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Reliable and Repeatable Microinjections Via Stereotaxic Procedures

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Charles E, Olmstead is an Assistant Professor of Psychiatry and a member of the UCLA Mental Retardation Research Center Research Group at Lanterman State Hospital, Pomona, CA. The work described here is continuing in the Laboratory ofJaime R. Villablanca with J.P. McAllister, II, B.J. Sonnier, J. W. Burgess and F. Gomez.

PRECISE INJECTION via stereotaxic procedures is a prerequisite of current neuro anatomical techniques using axonally transported tracer substances such as tritiated amino acids of horseradish peroxidase.

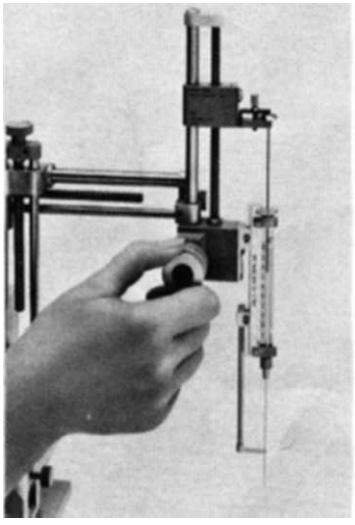
The procedures and apparatus described here evolved from our investigations of the neuroanatomical concomitants of recovery of function after large unilateral cortical ablations (2, 8, 9, 11, 12). Our basic procedure is to remove a single hemisphere, either in the adult cat or neonatal kitten. After a wait of some prescribed period, usually longer than 120 days, we then inject, either into the pericruciate cortex or deep cerebellar nuclei, tritiated amino acids for anterograde auto radiography. We are analysing interactive remodeling in those brain stem areas where cortical and cerebellar projections overlap.

When we first started our investigations we were somewhat hampered by the nature of our injection devices. Like others (1,3,4,7,10), our device consisted of a Hamilton or other available microliter syringe, mounted via a homemade clamp on a Kopf electrode carrier and was loosely coupled to a Kopf miniature microdrive. The device was, to say the least, clumsy to use and our injection volumes were quite variable. Once the stereotaxic measurements had been made for the deep cerebellar nuclei injections, or the mapping of the cortical injections sites was complete it was necessary to fill the syringe while it was mounted on the carrier. If one filled prior to these maneuvers there was the risk of evaporative loss from the tip. When angling into the deep cerebellar nuclei there was always the risk of drift on the needle. When injecting, since there was only a loose connection with the hydraulic drive it was necessary to read the amount injected from the syringe barrel. It was also necessary to use the Chaney adaptor to prevent bending of the syringe plunger. During the long injection sequences, often running several hours, there always seemed to be some drift of the plunger resulting in minute seepage at the end. If the procedure necessitates the reloading of the syringe, such as the case of doing multiple animals in a single day all of these factors combined to increase the variability in the size and location of injections.

We felt that the available syringes, Hamilton etc., were adequate for the job but modifications were needed. We then turned to the literature to see how others had solved the problem. Where precise methods were discussed they were highly individualized from laboratory to laboratory with very little consistency beyond the use of commercially available syringes adapted in various ways. This, of course, contributes a fair degree of variability between laboratories and their results The Kopf microinjector provides a means of standardizing the use of commercially available syringes for injecting submi-croliter amounts of tracer substances into the central nervous system.

The procedures we are now using describe some of the features of the Kopf Microinjector Model 5000.

The intent of the cortical injections was to fill the dorsal pericruciate areas 3a and 4 of Hassler and Muhs-Clement (6) and to study the projections to the various brainstem nuclei (5). Injections into the pericruciate cortex were aimed specifically at layer 5. To properly fill these areas 5-6 injections of 0.2 - 0.6 jul of tritiated leucine-proline (50 /xCi/^1) were spaced 1.5 and 1.0 mm anterior and posterior, respectively, to the cruciate sulcus. The cat receiving the cortical injections is placed in the Kopf stereotaxic frame using the standard solid ear bars and the 35 mm riser block. This results in a chin up 30° angle of the head and, when the skull and the floor of the frontal sinus are opened, results is a flat cruciate in a plane parallel to the stereotaxic frame. The needle already mounted on the microinjector is used to map out the dimensions of the injection sites and make surface measurements. This is a fairly good way to evaluate the amount of edema occurring during the procedure.



Following the mapping of the injection site, the syringe is removed from the holder and loaded with the leucine-proline cocktail from the BEEM capsule. Prior to the receipt of the Kopf micro-injector it was necessary to fill the syringe either before the measurements were made and take the risk of evaporative loss during the measurements or while mounted on the carrier and risk poor filling.

Cont. Page 2, Col. 2

A Letter from the President

IT IS MY PLEASURE to introduce the Carrier to those of you who are unfamiliar with the publication, and to reintroduce it to the avid readers, with the assurance that the publication will now be printed on a regular basis.

Response to the publication has proven most favorable and is evidenced by a plethora of letters from readers inquiring about Its absence.

The publication has served, and will continue to serve, as a vehicle for the dissemination of new techniques and older techniques which have not been explicated fully in other publications. The publication will also serve as a forum for exploration of ideas that are not readily available in the area of neuroscience.

I am pleased to announce the appointment of Michael M. Patterson, Ph.D, as Science Editor. Dr. Patterson is well-known for his continuing work in the areas of spinal cord functions, spinal conditioning and spinal fixation, as well as the neural substrates of conditioning in the intact animal.

Dr. Patterson is a Professor of Osteopathic Medicine and a Professor of Psychology for the College of Osteopathic Medicine and the Department of Psychology at Ohio University, in Athens, Ohio.

The following articles are a sampling of the many that have evolved from Dr. Patterson's research laboratory efforts in the past year:

Electrical Stimulation Research Techniques, edited by Michael M. Patterson, Ph.D. and Raymond P Kesner, Ph.D, published by Academic Press, 1981.

"Effects of Manipulating Stimulation, Intensity and Duration on Fixation of a Peripherally-Induced Spinal Reflex Alteration in Rats,"published by Physiology and Behavior, 1982.

"Associative Processes in Spinal Reflexes,' 'A Chapter in Conditioning: Representation of Involved Neural Functions, edited by Charles D. Woody, Ph.D, published by Plenum Press, 1982.

Dr. Patterson encourages other researchers and scientists to contact him, personally, regarding submission of published and unpublished techniques related to neuroscience. He can be contacted by telephoning: (614) 594-6401, or writing: Michael M. Patterson, Ph.D, College of Osteopathic Medicine, Ohio University, Athens, Ohio 45701.

We look forward to the continued success of the Carrier and invite our readers to continue their correspondence with us. Any new information or news pertinent to the field should be addressed to my attention, at: David Kopf Instruments, 7324 Elmo St., Tujunga, California 91042-0636. Please note our new Telex number: 215406 TBYT.

J. David Kopf

Microinjections - cont.

The syringe is then remounted and the injections are performed. In the cortex we routinely inject 0.05 μl increments at 5 minute intervals. We use a 10 minute equilibration to allow the brain to respond to the distortion caused by the insertion of the needle before the first increment is injected and wait 10 minutes after the last to allow the amino acids to diffuse away from the tip prior to moving on to the next injection site. All injections were made using a combination of reading the syringe barrel and the dial setting on the microinjector. Theoretically, with approximately 600 turns required for full piston travel increments as small as .002 μl could be injected from a Hamilton 7001 syringe. Figures 1 and 2 show a typical example of multiple injections into the pericruciate cortex with volumes ranging from 0.2 to 0.6 microliters. An important feature was that during these injections the 7001 syringe was refilled at least twice and returned to the precise position mapped earlier. We find that even the smallest injection volumes (0.2 μl) are reliably repeated

FIGURE 1

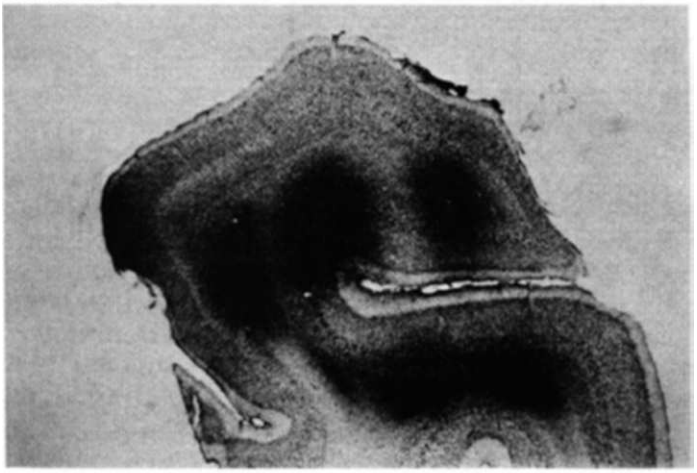


FIGURE 2

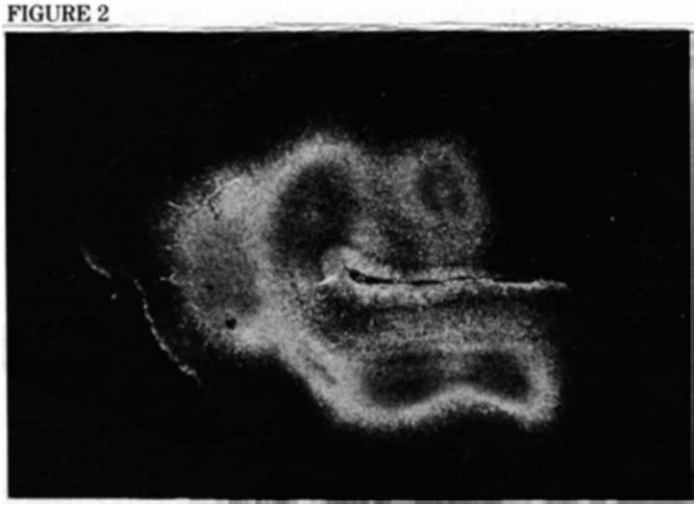


***FIGURE I & 2**, respectively. Bright and dark field photomicrographs of a series of pericruciate injections. The volumes of 50 µ Ci/nl leucine-proline varied (counter clockwise from, the top) from 0.2 pi to 0.6 pi (at 9 O'Clock) to 0.4 nl.*

Further, features are illustrated by the stereotaxic injections into the deep cerebellar nuclei which were aimed at either the anterior or posterior segments of the nucleus interpositus, the Medial or Lateral cerebellar nuclei. The needle was aimed at a 40° angle from the rear and, as mentioned above, it was desirable to have a rigid support for the needle to prevent drift. Likewise, it is necessary to have the plunger of the syringe rigidly fixed to prevent bending. Single injections of 0.2 /nl of 50:50 mixture of tritiated leucine-proline (SO^Ci/^ul) were injected in 0.05 (A increments at 3 minute intervals. 10 minute equilibration periods were interposed at the beginning and at the end, to permit diffusion of the final 0.05 fj,l and avoid drawing any of the tracer substance back up the needle track. The easy removability and accurate replacement without having to recalculate the stereotaxic settings considerably shortens the time necessary to do several animals on a given day. The capability to refill the syringe between animals reduces the risk of evaporative loss which could be disastrous with small injection volumes.

In summary, the Kopf Microinjector Model 5000 provides a straight forward means of accurate, repeatable delivery of minute amounts of tracer substances into the central nervous system. It permits one to standardize the use of commercially available syringes without extensive calibration. Although there are still some problems with relatively large size of the commercially available needles (4, 10), the system described here is excellent when the injection size is in the fractional microliter range.

ACKNOWLEDGEMENTS

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New Products

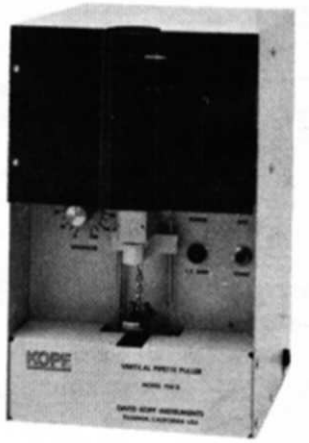
The recent introduction of the **Kopf Microinjector, Model 5000**, markedly improves the accuracy of localizing electrode placement within specific brain structures.

A common problem encountered by researchers using placement of syringe needles into the central nervous system of laboratory animals is the repositioning of the syringe. The quick-change syringe holders facilitate rapid refilling with accurate repositioning. Exact amounts of fluid can be injected repeatedly on the same site because of the adjustable foot support that stabilizes the needled tip.

The control knob allows precise dispensing of increments of one-thousandth of a micro liter of liquid. The Model 5000 is attachable to any Kopf manipulator. It is accompanied by a 5001 Syringe Holder designed for the 7000 series Hamilton Syringe.

A significant feature was recently added to our **Pipette Puller, Model 700D.** A cover over the front of the instrument prevents air currents from affecting the heater coil, resulting in better controlled pipettes.

The Model 700D was designed to pull a large variety of straight, concentric and repeatable micro -pipettes.



For Your Information

''The Rat Brain in Stereotaxic Coordinates'' by George Paxinos and Charles Watson, is the first stereotaxic atlas to detail all areas of the rat brain in all three cardinal planes. The book has been published by Academic Press. David Kopf Instruments has an updated list of all atlases published to date. Write us for a complimentary copy.

We recently updated our price list and order sheet and have developed a mini-catalog featuring those instruments ordered most often. Both are available upon request.

We are compiling a full-line catalog featuring indepth information about our equipment and accessories.

Several pieces of equipment have been updated to keep pace with technology. Please note the following: The Manual Micro-Drive, Model 607B, has been replaced by the Micro-Drive, Model 607C, which has a handwheel calibrated in microns.

The Mouse Adaptor, Model 922, has been changed to Model 921. The Rat Spinal, Model 981, has been changed to Model 980.

Electrode holders 1270 through 1279 are being replaced by new, sturdier electrode shafts and holders numbered 1770 through 1779.

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