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Brain Slice Preparation In Electrophysiology

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Brain slice preparations are becoming increasingly popular among neurobiologists for the study of the mammalian central nervous system (CNS) in general and synaptic phenomena in particular.

While the hippocampal slice is probably the single most used such preparation, slices of olfactory cortex, neocortex, hypothalamus, caudate nucleus, amygdala and other brain areas have also been studied in the past decade or so.

Brain slices are being used because they offer certain advantages over in vivo approaches to the study of the CNS. These are: 1) Rapid preparation, using relatively inexpensive animals (mouse, rat, guinea pig) where anesthetics are not necessary; 2) mechanical stability of the preparation, due to lack of heart beat and respiration pulsations, which permits intracellular recordings for long periods; 3) simple control over the preparation's condition, where pO^, pC02, pH and temperature can be maintained as desired; 4) direct visualization of the slice structure, allowing the accurate placement of both recording and stimulating electrodes in the desired sites; 5) slices have no blood brain barrier and thus their extracellular space is accessible to the perfusion medium and its content (ions, transmitters, drugs); 6) while simplified, the brain slice preparation maintains structural integrity, unlike cell cultures or tissue homogenates.

Some of the limitations of these preparations are: 1) lack of certain inputs and outputs normally existing in the intact brain; 2) certain portions of the sliced tissue, especially the top and bottom surfaces of the slice, are damaged by the slicing action itself; 3) the life span of a brain slice is limited and the tissue gets "older" at a much faster rate than the whole animal; 4) the effects of decapitation ischemia on the viability of the slice are not well understood; 5) Since blood-borne factors may be missing from the artificial bathing medium of the brain slice, they cannot benefit the preparation and thus the optimal composition of the bathing solution is not yet established.

PREPARATION OF SLICES

In general, rodents are the animals of choice for the preparation of brain slices. Of those, the rat and the guinea pig are the most used. After decapitation, the brain is removed rapidly from the skull and rinsed with cold artificial cerebro-spinal fluid (ACSF) (see Table 1) which has been equilibrated with 95% O2/5% CO-2 gas mixture. The brain area under study is then dissected out and sliced into slices of 100-500 um. Tissue choppers of different designs can be used for slicing; most of them utilize a razor blade for the actual cut. Slices are collected in a small beaker containing oxygenated ACSF and transferred with a pipette to the incubation/recording chamber. Although many brain slicers recommend keeping the time from decapitation until placement of slices in the chamber to a minimum (3-8 min.), results from our laboratory show that even when hippocampal slices were prepared 30 minutes after decapitation, CA1 excitatory synaptic function completely recovered.

INCUBATION/RECORDING CHAMBERS

Two types of chambers are used, each with its own advantages and limitations. Whether to use the static type, in which the bathing medium volume is large compared to the volume of the slice, or the continuous perf usion type, depends on the experiment one is

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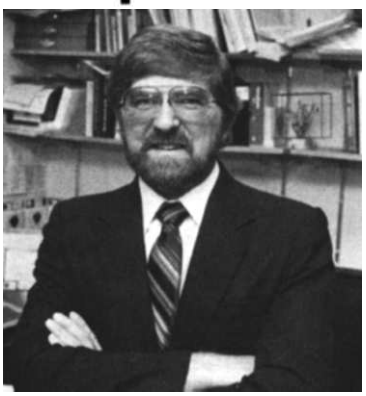

Editor's Column

The summer has by so rapidly and now we are looking forward to a new school year and the activities that time of year brings. One of these activities is the upcoming Society for Neuroscience meetings. It has been 15 years since I attended my first Society for Neuroscience meeting which was also held in Washington D.C. That fall of 1971, there were considerably fewer people attending the meeting than now attend. In fact, as I recall, there were about 1200 (750 members and 450 non-members) attending that first annual convention of the society. The attendance at the upcoming Washington meeting is expected to be about 9000. The society and the annual meetings have grown very rapidly in response to what has obviously been a great need among scientists in that area for a representative organization. This will truly be a very different meeting from that first one 16 years ago. However, despite the difference in size, there remains a feeling of excitement in the field of neuroscience that is felt at the meetings now as it was at the first one. The challenges of exploring the brain and its workings are now even more fascinating and exciting. We have more information now and some of the basic problems of information processing and brain mechanisms are beginning to look soluble. At the same time, there are more challenges opening up and more tools available to work with. Thus, the pace of the field is accelerating, while the puzzles we are dealing with are becoming even more fascinating. The technique described in this Carrier is one of the tools which is opening up more opportunities for defining brain function. The slice preparation is rapidly becoming a standard for investigating synaptic phenomena. It has given insights into long-term alterations which may have implications for learning and memory, and is being adapted for many different brain areas. Dr. Schurr's description of the preparation will provide the reader with a ready reference for the general uses and methods of the technique.

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The upcoming convention of the Society for Neuroscience will be another place where we will be able to see new instruments and techniques displayed and discussed. The Kopf Company, for instance, will have at its booth, several new electrode pullers which incorporate the new technologies developed for the 750 model. In addition, they will be displaying a new manual microdrive and other instruments. I will be at the convention and will be at the Kopf booth at times during the convention. I look forward to seeing you there. If you wish to contribute an article to the Carrier, I will be happy to talk to you about it there, or you can contact me at the address below for instructions and details.

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Brain Slice (continued)

planning. Oxygenation and heating could be problematic in the static chamber. Addition, and later washout, of drugs or ions is easy in the continuously perfused chamber but difficult in the static one. In both chamber types, slices can be either submerged below the bathing fluid surface or maintained at the fluid-gas interface. These two approaches each has its own advantages and limitations. The composition of the bathing solution of a fully submerged slice may be changed quickly, movement artifacts are minimized and dryness is avoided. However, field potentials are difficult to record because of current shunting by the ACSF, stimulus artifacts may be difficult to control and oxygenation can be inadequate. The partially submerged (interface) slice has a better oxygen supply and much less fluid shunting which gives rise to larger (1-20 mV) extracellular evoked field potentials. However, high humidity must be kept in the chamber atmosphere to prevent dryness and changing the composition of the ACSF for drug studies is much slower due to the low flow rate than with submerged slices. (For details of different chamber

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constructions the reader is referred to references 1-3.) Several designs are available commercially. Beside providing basic mechanical support and the necessary ingredients for the maintenance of viable tissue slices, chambers must be equipped with temperature control devices and provide clear and easy visualization of, and access to, the slices for accurate placement of recording and stimulating electrodes.

RECORDING AND STIMULATING ELECTRODES

Since, by its nature, the brain slice is a compromised system, any recording equipment must be of high quality. At the center of that equipment are the recording and stimulating electrodes. For extracellular recording of field potentials (population spike, field postsynaptic potential (EPSP), and pre-volley) borosilicate glass-micropipettes filled with either 4 M NaCl or ACSF, with an impedance of 1-5MC1 are most suitable. Impedance higher than 10 Mft makes background noise bothersome. For intracellular recordings, electrodes are pulled from fiber-filled borosilicate micropipettes filled with either 4 M potassium citrate, potassium acetate or potassium chloride with impedance of 70-150 MO. Lower impedance electrodes tend to seal poorly so recordings tend to be less stable while electrodes with impedances above 150 Mft are both noisy and prone to clog.

Several types of stimulating electrodes can be used. Bipolar electrodes made of insulated tungsten needles with tips of about 50 um and resistance of 0.5-2.0 MO are quite satisfying. Concentric bipolar stimulating electrodes are not recommended because their relatively large tip diameter damages the tissue. Other metals recommended for making of stimulating electrodes are silver and platinum. Stimulation can also be achieved with glass micro-pipettes filled with Wood's metal and plated at the tip with gold and platinum. In an interface chamber, healthy slices produce evoked responses (CA1 population spike in hippocampal slice) of 10 mV or more in amplitude with stimulation currents of 50-100 uAand a duration of 0.1 mS.

DIFFERENT ELECTROPHYSIOLOGICAL RESPONSES

Depending on the brain structure under study, placement of electrodes and the type of recording (extracellular or intracellular), different responses can be produced. Since the hippocampal slice preparation is the most popular of all slice preparations and all the different types of responses can be evoked in, and recorded from it, this section will deal with this preparation only (Fig. 1, A). The most common response is the population spike (Fig. 1, B). This response is recorded extracellularly and is obtained from the pyramidal cell layer by applying an orthodromic stimulation to afferent fibers in stratum radiatum or oriens. It is a summation of single action potentials of many neurons in the vicinity of the recording electrode. Population responses with shorter latency can be produced by stimulating pyramidal cell axons in the alveus. These are antidromic population spikes. Another extracellular response recorded frequently from the hippocampal slice is the field excitatory post synaptic potential (EPSP) (Fig. 1, C). This .negative potential is obtained by stimulating the same sites stimulated to induce an orthodromic population spike response. However, the recording electrode is placed at the site of the afferent input to the pyramidal cell dendrites. The EPSP response is believed to be the current flowing into the dendrites at, and near, the electrode site. The synchronous firing of the afferent fibers, appearing as a biphasic deflection preceding the EPSP, is known at the presynaptic volley or pre-volley (Fig. 1, C). Intracellular recordings of either spontaneously active or stimulated neurons are not different from those made in vivo, though pulsations due to heart beats and respiration are absent. Typical responses of a CA1 pyramidal cell recorded intracellularly are shown in Fig. 1, D.

THE USE OF BRAIN SLICES IN DIFFERENT STUDIES

Although the studies I will mention here all use electrophysiological techniques, it is obvious that the slice preparation can be used in numerous studies where electrophysiology is not employed. Here, however, I will briefly outline only some major research fronts in which the slice preparation has been used as the appropriate technique. Our own studies will be described in somewhat more detail.

The brain slice preparation is an excellent tool in the study of the fundamentals of neurophysiology at the cellular and simple circuit levels. While the neuronal membrane properties can be studied using intracellular recording, including the study of ion channels and putative neurotransmitters, the study of synaptic activity can be performed with extracellular recordings and specific stimuli. The nature of different synaptic connections has been studied by evoking excitatory

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and inhibitory postsynaptic potentials (EPSPs and IPSPs). For such studies, other electrophysiological techniques such as voltage clamp and iontophoresis can be very useful. Slice preparations, especially the hippocampal one, are being used extensively in plasticity studies exploring longterm potentiation (LTP), adaptation and kindling. Such studies can be performed either entirely with the slice preparation or by inducing these physiological changes in the intact animal and then studying the affected brain area as a slice. Endocrinological studies can profit by adopting the in vitro slice approach. The brain slice preparation is now used as a system for the study of brain metabolism, pharmacology of drugs in the CNS, and as a model for numerous pathological situations. In our laboratory, the rat hippocampal slice is being used for the study of cerebral hypoxia. Recognizing the advantages of the brain slice preparation as an in vitro system, we combine our electrophysiological measurements with morphological and biochemical correlates which are easy to include using slice tissue. To increase the accuracy of the system, we constructed a dual, linear-flow, interface chamber.(4) with two identical compartments. ACSF is supplied by a two-channel perstaltic pump while the gas atmosphere can be changed in each compartment independently. Slices from one hippocampus are placed in both compartments, one designated "control" and the other "experimental". Hypoxia is being produced in one compartment by changing the "normal" 95% O2/5% CO,; gas mixture to 95% N2/5% CO^. Continuous extracellular recordings of CA1 population spikes, evoked by stimulation of the Schaffer collaterals, in two slices, one in each compartment, are made automatically every minute, before, during and after hypoxia.

Our whole system is under computer control which is responsible not only for the smooth, automatic run of the experiment but also digitizes and stores the data and later analyzes it to produce parameters such as population spike amplitude and latency.(5) Each compartment of the dual chamber houses 6-10 slices, some of which can be used for morphological and biochemical studies. Using this set-up, we have standardized our hypoxic insult (10 min exposure to 95% N2/5% CO2) which allows us to evaluate the effect of certain pre-treatments of the slices on their resistance to hypoxia. We have demonstrated that

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TABLE 1

Composition of ACSF solutions used for maintaining electrical evoked responses in brain slices. Equilibration with 95% 0_2/5% CO_2 is needed to give a pH 7.2 to 7.4 at room temperature.

Compound	Solution 1 (mM)	Solution 2 (mM)	Solution 3 (mM)
NaCl	134.0	124.0	124.0
KCl	5.0	5.0	5.0
KH_2PO_4	1.25	1.25	—
NaH_2PO_4	—	—	3.0
$MgSO_4$	2.0	2.0	2.4
$CaCl_2$	1.0	2.0	2.5
$NaHCO_3$	16.0	26.0	23.0
Glucose	10.0	10.0	10.0

FIGURE 1

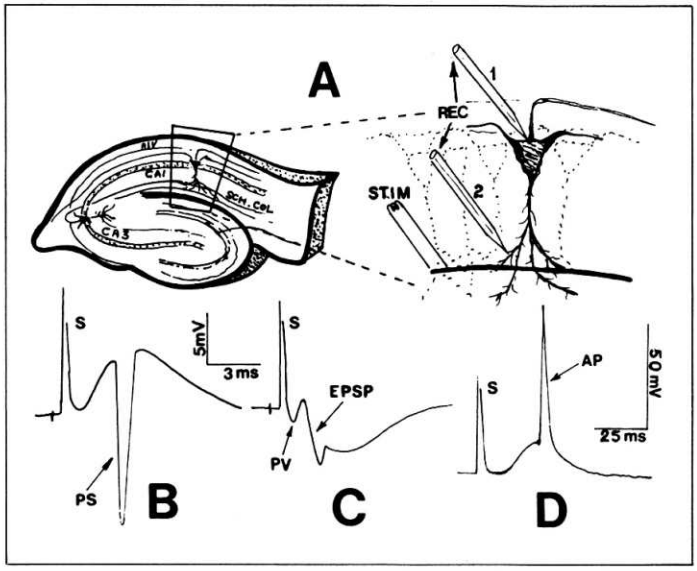

Figure 1. A diagram of the hippocampal slice preparation (A). The boxed-in area on the left shown enlarged on the right. Alv = alveus; CA1, CA3 = Cornu Ammonis, field 1 and field 3; SCH. Col = Schaffer collaterals of stratum radiatum; REC = recording electrodes 1 and 2; STIM = stimulating electrode. B shows a trace of population spike (PS) evoked by orthodromic stimulation of Schaffer collaterals and recorded by recording electrode 1. C is a trace of an excitatory postsynaptic potential (EPSP) and a prevolley (PV) evoked by orthodromic stimulation and recorded by recording electrode 2. D is a typical intracellular record from a CA1 pyramidal cell evoked by orthodromic stimulation and showing the rise of EPSP and a spike (action potential, AP). In all traces S = stimulus artefact.

hippocampal tissue has some capacity to adapt to hypoxia (5) and have shown that certain drugs can protect brain tissue against hypoxia. These are just a few of a long series of studies one can devise using the brain slice preparation.

The number of brain slicers has grown so rapidly in the past few years that several conferences have been held to share knowledge and ideas. On June 4-6, 1986, many "slicer" scientists will meet in Louisville, Kentucky to confer on "Brain Slices: Fundamentals, Applications and Implications". We slicers all believe that the brain slice preparation is an important tool for neuronal research. Several significant advancements have already been made and the possibilities for the future possibilities appear to be very promising.

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