Letter to the Editor

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Is frozen sections an advantage for unbiased stereological methods?

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The optical fractionator is one of the most used unbiased stereological methods. It involves counting of neurons with optical dissectors in a uniform systematic sample that constitutes a known fraction of the volume of the region being analyzed. In order to practice optical dissector for counting cell/neuron, both embedding (including, methacrylate resin, paraffin, paraplast and etc.) and unembedding (vibratome, frozen and etc.) methods can be utilized with thick sections after fixation and staining [1]. However, possible problems have to be considered for both tissue preparation methods. These common problems may include neuronal distribution differences between the core and margin, z-axis distortion and problems at providing thick sections. Here, I want to debate some issues regarding these problems. According to the optical dissector counting rules, it should be let guard zone at the top and bottom surface of the sections. It has been shown in a previous study that the number of the counting neurons at core is 25% lower than accurate neuron numbers at paraffin and plastic sections [2]. This may be due to the fact that neurons are accumulated at the margin in paraffin and plastic embedded sections, while this is not true in frozen section, because there is no significant neuron number difference between the core and margins of section [2, 3]. Furthermore, z-axis distortion is an important problem in optical dissector application. Vibratome sections showed a pronounced z-axis distortion, while cryostat sections were minimally distorted [4]. In another study, it has been found that cryostat sections of snap-frozen nervous tissue are reliably and successfully used for counting neuron numbers using optical dissector [5]. Tissue shrinkage problem has been overcome in frozen section since it is possible to get thick sections in spite of tissue shrinkage. As a result, I emphasize that frozen sectioning is more advantageous than paraffin, vibratome and methacrylate sectioning for counting of neurons using an unbiased stereological method/optical dissector. The disadvantageous of this method is that researcher needs an expensive apparatus for an application of stereological counting method. So, if someone plan to constructs a new laboratory, this point should be taken into account. In conclusion, during the planning of a neurostereological study in nervous system, investigators have to consider the proper method, i.e., embedding or unembedding methods, and their advantageous or disadvantageous.

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