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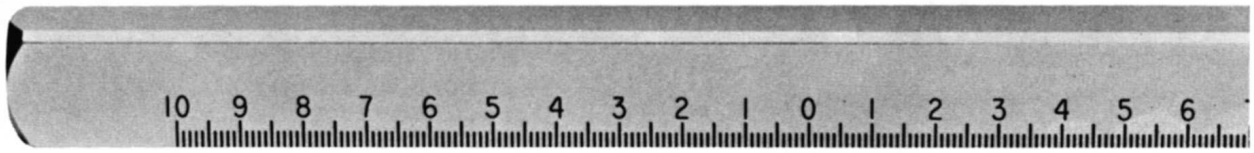

ELECTRODES FOR BRAIN SLICES

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As reviewed in numerous places (including the *CARRIER*, Oct. 1986), the brain slice is now established as a standard paradigm in neurobiology. This article and the one in the next *Carrier* will deal with only one aspect of brain slice neurobiology - the electrodes used for recording and stimulating. Whereas just about any recording and stimulating electrode can be used with brain slices, the unique aspects of the brain slice present the experimenter with recording and stimulating opportunities not present in intact systems. Thus this article will emphasize electrodes specialized for brain slices.

RECORDING ELECTRODES

The most common recording electrodes are the pulled glass microelectrode and the stainless-steel or tungsten metal electrodes. The former are generally pulled to have a tip diameter of 5-10 um for extracellular field potential recordings (a corresponding resistance of 2-7MOhm) or a tip diameter of 0.5 um with subsequent beveling for intracellular recordings (a bevelled resistance of 30-60MOhm in 2MK Acetate). Convenient stainless-steel electrodes are made from either insulated wire (50-75 um diameter) which is simply cut or from small (00 or 000 gauge) uncoated insect pins. The insect pin is already tapered to a fine point, thus eliminating the need to etch the metal to a point, as is the case with tungsten. Metal electrodes are sometimes preferred over glass when seeking extracellular unit recordings.

Glass micropipettes are generally preferred over metal microelectrodes for recording field potentials since they are smaller and appear to do less damage to the tissue, and, of course, are mandatory for intracellular recordings. However, the glass electrodes are difficult to see, leading some to fill the electrode with a dye such as Fast Green, which does help. Unfortunately some dyes can be toxic - in our hands tissue stability is compromised when using Fast Green, for example.

Most brain slices are nominally 400 um thick, thereby posing the problem of how deep to place the recording electrode. Potentials are not uniform across the thickness of the slice due to variations in tissue homogeneity, orientation of the section, trajectory of the afferents being stimulated and surface damage suffered by the tissue when cutting. Thus, each slice must be mapped or else a standard recording location (say, 100 um deep) should be adopted. The disadvantage of mapping each slice is in the attendant damage done by the mapping electrode.

Marking the recording site for histological verification can be accomplished with several kinds of recording electrodes. The most common are the Prussian Blue reaction to deposited metal from current passed through the stainless steel electrode (with possible damage to the tip in the process), the deposition of a dye from a pipette (either deposited by diffusion or actively with current or pressure), and HRP marking either using HRP in solution and ejecting it with current or pressure, or with crystallized HRP at the electrode tip.

Gad Vaknin in our laboratory has been experimenting with a carbon filled micropipette for use in brain slices. A glass capillary tube (not an electrode blank which is unsuitable due to the presence of the microfilament - we use a Drummand Microtrol tube) is pulled to a conventional profile in a standard Kopf puller. Carbon black is mixed

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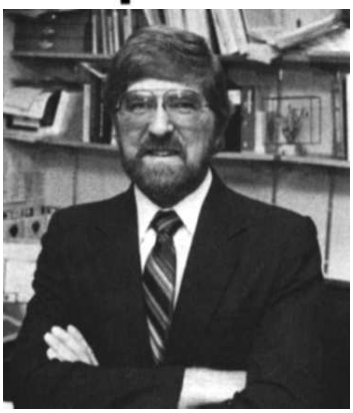

Editor's Column

It is a beautiful fall here in southeast Ohio. Fall means that we are back in the swing of the academic year and looking toward the travel and excitement of the next Neuroscience meeting, several other society gatherings and before we know it, to the upcoming holidays. This newsletter will get to most of you just before the Society for Neuroscience convention. There are many new things to look forward to this year at the meetings. Be sure to stop by the Kopf booth to see the instruments which will be on display and chat with the Kopf people. If you have an idea for an article for the Carrier stop by to chat with me about it. With a mailing of over 10,000, the Carrier is an excellent way to share your ideas with your colleagues.

John Moore has written to tell me of a very nice book just for those of you who are interested in rabbits. The book was uncovered by Marcy Ro-senfield, his technician, and is entitled The Rabbit: A Model for the Principles of Mammalian Physiology and Surgery, by Harold M. Kaplan and Edward H. Thomas (Academic, 1979). It looks like a good resource for us bunny runners. Thanks, John and Marcy, for the tip!

An important announcement appears on the bottom of the back page of this issue of the Carrier. This notice has to do with the return of instruments, especially of stereotaxic frames and accessories, and carriers to the factory for repair or adjustment. It may seem suprising, but often instruments are returned in very dirty condition. Therefore, it has become necessary to institute the policy announced on the back page, of requesting that returned instruments be clean when returned. This is necessary for the protection of the machinists who work with the instruments. It would be inordinatly expensive for the company to install sterilizing equipment. So, please cooperate with this policy. See you at the Neuroscience meeting.

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in mineral oil and injected into the pipette. A snugly fitting plunger connected to a solenoid is then used to "hammer" the carbon slurry into the tip of the electrode and to pack the carbon, eliminating most of the oil out the tip. Such an electrode makes a fine recording electrode, can be seen easily, can leave a carbon spot behind as a mark, makes a good monopolar stimulating micro-electrode, and, we believe, will perform adequately as an in vivo/in vitro electrochemistry voltam-metry microelectrode.

A limiting aspect of current neurophysiological recordings in vivo is in the relatively small number of sites from which recordings can be made. Such capabilities are important for the study of distributed networks and for specialized measures, such as the Current Source Density (CSD) analysis. The brain slice offers a unique preparation in which numerous parallel recordings can realistically be made at reasonable effort. The simplest multiple recording electrode array we refer to as a "RAKE." A RAKE is simply a linear array of recording electrodes, usually spaced from 100-500 um apart (Fig. 1). While RAKES can be made of any recording electrode material, the small interelectrode distances usually employed call for a novel solution. The simplest RAKE is made by placing (or winding) Teflon insulated 25-20 um stainless steel or Ptlr wire on a gluing form, such that adjacent wires touch (thus determining the electrode spacing). While useful, such electrodes damage the slice when used at the standard recording electrode depth (about 100 um). A smaller, less traumatic, RAKE electrode can be constructed from 7 um graphite fibers. While difficult to handle due to their tiny diameter, these fibers can be laid down in a parallel array and epox-ied (Learjets are also made of epoxied graphite fibers!). A ten electrode linear array of 7 um fibers spaced 100 um apart is only 1 mm long and can be manipulated in a manner similar to a conventional electrode. The graphite fibers are uninsulated, which is of little concern for most field potential and CSD recordings in brain slices, however the entire array could be dipped into an appropriate insulating liquid if needed. Since you cannot solder or crimp to graphite fibers (particularly 7 um ones!), electrical connections can be made using silver conductive paint (available from scanning electron microscopists).

All parallel recording electrode arrays require a like amount of amplifiers. A ten channel electrode requires ten identical amplifiers - a difficult proposition both in terms of matching the performance

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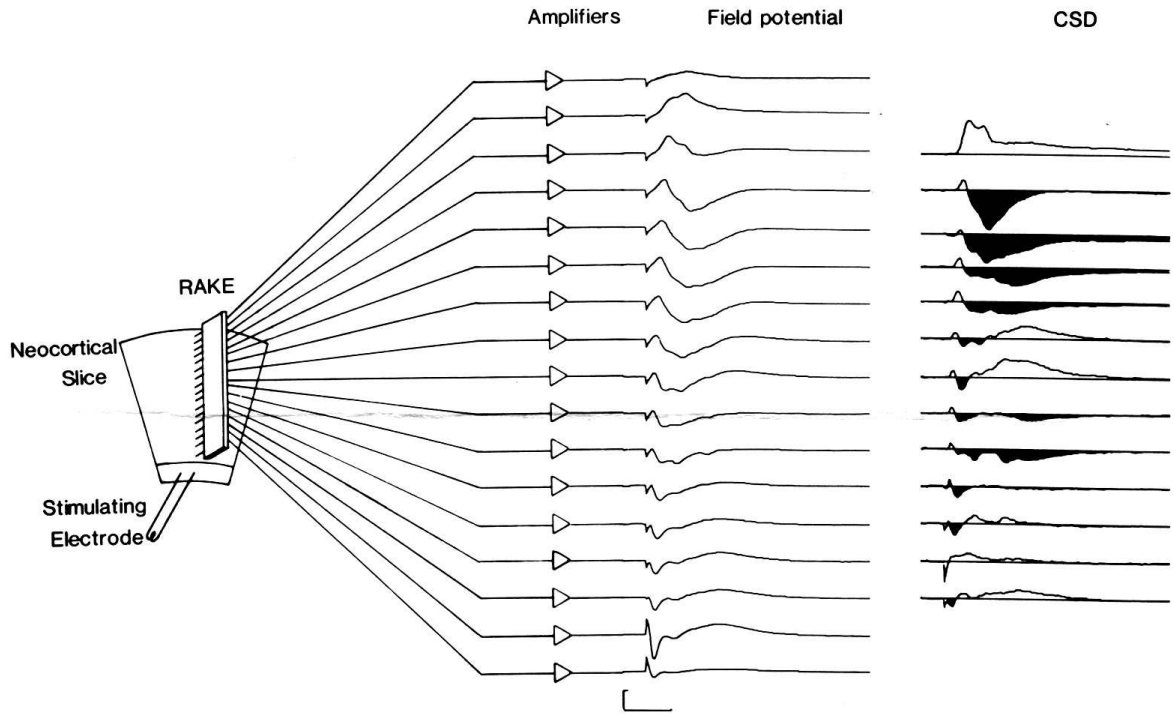

Figure 1. A RAKE electrode composed of individual recording electrodes (100mm spacing) capable of sampling activity from the pial surface to the white matter of an area OC1 neocortical slice from 15 day old rat. Each electrode supplies its associated amplifier with a field potential, from which a Current Source-Density (CSD) analysis can be made (2nd nearest neighbor solution). These data were not collected with a RAKE, but with a single electrode making sequential observations. A significant advantage of the RAKE electrode is that all the data shown here would be collected simultaneously, eliminating the time variable. (Data from T. Perkins, unpublished)

of the amplifiers and affording the cost of ten amplifiers. Larry Cauller in our laboratory has devised a high-quality multiple-channel amplifier ex-~ pressly for this application. The parallel output of the amplifiers must then be saved, preferably digitally, using a high-speed (5 KHz per channel), 12-16 bit multiplexed, Analog-to-Digital Converter (ADC).

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(Editor's note: The second part of this article will appear in the next *Carrier)*

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