Video Article

Horizontal Slice Preparation of the Retina

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Abstract

Traditionally the vertical slice and the whole-mount preparation of the retina have been used to study the function of retinal circuits. However, many of retinal neurons, such as amacrine cells, expand their dendrites horizontally, so that the morphology of the cells is supposed to be severely damaged in the vertical slices. In the whole-mount preparation, especially for patch-clamp recordings, retinal neurons in the middle layer are not easily accessible due to the extensive coverage of glial cell (Mueller cell) s endfeets. Here, we describe the novel slicing method to preserve the dendritic morphology of retinal neurons intact. The slice was made horizontally at the inner layer of the retina using a vibratome slicer after the retina was embedded in the low-temperature melting agarose gel. In this horizontal slice preparation of the retina, we studied the function of retinal neurons compared with their morphology, by using patch-clamp recording, calcium imaging technique, immunocytochemistry, and single-cell RT-PCR.

Video Link

The video component of this article can be found at https://www.jove.com/video/108/

Protocol

1. Prepare an agar block (30 mg/ml, ie 3%, in distilled water). Dissolve and microwave it first and pour it into a glass dish. Keep the dish in a refrigerator at 4 ^{o}C.
   Tip: The agar block should be prepared in advance.
2. Prepare low-temperature-melting agarose gel (25 mg/ml, ie 2.5%, in medium). Dissolve and microwave it. Keep it in the hot water bath at 35 ^{o}C.
   Tip: Keep the agarose gel NOT too hot. If it is too hot, it takes a long time to be solidified, and the gel may kill the retina
   .
3. Euthanize the animal, enucleate an eyeball and open it as an eyecup. To liquefy vitreous humor, the opened eyecup is soaked for 10 min in hyaluronidase in the medium (0.07 mg/ml), if necessarily.
4. Cut the agar into a block (eg., 1 cm x 1.5 cm for mouse retina) and firmly attached on a vibratome stage by instant glue. Keep the agar block wet.
5. Place the blade at 5 degree at the slicer. Gently cut the top surface of the agar block at the speed of approx. 50 µm /sec, freq. 50 Hz.
   Tip: Ensure that the top surface of the agar block is totally smooth and flat. Several cuts (at least 3 times) may be needed to get the smooth and flat surface of the agar block.
6. Rinse the eyecup with the medium without hyaluronidase. Isolate the retina from a pigment epithelium and place it photoreceptor-side down on a piece of filter paper. To firmly attach the retina to the filter paper, vacuum the retina on the filter paper several times.
   Tip: Ensure that the retina is attached to the filter paper completely flat. After you put a retina on the filter paper, cut the excess filter paper surrounding the retina (Fig.2A). The excess filter paper often prevents a blade from going into the appropriate layer of the retina.
7. Gently wipe out the excess medium on the agar block and carefully place the retina on the filter paper on the agar block. To firmly place the filter paper on the agar block, attach instant glue on the edge of the filter paper.
   Tip: To avoid unexpectable crack, place the retina as front as possible on the top surface of the agar block.
8. Gently pour the low-temperature-melting agarose gel over the retina. Wait for 1 min and gently pour the medium on it to cool it down. The agarose gel becomes solidified quickly.
   Tip: Too hot agarose gel kills the retina.
9. Lift the blade position to several hundreds µm above the top surface of the agar block and gently cut the top of the solidified agarose gel.
10. Lower down the blade position. Cut the retina at the level of the proximal one-third of the inner nuclear layer (approx. in between 200 and 250 µm above the top surface of the agar block).
    Tip: Too much speed of the blade will damage the retina severely. Approx. 50 µm/sec (50 Hz) is appropriate.
11. Notice that the amacrine cells are now face-down. Carefully pick up the slice of the retina together with the solidified agarose gel and place it face-up in a chamber or a dish.
    Tip: Because the mouse retina is tiny and wavy, it is difficult to obtain a perfect horizontal slice at an appropriate layer. An "oblique" slice may be an alternative method to get the relative flat slice of the retina. To get the oblique slice, put another filter paper in between the retina on the

filter paper and the agar block as shown in Fig.2B. Although you can't expect a perfect horizontal slice, there are always some areas where amacrine cell's soma is on the surface of the slice.

Discussion

There are several important advantages of the horizontal slice preparation of the retina.

First, the morphology of the cells that expand dendrites horizontally, such as amacrine cells and horizontal cells, is preserved. It has been shown that there are over 20 types of amacrine cells in the retina (MacNeil and Masland, 1998), so that it is quite important to compare the morphology of the cells with the physiological property.

Second, in the whole-mount retina, the extensive coverage of glial cell s endfeets prevents the access of patch pipettes to amacrine cells. In the horizontal slices, the soma of amacrine cells is exposed to the surface of the slices.

Third, chemical reagents can easily reach to targeted cells and their dendrites, because these are located on the surface of the slices. Wash-in and wash-out of chemicals are fast and easy.

Fourth, fluorescent ion imaging such as conventional calcium imaging is performable. In the traditional vertical slices or the whole-mount retina, excitation light for imaging itself activates photoreceptors. In the horizontal slice preparation, photoreceptors and photopigments are totally removed so that there is no contamination of excitation light-evoked artifacts. In addition, the signal-to-noise ratio of imaging is much higher because the background activity of photoreceptors is eliminated. The biggest disadvantage of the horizontal slice preparation is that vertical connection between photoreceptors and ganglion cells is cut so that there is no visual information processing within the slice.

This technique is applicable to the retina of any standard animals such as goldfish, mouse, and rabbit (Fig.1B). The method of the horizontal slice may offer an alternative way in investigating the function of neurons and neural circuits in the retina.

Disclosures

All experimental protocols were conducted according to the National Institutes of Health guidelines for animal use.

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References

1. Azuma T, Enoki R, Iwamuro K, Kaneko A, Koizumi A. Multiple spatiotemporal patterns of dendritic Ca2+ signals in goldfish retinal amacrine cells. Brain Res. 2004, 1023(1):64-73.

2. Koizumi A, Jakobs TC, Masland RH. Inward rectifying currents stabilize the membrane potential in dendrites of mouse amacrine cells: patch-clamp recordings and single-cell RT-PCR. Mol Vis. 2004, 10:328-40.

3. MacNeil MA, Masland RH. Extreme diversity among amacrine cells: implications for function. Neuron. 1998, 20(5):971-82.

