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Journal of Neuroscience Methods 80 (1998) 13–17

**JOURNAL OF
NEUROSCIENCE
METHODS**

A new slicing method for the lower vertebrate brain: Brain mould and adjustable stage

Jun Xiao, Shu-Rong Wang *

Laboratory for Visual Information Processing, Institute of Biophysics, Academia Sinica, 15 Datun Road, Beijing 100101, PR China

Received 4 March 1997; received in revised form 17 October 1997; accepted 19 October 1997

Abstract

This paper describes a new method for slicing the lower vertebrate brain which is too small and soft to be sliced using conventional methods. The brain is sliced in a pre-prepared agar mould glued to a special stage placed in a conventional vibratome. The mould is constructed from a plaster model prepared by embedding a paraformaldehyde fixed brain in a paraffin and vaseline mixture. The brain to be sliced is placed within two prepared agar half cylinders which are in turn placed in a pre-prepared larger agar collar glued to a special stage. The study describes in detail the preparation of the interlocking collar and inner cylinders of agar. The plexiglass stage allows the agar block containing the brain, to be rotated and inclined to improve the angle of cut. By using this method, small and soft brain even with thin walls and large ventricles could be sliced coronally, sagittally, horizontally or obliquely. Brain slices obtained by this method have good viability showing spontaneous and evoked activity. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Brain slice; Brain mould; In vitro; Nucleus isthmi; Decussatio veli

1. Introduction

Brain slices maintained physiologically in vitro have several advantages for neurobiological experimentation, including visual and physical access for stimulating and recording electrodes, bath application of neuroactive substances and freedom from anaesthetic. They are therefore widely used for investigating electrophysiological properties of neurons (Hardy et al., 1987; Bradley et al., 1996; Zaykin and Nistri, 1996a,b), synaptic transmission and plasticity (Bradley et al., 1991; Malinow, 1991; Callaway and Katz, 1993; Katz and Dalva, 1994; Zhang and Trussell, 1994), neuropharmacology (Horunou et al., 1995; Kita et al., 1995; Wickens et al., 1996) and neuropathology (Raley-Susman and Lipton, 1990; Richerson and Messer, 1995). These slice preparations are easiest to use in mammalian (rats, guinea-

pigs) and avian (pigeons, chicks) brains which are larger and more rigid than amphibian brains.

In lower vertebrates, en bloc brain preparations with peripheral sensory nerves or organs attached have been used for the in vitro studies of the central nervous system (CNS). Electrophysiological responses in the CNS neurons have been recorded during stimulation of the peripheral sensory nerves or organs to study visual circuits (Kriegstein and Connors, 1986; King and Schmidt, 1993; Atzori and Nistri, 1994; Fan et al., 1995), auditory tonotopy (Hailey et al., 1991) and activity evoked by odour (Delaney and Hall, 1996). However, little information has been obtained using brain slices of lower vertebrates. This may be partly due to the difficulty of preparing brain slices because of the small size and/or softness of the brain. To prepare spinal cord slices, some authors placed a piece of the cord in a shallow groove formed in an agar block (Shimizu et al., 1995) or embed it in fresh, cool agar (Takahashi, 1990). In our hands, these slicing methods

* Corresponding author. Tel.: +86 10 62022010; e-mail: wangsr@mimi.cnc.ac.cn

did not work when preparing midbrain slices of amphibians. Therefore, we developed a new method using a brain mould and an adjustable stage. Slices prepared with this method have been used successfully in investigating the effect of electrical stimulation of the DV on isthmic neurons in the toad midbrain.

2. Method

2.1. Brain mould

The whole brain was removed from the Chinese toads (*Bufo bufo gargarizans*) with a body length of 7.0–10 cm and fixed in 4% paraformaldehyde for 1–4 h. The fixed brain was oriented vertically, obliquely or horizontally and pinned with a tungsten electrode in a paper box (1.5 × 1.0 × 1.0 cm) (Fig. 1A). A mixture of paraffin and vaseline (1:0.6) was melted and then poured into the box around the brain to be embedded. The brain-containing block was cut in half (Fig. 1B) and the two pieces of brain tissue were removed from the paraffin moulds, which were then filled with gypsum plaster (calcium sulfate) (Fig. 1C). The two half-models were immersed in acetone-celluloid solution (1 g celluloid in 15 ml acetone) and then air-dried.

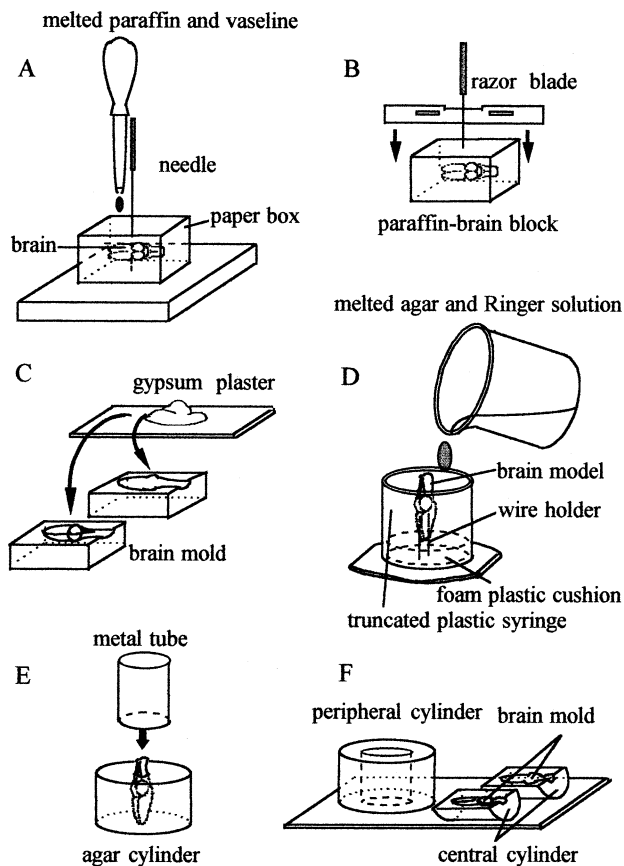


Fig. 1. Schematic drawings to describe the procedures for making an agar mould of the amphibian brain. For details see text.

A cylinder was cut from a disposable 60 ml syringe and placed on a foam plastic cushion. The brain model was positioned with a clip made of fine metal wires (0.4–0.5 mm thick) at a desirable angle in the plastic cylinder. A quantity of 6 g of agar was melted in 200 ml Ringer solution (pH 7.3), which was made by dissolving 6.5 g NaCl, 0.14 g KCl, 0.12 g CaCl₂, 0.20 g NaHCO₃, 0.01 g NaH₂PO₄ and 2 g glucose in 1000 ml of distilled water. The melted agar solution was gently poured into the cylinder (Fig. 1D). After it solidified, the agar block containing the brain model was taken out of the cylinder. This agar cylinder was trimmed using a thin metal tube (diameter was greater than that of the oriented brain model) to a small central cylinder containing the brain model. The peripheral cylinder was set on a slide for future use (Fig. 1E). The central cylinder was cut and the two halves of the brain model were removed from their moulds (Fig. 1F). Several brain moulds were made prior to each experimentation and kept in a water-vapor saturated box to prevent shrinkage. By positioning the brain appropriately at step Fig. 1D various formats of the brain moulds can be made for coronal, sagittal or horizontal slicing.

2.2. Adjustable stage

Most commercial microtome stages cannot be adjusted in the *X–Y* plane and are inconvenient for slicing at a variety of tilted angles. An adjustable stage with two degrees of freedom was made of plexiglass (Fig. 2A). Knob (7) rotates the *X–Y* plane of the stage around *X* axis (12) and knob (8) controls incline of the plane around *Y* axis (13). Thus, the stage can be adjusted by about $\pm 20^\circ$. The adjustable stage is screwed tightly onto a H-shaped metal holder (16), which can be removed by securing knobs (17) from the vibroslicer bath (Campden Instruments 752M) (Fig. 2B).

2.3. Brain slicing

The Chinese toad (*Bufo bufo gargarizans*) was anesthetized with MS222 and then decapitated. Its brain was removed from the skull and placed in cold (4°C) Ringer solution oxygenated with 95% O₂ and 5% CO₂. The brain was fitted in a brain mould, where a few drops of Ringer solution were added. This central cylinder containing an *in vitro* brain preparation was gently inserted into the peripheral cylinder, which had been glued with superglue (cyanoacrylate) on the adjustable stage. Then the stage and H-shaped metal holder were fixed to the vibroslicer and the agar cylinder containing the brain was immersed in cold (4°C) Ringer solution bubbled with the gas mixture. The slicing angle was adjusted so that slices could contain, for example, both the nucleus isthmi (NI) and the

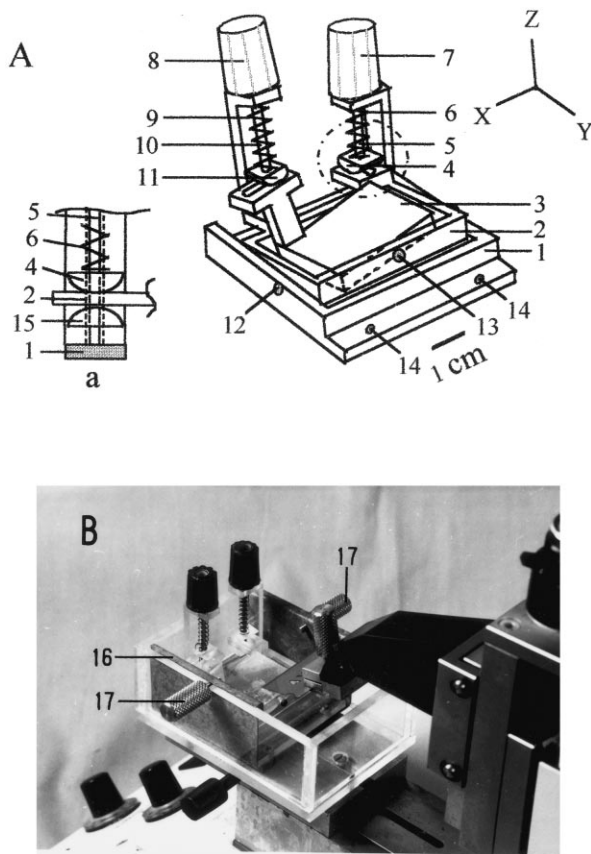


Fig. 2. Design of an adjustable stage (A) fixed to a vibroslicer (B). A: Adjustable stage. 1, Stage base; 2, frame that can be tilted around the X-axis 12; 3, platform that can be tilted around the Y-axis 13; 4 and 11, upper blocks that are depressed by springs 6 and 9, respectively; 5 and 10, lead screws that drive the lower blocks when the knobs are rotated; frame 2 and platform 3 can be tilted by rotating knobs 7 and 8, respectively; 14, screws fixed to vibroslicer. A broken line circle shows details in inset a, numerals correspond to those in A. 15, lower block that is driven by lead screw 5. B: The adjustable stage is fixed to a vibroslicer. 16, H-shaped metal holder; 17, securing knobs.

decussatio veli (DV). The slice thickness was 250–400 μm . These slices were immediately transferred with a large-diameter pipette into a storage container filled with Ringer solution bubbled with 95% O_2 and 5% CO_2 at room-temperature and incubated for about 1 h.

As shown in Fig. 3, both NI and DV are clearly visible on the midbrain slices. By using different brain moulds and adjusting slicing angle, we obtained coronal, sagittal or horizontal slices from the toad brain. All these slices showed clear appearance of NI and DV. It is our belief that this method can provide slices for electrophysiological and neuroanatomical studies of neuronal structures located in coronal, sagittal, horizontal or any oblique planes of the brain.

2.4. Chamber recording

One slice was transferred from the storage container into an interface-type chamber (BSC-MT, Medical Sys-

tem) perfused with Ringer solution bubbled with 95% O_2 and 5% CO_2 , and overflowed (0.5 ml/min). The slice was held by a nylon mesh and pieces of cotton fiber. A micropipette (2–3 μm tip diameter) filled with 2 M NaCl solution was advanced into NI under visual control with a hydraulic microdrive for extracellular recordings. A metal bipolar electrode with poles 300–400 μm apart was positioned at DV for delivering electrical stimulation comprised of rectangular pulses of 0.4–0.5 mA intensity, 0.6–1.0 ms duration and 0.1–0.05 Hz in frequency (WPI Pulsemaster A360).

Spontaneous and evoked activities can be recorded extracellularly for about 10 h from a number of isthmus neurons in slices maintained under these conditions (Fig. 4). One finding was that the DV mainly exerts an inhibitory influence on isthmus neurons. This gives strong support to the previous suggestion that the optic tectum inhibits the contralateral NI through the DV (Wu and Wang, 1995).

3. Discussion

Many in vitro slice studies have been developed for cellular and synaptic study in mammals (Malinow, 1991; Callaway and Katz, 1993; Kita et al., 1995;

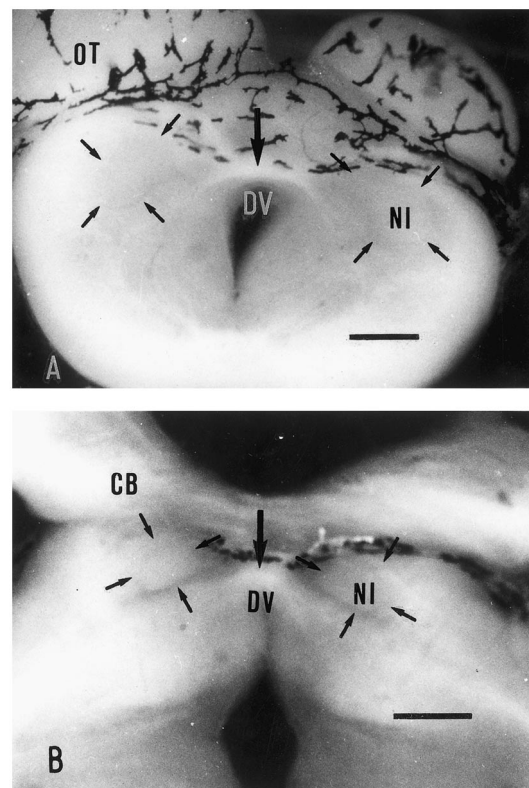


Fig. 3. Photographs of slices showing location of the decussatio veli (DV) and the nucleus isthmi (NI) in a coronal (A) and horizontal (B) sections of the toad midbrain. Thin arrows circumscribe NI, thick point to DV. CB, Cerebellum; OT, Optic tectum. Scale bars: 500 μm .

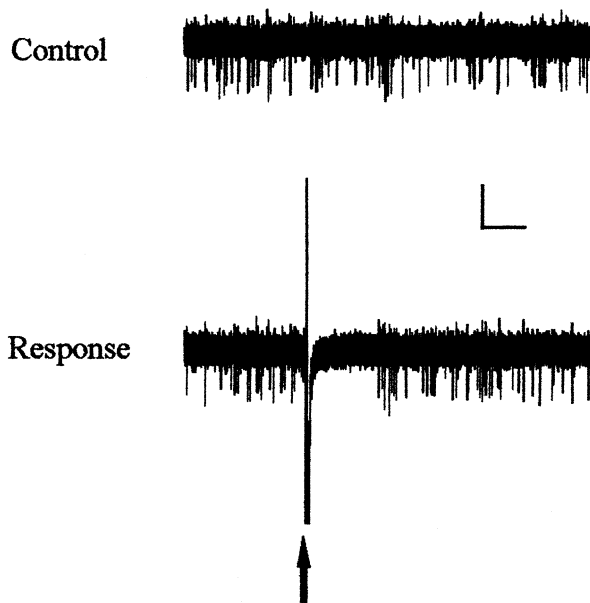


Fig. 4. Extracellular responses of isthmic neurons to electrical stimulation of the decussation, recorded 5 h after slicing. Note that the cells were inhibited by electrical stimulation of the DV. Superimposed sweeps (30). Arrow point to the stimulus artifact (fast sweeps showed no brief excitation preceding inhibition). Scale bars: 50 mv, 50 ms.

Wickens et al., 1996) and in birds (Hardy et al. 1987; Bradley et al., 1991; Zhang and Trussell, 1994; Bradley et al., 1996), whereas in the lower vertebrates including fish, amphibians and reptiles, en bloc brain preparations maintained in vitro are usually used. Sometimes, these preparations are attached with peripheral sensory nerves or organs and they are considered to be good models for studying central information processing (Kriegstein and Connors, 1986; Hailey et al., 1991; King and Schmidt, 1993; Atzori and Nistri, 1994; Fan et al., 1995; Delaney and Hall, 1996). Until recently, only a few studies have used slice preparations to investigate the electrophysiological properties of brain neurons in the lower vertebrates (Zaykin and Nistri, 1996a,b) and the lower vertebrate brain is too small and/or soft to be sliced in a conventional way. For example, at the very beginning of the experiments in which the effect of electrical stimulation of the DV was studied on neurons of the NI of toads, we tried conventional slices of the amphibian brain and failed completely. Attempts to slice the brain circumscribed by agar, similar to the spinal cord placed in a shallow groove formed in an agar block (Shimizu et al., 1995), were also unsuccessful. Our new method has three advantages: First, it guarantees viability of slices, indicated by readily finding cells and electrical activity. Second, the slicing angle can be adjusted by orienting the brain mould and/or adjusting the stage, so that coronal, sagittal, horizontal, oblique slices, or even symmetrical slices of both hemispheres, could be obtained. Third, the brain block, even the forebrain with

thin walls and large ventricles, is not deformed by the vibrating blade and the slices are homogeneous in thickness.

The present study describes for electrophysiologists and neuroanatomists a new method for brain slice studies using brain moulds and an adjustable stage. This improvement in brain slicing technology may promote more detailed in vitro studies of cellular and synaptic mechanisms of the brain in the lower vertebrates.

Acknowledgements

This work was supported by both the National Natural Science Foundation of China and the Chinese Academy of Sciences.

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