THE *IN VITRO* HIPPOCAMPAL SLICE TECHNIQUE AS A TOOL FOR STUDYING THE CENTRAL NERVOUS SYSTEM

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ABSTRACT: The use of brain slice preparations has become increasingly popular among scientists of different disciplines in recent decades for the study of the mammalian central nervous system (CNS) in general and of synaptic phenomena in particular. The *in vitro* hippocampal slice may be the single most used preparation, among other slices of different parts of the brain areas. The use of brain slices in different experimental work offers certain advantages over the *in vivo* approaches to the study of the CNS; however, such preparations may have some limitations. This review describes the hippocampal slice technique, explores some of the different types of studies in which it was employed and points out the advantages and limitations of its use. *(JUMMEC 2000; 1: 17-23)*

KEYWORDS: Hippocampal slices, brain slices, technique, synaptic function, electrophysiology, *in vitro*.

Introduction

The brain slice preparation was first used for neurochemical studies by McIlwain *et al.* in 1951 (1). Such a preparation has developed into one of the most widely used *in vitro* techniques in neuroscience, especially after the discovery that about 0.4 mm thin slices of the hippocampal tissue that are cut in the appropriate plane maintained synaptic function (2,3). While the hippocampal slice is probably the earliest and single most used preparation, slices of olfactory cortex, neocortex, hypothalamus, caudate nucleus, amygdala and other brain areas have also been studied in the past two decades.

Slices are taken from various parts of the brain, maintained in a chamber in the presence of a bathing medium and used for many purposes (4). The available literature pertaining to this subject is very vast and diverse. The aim of this review is to briefly describe the technique, explores the many different types of studies in which it was employed and discusses its advantages and limitations. For further details about the different aspects of this technique and its uses are found in the references provided herein and in many other resources.

Preparation of slices

In general, rodents are the animals of choice for the preparation of hippocampal and other brain slices. 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 cerebrospinal fluid (ACSF) which has been equilibrated with 95% O_2/ 5% CO_2 gas mixture. The composition of ACSF may vary (5); however, it contains the following basic ingredients: NaCl, KCl, NaH_2PO_4, $CaCl_2$, $MgSO_4$, $NaHCO_3$ and glucose (6-8). In some studies, NaH_2PO_4 is substituted for KH_2PO_4 (9). The brain area under study is then dissected out and sliced into slices of 100-500 µm. Tissue choppers of different designs can be used for slicing; most of them utilize a razor blade for the actual cut. Slices are then collected in a small beaker containing oxygenated ACSF and transferred with a pipette to the incubating/ recording chamber. The detailed procedure for the preparation of the rat hippocampal slices has been described (4). A schematic representation of the rat hippocampal slice, with its CA1 and CA3 regions and connecting synapses identified, is shown in Figure 1.

Although some investigators recommend keeping the time from decapitation until placement of slices in the chamber (Figure 2) to a maximum of 8 min, it has been shown that even when hippocampal slices were prepared 30 min after decapitation, CA1 excitatory synaptic function completely recovered (10).

Incubating/recording chambers

Slices require an environment that enables the maintenance of their metabolic activity and electrophysiological function. The minimum

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requirements for this purpose are: a suitable ionic environment, provision of oxygen and glucose, an appropriate temperature and removal of metabolic wastes. This environment is normally provided by incubation chambers, many of which have been described (3,11–13).

Three types of chambers are used: the interface, the submerged and static - each with its own advantages and limitations (5). In the interface chamber (3,12), the slices are placed on a fine mesh through which ACSF is flowing (Figures 2 and 3) and a thin layer of a humidified 95% O_2/ 5% CO_2 gas mixture. Those who were not satisfied with the limitations of the interface chamber, for their experimental purposes, developed the submerged chamber (14–16). In such a submerged chamber, the slices are held between two mesh grids and are totally submerged in an oxygenated bathing medium. The static chamber was developed for the purpose of studying metabolic products of the slice (13). It can be regarded as a variant of the interface chamber in which the flowing medium is replaced by a static pool (5). The pool must be flushed at regular intervals to provide more glucose and remove waste products. Oxygenation is almost entirely via the upper surface. Whether to use the static type, in which the bathing medium volume is large compared to the volume of the slice, or the continuous perfusion type, depends upon the plan of the experiment. 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 these two basic chamber types, slices can be either submerged below the bathing fluid surface or maintained at the fluid-gas interface. These two approaches, each has it own advantages and limitations. The composition of the bathing medium (ACSF) 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. Several designs are available commercially; however, those who wish to construct such chambers can find instructions and guidance (17-19). Besides 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 positioning of the 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 post-synaptic potentials (EPSP) and pre-volley; borosilicone glass-micropipettes filled with either 4 M NaCl or ACSF (with impedance of 1-3 meghoms) are most suitable. Impedance higher than 10 meghoms makes background noise bothersome. For intracellular recordings, electrodes are pulled from fiber-filled borosilicate micropipettes filled with a solution of 4 M potassium citrate, potassium acetate or potassium chloride with impedance of 70-150 meghoms. Lower impedance electrodes tend to seal poorly so recordings tend to be less suitable while electrodes with impedance above 150 meghoms are both noisy and prone to clog.

Several types of stimulating electrodes can be used (9). Bipolar electrodes made of insulated tungsten needles with tips of about 50 μm and resistance of 0.5-2.0 meghoms are quite satisfying. Concentric bipolar stimulating electrodes are not recommended because their relatively large tip diameter damages the tissue. Other recommended metals for making of stimulating electrodes are silver and platinum. Stimulation can also be achieved with a glass micropipette 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 μA and a duration of 0.1 msec (Figures 4 and 5).

Data acquisition system

Responses to the electrical stimulation of slices are recorded, stored and retrieved for future analysis and plotting. The data acquisition system being commonly in use consists of a digital oscilloscope, which is connected to a personal computer (7,8). Positioning of both the stimulating and recording electrodes is done manually and with precision (Figure 3). However, recording and storing of responses can be done manually if many slices are checked or automatically when responses within a single slice are followed on a continuous basis for a long duration. Using appropriate software computer programs (20), stored responses can be analyzed to produce parameters such as population spike amplitude and latency and further characterize them (8,9).

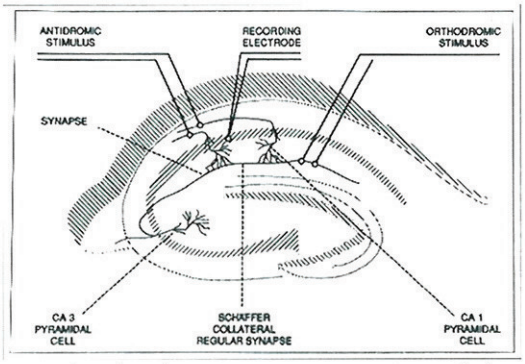

Figure 1. A schematic representation of the rat hippocampal slice, with its distinct CA1 and CA3 regions identified. Locations for placements of electrodes for orthodromic and antidromic stimulation and the site for the placement of the recording electrode are shown.

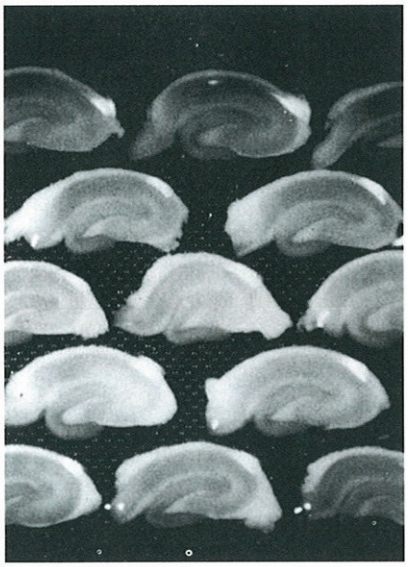

Figure 2. Arrangement of actual rat hippocampal slices in an interface incubating/recording chamber. Slices are placed on a fine mesh through which oxygenated ACSF flows gently.

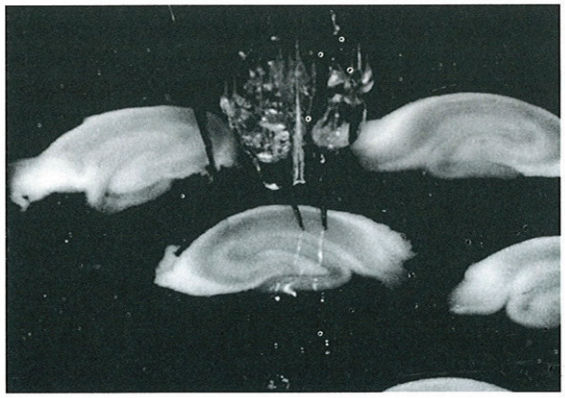

Figure 3. A photograph showing an arrangement of actual rat hippocampal slices on the mesh of the *in vitro* incubation chamber. Placement of the stimulating electrode (double metal bars) and the recording electrode (fine tipped micropipette) are also shown.

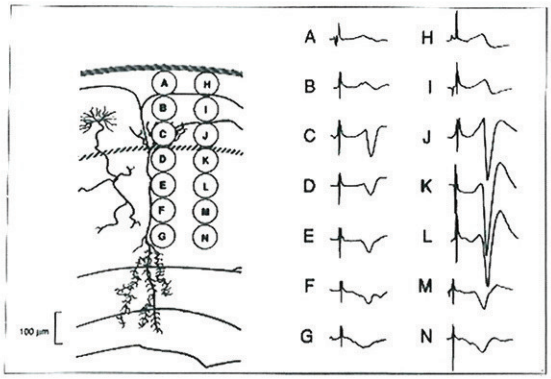

Figure 4. Variation of CA1 population spike potential (PSP). The stimulating electrode is placed at a point slightly above the measure scale (100 µm, in the lower left corner) and the recording electrode is positioned in CA1. There is a characteristic change in the shape of PSP related to the position of the recording site. For comparative responses to treatment(s), consistency in placing such a recording electrode is of significance - especially at the sites that produce the largest PSP's (positions J, K and L).

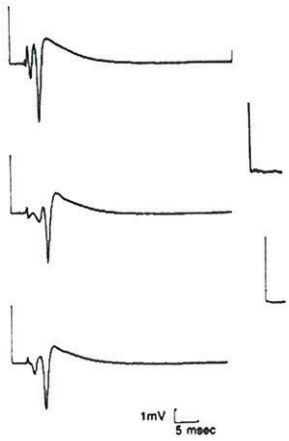

Figure 5. Three actual single recordings of PSP from the CA1 region of 3 different rat hippocampal slices. Placement of the recording electrode was around the J, K and L sites (shown in Figure 4).

Different electrophysiological recordings

Depending on the brain structure under study, placement of electrodes and the type of recording (extracellular or intracellular), different responses can be produced. Different types of responses can be evoked in, and recorded from the hippocampal slice preparation. The most common response is the population spike (Figure 5). This response is recorded extracellularly and is obtained from the pyrimidal cell layer by applying an orthodromic stimulation to afferent fibers in the stratum radiatum or oriens (Figures 1 and 3). 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 pyrimidal cell axons in the alveus. These are also population spikes that result from an antidromic stimulus (Figure 1). Another extracellular response recorded frequently from the hippocampal slice is the field excitatory post synaptic potential (EPSP). 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 as the pre-synaptic volley or pre-volley (9). Intracellular recordings of either spontaneously active or stimulated neurons are not different from those made *in vivo*, though pulsations due to heart beat and respiration are absent.

Use of slices in different studies

The use of the hippocampal slice technique in experimental work has been increasing rapidly over the last three decades. At present, the available literature pertaining to the use of this technique is very vast and diverse, thus attempting to cover all would be almost impossible. A brief mention of the major types of studies in which this *in vitro* method was employed would be appropriate for the purpose of this review. For further details, the reader should investigate the particular field or research problem of interest. Although most of the studies mentioned herein use electrophysiological techniques, as this preparation proved to be a tool in the study of the fundamentals of neurophysiology at the cellular and simple neuronal circuit level, others may not employ electrophysiology. More of the major research fronts in which the slice preparation has been used as the appropriate technique are still forthcoming, such as endocrinological and pharmacological studies as well as those which involve pathological situations of the CNS.

While the neuronal membrane properties can be studied using intracellular recordings, 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 and inhibitory post-synaptic potentials (EPSPs and IPSPs). For such studies, other electrophysiological techniques such as voltage clamping and ionophoresis can be very useful. Electrophysiological measurements can also be combined with morphological and biochemical correlates (9).

The hippocampal slice preparation has been widely used in studying the physiology and pharmacology of many aspects about neurons. Such studies were performed either entirely with the slice preparation or by inducing changes or treatments in the intact animal and then studying the affected brain area as a slice. Many studies endeavored to understand the cellular mechanisms underlying modulation of electrophysiological activity in neurons (21–26), to study the effects of temperature on excitatory transmission (27,28), to understand cerebral energy metabolism (29–31) and study the physiological and molecular mechanisms of age-related memory loss (32). The hippocampal slice preparation was also used extensively in plasticity (the strengthening or weakening of synaptic connections due to internal and/or external stimuli) and in neurotoxicity studies (33-35). Pharmacological studies involving neurotransmission (36-40), investigating the effects of various classes of antidepressant drugs on the electrically evoked release of neurotransmitters (41-45) and the effects of certain drugs on neuronal activity (46,47) utilized this slice preparation.

The utilization of the slice technique in understanding the mechanisms involved in certain pathological situations and in searching for means to alleviate or prevent them has been extensive. The slice preparation provided a model for studying epileptiform activity (4,6,48,49). This proved valuable in understanding the mechanisms and effects of anticonvulsant drugs, those which increase inhibitory synaptic transmission in the CNS and may be used in the treatment of seizures. Theoretically, substances that block the uptake of inhibitory transmitters such as gamma-aminobutyric acid (GABA) into intracellular compartments should increase inhibition and thus have potential value as antiepileptic drugs. The hippocampal slice preparation has been employed in studies geared to understand the different cellular mechanisms involved in anticonvulsant effects of these drugs (50-53). The hippocampal slice has also been extensively used as a model for investigating the effects of hypoxia, the physiopathology of cerebral ischemia and for finding possible protective remedies for such (8, 54-71).

Advantages and limitations of the slice technique

Brain slices are used because they offer certain advantages over the *in vivo* approaches to the study of the CNS. Such advantages include:

1. Rapid preparation, using rather relatively inexpensive animals (mouse, rat, guinea pigs...etc.) where anesthetics are not necessary.
2. Mechanical stability of the preparation, due to lack of heart beat and respiratory pulsation. Such conditions permit intracellular recordings for long periods.

3. Simple control over the condition of the preparation, where PO_2, PCO_2, pH and temperature can be controlled and maintained as desired.

4. Direct visualization of the slice structure, thus allowing the accurate placement of both recording and stimulating electrodes in desired sites.

5. Slices have no blood-brain barrier, thus their extracellular space is accessible to the perfusion medium and its content (ions, transmitters, drugs...etc.).

6. While simplified, the brain slice preparation maintains structural integrity, unlike cell cultures or tissue homogenates.

On the other hand, there are some limitations for these preparations, which include:

1. Lack of certain inputs and outputs which normally exist 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 brain slice deteriorates with the passage of time and the tissue gets "older" at a much faster rate than that of the brain in the intact animal.

4. The effects of decapitation ischemia on the viability of the slice are not yet well understood.

5. Since blood-borne factors may be missing from the artificial bathing medium of the brain slice, they can not benefit the preparation and thus the optimal composition of the bathing solution has not been fully assessed.

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