 0.1 µL/min. although others have successfully used speeds as fast as 0.2 uL/min. [8] or as slow as 45 µL/min. [9]. Injection of 0.1 µL over 5 min. has been recommended for the discrete placement of a drug solution within a specific subnucleus [7].

We use 32-33 gauge (0.23-0.20 mm outside diameter) injection cannulas with 26-gauge (0.45 mm) guide cannulas to produce as little disruption of the brain tissue as possible. Others successfully use 28 gauge (0.36 mm) injection cannulas [9]. The larger injection cannulas require larger 22-gauge guide cannulas (0.71 mm) which also induce a greater degree of tissue damage.

The osmolarity, ionic concentration, and pH of the drug solution may affect microinjection results [1,2]. Saline or artificial cerebrospinal fluid (CSF) are typically used as the drug vehicle but distilled water may be used if a particular concentration of test compound would otherwise cause a hypertonic solution. Most investigators attempt to microinject solutions as close to pH 7.4 as possible [1] although pH values ranging from 5.0 to 8.0 have been reported. It is critical to test microinjections of vehicle control solutions that are identical to the drug solution in all possible ways except for the drug presence. Interpretation When performing microinjection experiments it is possible to conclude that a drug is acting within a specific brain region if the following conditions apply: 1) The microinjected drug induces a dose-response effect; 2) The effect of the drug is inhibited by a specific antagonist. The antagonist may be administered by microinjection or systemically; 3) The drug effect is site specific. This means that drug microinjections 1 mm beside, above or below the hypothesized site of action have no effect [10, 11]; 4) Microinjected drugs that are chemically unrelated do not have the same effect. Implementation This technique has been used in my laboratory to demonstrate a potential role of the brainstem in the mechanism of action of anti-convulsant drugs. We have shown that bilateral microinjection of N-methyl-D-aspartic acid receptor antagonists in the n. reticularis pontis oral-is inhibits the tonic hindlimb component of maximal electroshock seizures in rats [11, 12]. Inhibition of tonic hindlimb extension is the end-point taken for anticonvulsant efficacy in generalized tonic-clonic (Grand Mal) seizures in epileptics. Microinjection has played a critical role in the determination of neuronal networks involved in several experimental models of epilepsy [8, 9, 10, 11, 12]. Others have microinjected inhibitors of muscarinic receptor linked G-proteins to characterize further the intracellular mechanisms associated with rapid eye movement sleep [13]. The use of microinjection to administer selective signal transduction inhibitors to specific brain sites in conscious, behaving animals has significant scientific potential.

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References

1. **Carvey, P.M, Maag, T.J.** and **Lin, D.** (1994) *Injection of Biologically Active Substances into the Brain. In Providing Pharmacological Access to the Brain: Alternate Approaches.* San Diego: Academic Press, Inc.

2. **Myers, R.D.** (1966) Injection of Solutions into Cerebral Tissue. Relation between Volume and Diffusion. *Physiol Behav. 1(2)*, 171-174.

3. **Lomax, P.** (1966) The Distribution of Morphine Following Intracerebral Microinjection. *Specialia, 15,* 249-250.

4. **Myers, R.D.** (1974) *Experimental Methods for Stimulating the Brain with Chemicals. In Handbook of Drugs and Chemical Stimulation of the Brain.* NYC: Van Nostrand Reinhold Co.

5. **Bondareff, W., Routtenberg, A., Narotzky, R.** and **McLone, D.G.** (1970) Intrastriatal Spreading of Biogenic Amines. *Experimental Neurology, 28,* 213-229

6. **Rech, R.H.** and **Domino, E.F.** (1959) Observations on Injection of Drugs into the Brain Substance. *Arch. Int. Pharmacodyn., 3*-4,429-442.

7. **James, TA.** and **Starr, M.S.** (1978) Effects of the Rate and Volume of Injection of the Pharmacological Response Elicited by Intranigral Microapplication of Drugs in the Rat. *J Pharmac. Meth. 1,* 197-202.

8. **Faingold, C.L., Marcinczyk, M.J., Casebeer, D.J., Randall, M.E.,** *et al.* (1993) GABA in the Inferior Colliculus Plays a Critical Role in Control of Audiogenic *Seizures. Brain Res., 640*, 40-47.

9. **Browning, R., Maggio, R., Sahibzada, N.,** and **Gale, K.** (1993) Role of Brainstem Structures in Seizures Initiated from the Deep Prepryiform Cortex of Rats. *Epilepsia, 34(3)*, 393-407.

10. **Iadarola, M.J.,** and **Gale, K.** (1982) Substantia Ni-gra: Site of Anticonvulsant Activity Mediated by y Amino-butyric Acid. *Science, 218,* 1237-1240.

11. **Peterson, S.L.** (1994) Localization of an Anatomic Substrate for the Anticonvulsant Activity Induced by D-Cycloserine. *Epilepsia, 35(5),* 933-938.

12. **Peterson, S.L.** (1995) Infusion of NMDA Antagonists into the Nucleus Reticularis Pontis Orals Inhibits the Maximal Electroshock Seizure Response. *Brain Res., 702,* 101-109.

13. **Shuman, S.L., Capece, M.L., Baghdoyan, H.A.,** and **Lydic, R.** (1995) Pertussis Toxin-Sensitive G Proteins Mediate Carbachol-Induced REM Sleep and Respiratory Depression. *Am. J Physiol., 269*, R308-R317.