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A Stereotaxic Modification for Retinal Neurophysiology

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The vertebrate retina is in effect an anatomically isolated compartment of the central nervous system which has been a popular subject of neuroanatomical study for over 100 years. [Some of the earlier work on this structure is summarized by Slonaker (1897).]The

reasons for this are many. Apart from the practical advantages of accessibility and the ease with which it can be dissected and handled during histological pro cedures, the analysis of the retina is both an important and tractable problem: important because the retina is the peripheral source of the sense of vision; tractable because it is a structure of only moderate synaptic complexity. The complete transformation of the activity in the photoreceptors into the neural signals which are transmitted to the brain is accomplished by only five distinct types of retinal neurons, enough to be challenging without being unmanageable.

Neurophysiological studies of mammalian retinal neurons began about 30 years ago (Kuffler, 1953). Much of this work has been done in isolated eye cup preparations, but a great deal of information, particularly about retinal ganglion cells, has been derived from recordings in the intact eye. In this note, I will describe a



Fig. 1 An overall view of the apparatus described in the text.

tway to combine two of the available accessories for the standard Kopf stereotaxic, thereby enabling it to be adapted for use in retinal physiology.

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A fundamental requirement for all visual physiology is an unobstructed visual field. This can be partially achieved using the standard optical investigation unit, in which the animal faces the rear of the instrument. However, in this configuration, the zero horizontal meridian is still somewhat obstructed. This can be remedied by using the Kopf optical investigation unit (875) in conjunction with the offset earbars and raised eyepieces (866), as shown in Fig. 1. The raised earbars place the zero horizontal meridian 35mm above the main support bars of the apparatus resulting in a visual field that is significantly less obstructed. The raised eyebars can be easily attached to the optical investigation assembly, and since they are also designed to raise the lower orbital margin by 35mm, the animal's head remains in stereotaxic orientation (even though proper stereotaxic orientation is not strictly required for retinal physiology, it is useful whenever it is necessary to place stimulating electrodes in central visual structures such as the optic tract, and visually important axes and planes of reference are commonly expressed in terms of standard Horsley-Clark planes (Bishop, Kozak and Vakkur, 1962)]. The mouth bar can also be inverted to ensure that the head is firmly held in the apparatus. It is convenient, when the animal faces the rear of the stereotaxic, to place the animal's body under the frame of the stereotaxic, with the body aligned at an angle of about 45 deg. to the sagittal plane of the skull. The height of the instrument can be easily adjusted to suit the size of the animal.

In addition to greatly expanding the amount of unobstructed visual field, this arrangement has the advantage of making the eye itself freely accessible by raising it well above the main parts of the apparatus. The supports for the optical investigation unit can now be used to mount any additional apparatus that is required to support and position a recording electrode within the eye itself. Such devices are typically modified versions of the one originally described by Kuffler (1953). The particular device shown in Fig. 1 was designed and built by Dr. Jonathan Stone and Mr. David Solomons of the University of New South Wales, in Sydney, Australia, and incorporates the accumulated experiences and ideas of dozens of investigators from all over the world. It consists essentially of a 17gauge stainless steel hypodermic needle which can be inserted into the vitreous chamber of the eye and through which a microelectrode can be introduced (Fig. 2). A small brass ball, approximately 4mm in diameter has been fitted on to the needle and soldered in place about 5mm from its bevelled end.





Editor's Column

It is a real pleasure to bring you this first issue as Science Editor of the Kopf Carrier. The Carrier has served for several years as a means of information exchange for techniques and methods not readily found elsewhere. The

decision by David Kopf to put the publication on a regular basis and to standardize its format is a_ confirmation of the excellence to which Kopf Instruments has been dedicated over the years. The fact that the publication has served for so long in its role is due to the continued efforts of Dan Nichols of Kopf Instruments. He and David Kopf are to be commended for starting this service and for deciding to continue providing it to the scientific community. Under the present plans, the Carrier will appear four times a year.

The purpose of the publication was expressed well by David Kopf in the last issue as "... a vehicle for the dissemination of new techniques and older techniques which have not been explicated fully in other publications ... (and) as a forum for exploration of ideas that are not readily available in the area of neuroscience." We plan to pursue this policy for the Carrier in the coming years. There will be two means for meeting this goal; the regular article in each issue, and through reader questions and comments.

The Editor invites and will continuously accept articles from readers for publication in the Carrier. Such articles need not be related to stereotaxic methods or equipment, but only to the broad range of neuroscience. The following guidelines should be considered in planning articles: 1. Write or call the Editor to discuss ideas for an article. 2r Articles should be ab~out 1800 words" with one figure (subtract 200 words for each additional figure). 3. Articles should detail techniques or methods not generally available in other sources. 4. The author or authors of all articles accepted by the Editor will receive a fee of \$200 (or comparable equipment credit with Kopf Instruments).

In addition to articles, the Editor will publish short (about 100 words) comments by readers on methods, previous articles or areas of general interest in the Editor's Column. Also, questions of general interest and answers solicited from readers or supplied by experts will be published in the Column. Readers are encouraged to send such comments or questions to the Editor at any time.

Correspondence with the Editor regarding articles, comments or questions should be sent to the following address or the Editor may be contacted by phone. Michael

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Fig. 2 Details of the microelectrode holder and supporting rod for the miniature hydraulic microdrive slave unit.



Fig. 3 Close-up of the ball and socket mount for the microelectrode holding tube.

The tube is mounted by clamping the ball in a socket located at one end of a brass tube (Fig. 3). The socket consists of a transverse hole near the end of the tube. The size of this opening is varied by means of a moveable rod within the brass tube. Movement of this rod, and the pressure exerted on the ball, is controlled via a bolt threaded into the other end of the brass tube (visible in Fig. 1). The ring suspended below the tube assembly is for conjunctival attachment which is accomplished by dissecting the conjunctiva from the eyelid, pulling it forward through the ring and sewing it in place. The entire assembly is mounted on a vertical rod such that it can be rotated in all three planes. The vertical rod itself is attached to the supports for the optical investigation unit by means of a bolt threaded into its lower end.

Mounted parallel to the microelectrode tube, and clamped firmly to it, is a long rod on which can be mounted the slave unit of a miniature hydraulic micro-drive (Fig. 2), allowing precise movements of the micro-electrode through the retinal tissue. Relatively precise lateral positioning of the steel tube and hence the micro-electrode can be easily achieved using a standard electrode carrier (Fig. 1). The normal stainless steel rod of the carrier is replaced by a brass rod which is attached to the retinal apparatus by means of a sliding universal joint which permits a free change of angle between the connecting brass rod and the rod supporting the microdrive slave unit. This arrangement allows the microelectrode to be directed anywhere within a large area of retina, and also provides additional mechanical support and stability for the microelectrode holder.

The apparatus described here easily provides enough stability for extracellular single unit recording, even where prolonged isolation of a particular cell is required. Healthy animals paralyzed with flaxedil and anesthetized with nitrous oxide (70% N20,30% O2) supplemented with intravenous nembutal (1 mg/kg/hr) will remain in good condition for at least 48 hours and often as long as 96 hours. The activity of single ganglion cells can be isolated and recorded for hours with either metal or glass capillary microelectrodes.

REFERENCES

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Correction

The price list dated December 1, 1982 described the 5003 as a "Syringe/Pipette Holder." Early literature stated "a 5003 holds a Model 701,10 microliter Hamilton Syringe." Both of these statements are incorrect. The 5003 is a dovetail clamp with holder to accept glass pipettes up to 2.3mm in diameter. The guide foot has a set screw to hold the pipette. The 5003 Pipette Holder may be attached to either the 5001 or the 5002 Syringe Holder.

The innerface between syringe and pipette must be user supplied.

Model 5000 Microinjection Unit is equipped with a 5001 Syringe Holder that will accommodate the 7000 Series Hamilton Syringes.

The 5002 is an accessory that will hold 5 microliter and 10 microliter, 700 Series Hamilton Syringes. Syringes should be ordered from a Hamilton dealer.

Design Change

Removable zero point, Model 950. The vertical distance of this zero point has been raised from 20mm to 30mm above ear bar zero (Fig. 4) and may now be used with larger animals.

Designed exclusively for the Model 900 Small Animal Stereotaxic. This zero point allows one to set electrodes while the animal is in the instrument.



Fig. 4

Special Rat Spinal

The standard 980 Rat Spinal Unit is pictured in Fig. 5.

This instrument consists of a 912 Base Plate, three 982 Adjustable Base Mounts with Post and Clamp, one pair 985 Hip Spikes and one 986 Vertebrae Clamp.

Several requests for a special combination Rat Stereotaxic/Spinal Unit have been answered by supplying a special 24 inch 912 Base Plate and mounting a 900 Small Animal Stereotaxic "U" Frame at one end of this plate with the above mentioned spinal parts mounted on the posterior end of the plate.

Also available are the following accessories for the Rat Spinal:

- 987 V-Notch Spikes, pair
- 988 Retractors, pair
- 923 Head Holder with Ear Bars
- 992-2 Calibrated A-P Bar and Brackets
- 992-3 Calibrated A-P Bar and Brackets



Announcing New 700D Accessories

#701 Patch Clamp Bracket

The patch clamp micro-electrode accessory attaches to the slide block assembly of the Vertical Pipette Puller. It has a vertically adjustable rod with swing away bracket to preset the first free fall stage. For repeatable patch clamp micro-electrodes. Takes all the guess work out. Comes complete with alien wrench. Easily attachable to all 700 Series Kopf Vertical Pipette Pullers.

710-UN New U-shaped nichrome heater element.

710-UPS Platinum indium heater elements will also be available shortly.

These heater elements will require the new #701 Patch Clamp Bracket.

For Your Information

In the last newsletter, we indicated a new catalog was being made. The following new catalog sheets are now available:

700D - Vertical Pipette Puller

900 - Small Animal Stereotaxic Instrument 5000 - Microinj ector

RFG-4 and RFG-4A - Lesion Generators

Catalog sheets on additional products will be available in August.

Quality Research Requires Quality Electrodes

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If you have a requirement that is not met by the electrodes shown in this catalog, we would welcome the opportunity to quote on your specific application.

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Address Correction Requested

For Your Information (continued)

A number of investigators use x-rays to verify electrode placement. Fig. 6 shows an excellent new product introduced by Polaroid, an instant 8 x 10 radiographic system. Benefits:

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Fast. Radiographs are processed and ready for analysis, in little over a minute.

High Quality. The images are produced with low radiation dosages and meet or exceed the resolution of conventional systems (see Fig. 7). It is compatible with all x-ray generators now in common use.

For more information write or call Polaroid Corporation, Medical Marketing, 575 Technology Square, Cambridge, Massachusetts 02139. In U.S., phone (800) 225-1618, Massachusetts (617) 547-5177 collect.



Fig. 6