Video Article

Brain Banking: Making the Most of your Research Specimens

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URL: http://www.jove.com/details.php?id=1260

DOI: 10.3791/1260

Citation: Burke M.W., Zangenehpour S., Ptito M. (2009). Brain Banking: Making the Most of your Research Specimens. JoVE. 29. http://www.jove.com/details.php?id=1260, doi: 10.3791/1260

Abstract

Unbiased stereology is a method for accurately and efficiently estimating the total neuron number (or other cell type) in a given area of interest[1]. To achieve this goal 6-10 systematic sections should be probed covering the entire structure. Typically this involves processing 1/5 sections which leaves a significant amount of material unprocessed. In order to maximize the material, we propose an inexpensive method for preserving fixed tissue as part of a long-term storage research plan. As tissue is sliced and processed for the desired stain or antibody, alternate sections should be systematically placed in antigen preserve at -20°C for future processing. Using 24-well plates, sections can be placed in order for future retrieval. Using this method, tissue can be stored and processed for immunohistochemistry over the course of years.

Protocol

Part 1: Pre-processing of tissue

1. Tissue should be well perfused with paraformaldehyde, glutaraldehyde, or formalin. This can be achieved through standard transcardial perfusion typically used to harvest other organs. In the present study the subject was deeply sedated with ketamine hydrochloride (10 mg/kg, i.m.), euthanized with an overdose of sodium pentobarbital (25 mg/kg, i.v.) and perfused transcardially with 0.1 M PBS until completely exsanguinated. This is followed by a 4% paraformaldehyde solution in PBS for 5 min (~1 liter).
2. The brain should be stereotaxically blocked, removed from the skull, weighed, and volume determined[2]. The tissue should then be cryoprotected in graded sucrose solutions with a final concentration of 30% sucrose in phosphate buffer. Blocks of tissue should then be frozen in isopentane at -65°C and stored at -80°C until you are ready to slice.

Part 2: Systematic Sampling

1. Systematic sampling relies on a proper plan prior to sectioning. The entire region of interest in both the rostral-caudal and dorsal-ventral extents should be well defined. A stereotaxic atlas of the species in use is helpful to define the region of interest and to determine the length of the region of interest. Once the length of the region of interest is determined it is necessary to establish the sampling interval. For example, from the stereotaxic atlas you determine that the entire rostro-caudal extent of the region of interest is approximately 3mm in length when sectioning in the coronal plane. You then decide to set the cryostat to section at 50µm, at this rate you will have 60 sections covering the rostral-caudal extent of the region of interest. For a typical unbiased stereological study 6-10 systematically sampled sections are required to obtain reliable results. In this example 10 sections equally spaced throughout the rostro-caudal extent result in a sampling interval of 1/6 sections.

Part 3: Creation of the brain bank

1. Once the section sampling frequency has been determined, the next step is to determine which stains will be performed immediately. For example, if you are staining with cresyl violet you will capture 1/6 sections on a slide. This will be series 1. If you know there maybe several potential antibodies that you would like run, but either want to see the data that your primary stain provides or simply do not have the time to perform the immunohistochemistry, place the remaining 5 series of sections in the order that they were sliced into wells containing **antigen preserve** (1% polyvinyl pyrrolidone, 50% ethylene glycol in o.1M PBS, Ph 7.4). The table below provides a sampling scheme consisting of 1 slide and 1 plate (standard 48-well plate) of sections as part of a brain bank (Table 1).

Placement of sections 1-60 to obtain a section sampling interval of 1/6						
	Slide 1	1	7	13	19	25
	Series 1	31	37	43	49	55

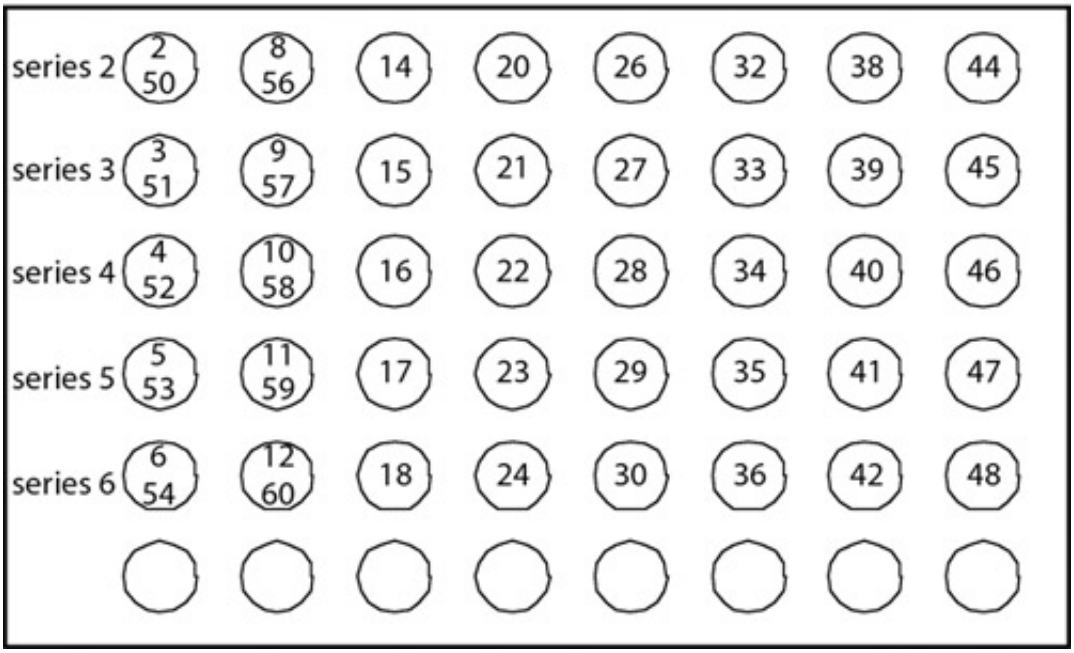

Table 1. The section sampling frequency here is 1/6. The first section is placed on a slide for cresyl violet staining and the next 5 sections are placed into antigen preserve. Section number 7 is then captured on the slide and sections 9-12 are placed in antigen preserve. This cycle is repeated until the region of interest or block of tissue is exhaustively sectioned.

2. The next step is to exhaustively section the block of tissue. Place a small amount of embedding medium on the chuck and let it freeze. Place the chuck into the microtome head and shave off enough of the frozen medium to have a flat surface. Remove the chuck from the microtome head and place flat within the cryostat. Pour embedding medium on the chuck and firmly place the brain block with the stereotaxically cut side positioned flatly on the chuck. Slowly and completely embed the brain in mounting medium. Place the chuck with the brain into the microtome head and section using the pre-determined parameters for the section sampling frequency.
3. Once the block of tissue has been exhaustively sectioned and sections are placed into wells, cover the plate with the lid, wrap the lid with parafilm, and place in a -20°C freezer. Log the total number of sections taken for each series, the section-sampling interval, and the number of series for each animal. This log will be vital to keep track of subsequent removal of sections from the brain bank for future immunohistochemistry.

Part 4: Representative Results:

Systematical sampling in this manner has been a standard practice in our laboratory for the past 3 years. We have had a great deal of success performing immunohistochemistry on material that has been stored in antigen preserve three years after it was sliced without deterioration of the signal (Figure 1). Furthermore, as part of our brain bank we have logged close to 20,000 systematic sections of the non-human primate brain (Figure 2) as part of our long-term research plans.

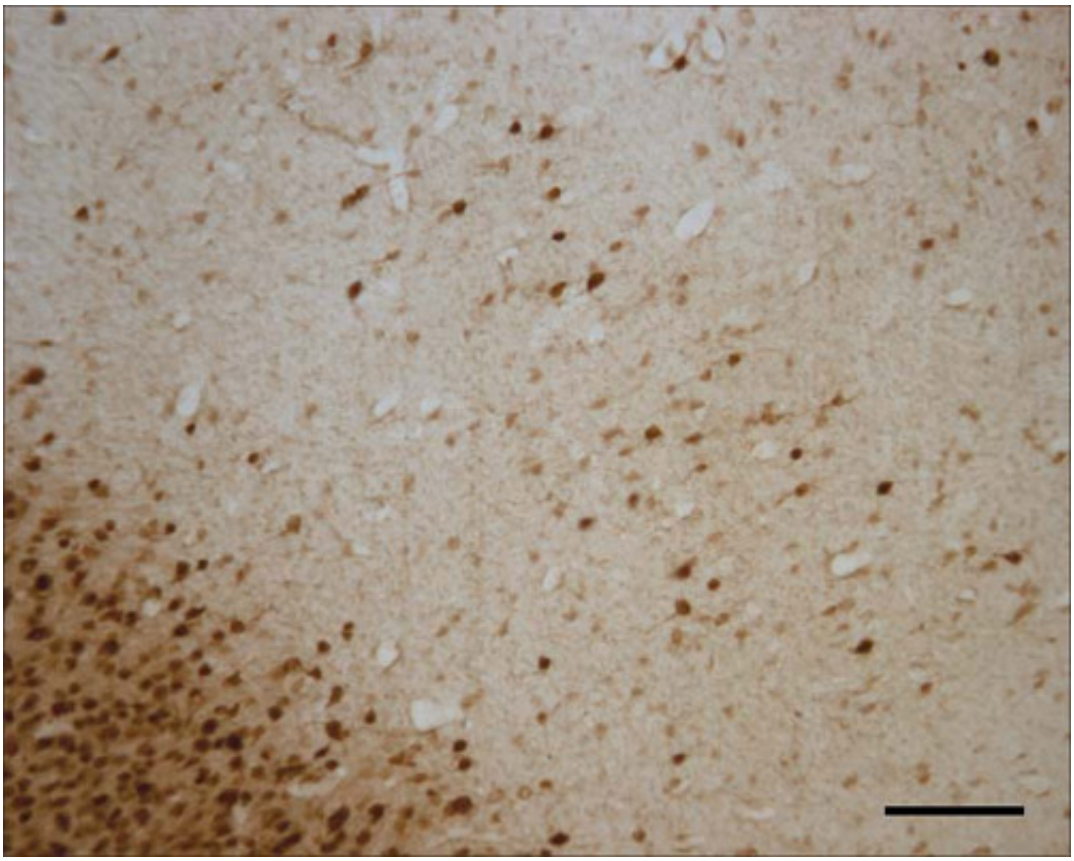

Figure 1. This is a section from the occipital pole of the non-human primate immunostained for NeuN showing both layer VI and interstitial white matter neurons. This particular section was stored in antigen preserve at -20°C for a period of 2 years. Scale bar = 100µm.

Figure 2. Our vervet brain bank now consists of close to 20,000 systematic sections from over 30 monkeys.

Discussion

Systematic sampling is an inexpensive method intended to maximize research material. This sampling strategy is designed to comply with the rules of unbiased stereology that requires systematic sampling throughout the region. It is critical that the order of sections for each series is maintained. In our laboratory we have successfully used this method for banking both hamster and non-human primate brain sections. So far, we have collected close to 20,000 vervet monkey (*Chlorocebus aethiops sabeus*) brain sections and routinely perform immunohistochemisty on sections that have been stored for over a year. The benefits of a well-characterized brain bank include the possibility to collect data between funding decisions, ability for new students to rapidly collect data, and minimize the use and treatment of new animals thereby maximizing research funds.

Acknowledgements

The authors would like to thank Ikiel Ptito for his continued technical support.

References

1. West, M., Slomianka, L., & Gundersen, H. Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. Aat Rec. 231, 482-497, (1991).
2. Burke MW, Zangenehpour S, Boire D, Ptito M (2009). Dissecting the Non-human Primate Brain in Stereotaxic Space. JoVE. 29. http://www.jove.com/index/details.stp?id=1259, doi: 10.3791/1259

