

## High Pressure Freezing of Brain Tissue Slices

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High pressure freezing when leading to vitrification has the potential to preserve cellular and tissue structure at the atomic level. However in the case of native tissues (not pretreated with cryoprotectants) vitrification is proved only for cartilage and leaves [1,2]. Partially also liver could be vitrified [3]. Brain tissue samples have to be incubated with a minimal concentration of 5% sucrose (cryoprotectant) to be vitreous after high pressure freezing [4]. The goal of our efforts is to characterise brain ultrastructural details as close as possible to the native state and we hope that synapse plasticity is much better revealed than with classical methods.

We use slice cultures of hippocampus. The specialized mossy fiber synapses herein are nicely preserved and display all structural characteristics of the particular synaptic connection [5]. Slice cultures of hippocampus are prepared as described previously in detail [5]. Following a few days of incubation the structural components of the tissue have developed in an organotypic manner. Cells and fibers damaged by the transverse dissection of the hippocampal formation were removed. Using conventional electron microscopy of recorded and biocytin-filled cells, an excellent fine structure was observed in these slice cultures [6]. Even structural abnormalities of the hippocampus in mice mutants are preserved in great detail [7].

In our study we compare culture slices chemically fixed, high pressure frozen with and without the addition of a cryoprotectant. We so far optimized the handling of the culture slices to minimize the influence of preparation prior to fixation. The slices about 200 micrometer in thickness are punched (diameter 1mm) and transferred with the help of a very fine hairbrush into commercial platelets of the EMPACT high pressure freezer (Leica-Microsystems, Vienna). Punching and transfer are performed with the help of a stereomicroscope. Platelets are then introduced to the high pressure freezing machine using a pod and transfer tool. Once the sample is punched it is high pressure frozen in less than one minute. The samples pretreated with 10% sucrose as a cryoprotectant were incubated for 10 minutes prior to preparation. Frozen samples were processed as described in [1].

Preliminary results will be presented. We have some indications that the three different preparation protocols lead to different ultrastructural features in the hippocampus slices. The synapses in unpretreated high pressure frozen samples for example are, as shown in the figure, not as clear as in chemically fixed ones. The different structural aspects will be discussed.

### References

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The figure shows a typical detail of an unpretreated high pressure frozen and freeze-substituted hippocampus slice. Boutons (B) in close apposition with dendrites (D) do not show the typical synapse (S) features for example postsynaptic densities are not revealed.

