# Preparation of high quality rodent hippocampal brain slices for experimental studies of synaptic plasticity and epileptiform activity

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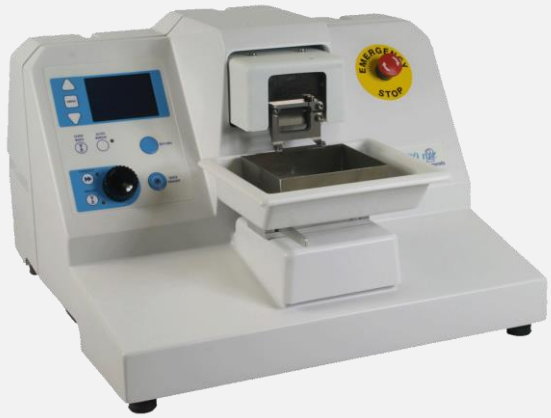
## Introduction

The hippocampus occupies an important role in the mammalian brain in terms of its involvement in learning and memory, as a site of epileptic activity, and its propensity to damage following conditions such as stroke and Alzheimer's disease. Accordingly, there is much interest in understanding hippocampal physiology and pathology, and the hippocampal slice preparation provides a very convenient and amenable *in vitro* preparation for such investigations. Hippocampal slices readily lend themselves to study a wide range of neuronal activity ranging from the basic properties of synaptic transmission, to activity-dependent synaptic plasticity, to the cellular events underlying pathological activity such as epileptic seizures, stroke and neurodegenerative conditions. These studies are greatly facilitated by the laminar appearance of the hippocampal slice such that individual neuronal populations and afferent fibre pathways can be identified and selectively stimulated and recorded, and which persist even after long-term culturing of slices. This lamination also facilitates immunohistochemical or immunofluorescence studies to be performed, as well as other biochemical or molecular biological techniques such as western blotting or PCR in defined subfields. Moreover, such slices can be prepared after *in vivo* treatments, such as stress or enrichment protocols, and the ensuing behavioural adaptations can be observed in cellular correlates in the *ex vivo* slice.

## Procedures

Typically, rodents (mice or rats), either wild type or harbouring various genetic mutations or inclusions, are used in the preparation of hippocampal slices. Animals are humanely killed using an approved method and the brain is quickly removed and placed into cold, slushy, artificial cerebral-spinal fluid (aCSF). Following trimming of the hemispheres into two blocks containing the hippocampus, the trimmed hemispheres are super-glued to a chilled (2 – 3°C) metal chuck and placed into the metal specimen tray of the Vibratome. Slushy aCSF is added to the specimen tray to cover the hemispheres, and, in order to maintain the cold temperature throughout the cutting process, we place a pre-chilled (-20°C) U-shaped custom-made brass block into the tray around the chuck. Slices are cut at slow speed (0.08 mm/s) and at 200-300 µm in thickness for patch clamping or immunofluorescence, or 400 µm for extracellular recordings (**7000smz-2** vibrotome, Campden Instruments). Once cut, slices can be further trimmed using the brass block as a

## Product Focus: 7000smz-2 Vibrotome




Our top of the range high precision, vibrating microtome (vibrotome for short), this is the finest tissue slicer in the world for preparations for visual patch clamping or high-resolution imaging.

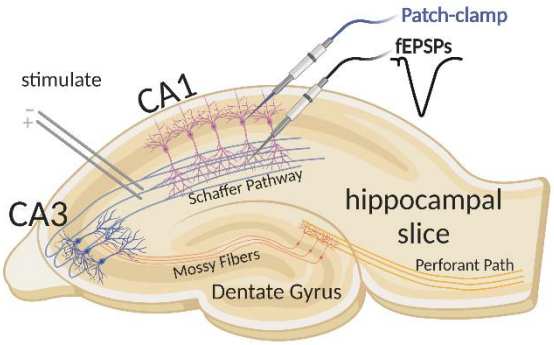
Using our microtomes, research detailing sectioning for visual patching of neurological tissue, heart, lung, and tissue scaffolds have all been published. The **7000smz-2** microtome represents significant advances with higher precision at a lower cost.

| Application Parameters | |
|---|---|
| **Amplitude** | 1mm |
| **Frequency** | 70Hz |
| **Advance Speed** | 0.08mm/s |

For more information click visit https://campdeninstruments.com/products/7000smz-2-vibrotome

cutting surface. Thereafter, slices are moved to an incubation chamber that contains circulating oxygenated regular aCSF (1 mM $MgSO_4$) at 32°C. Experiments are performed using this aCSF at a similar temperature (32 – 33°C).

In our lab the slice preparation aCSF contains additional $Mg^{2+}$ (10 mM $MgCl_2$), but the exact composition of the cutting aCSF various across labs, and may contain lowered $Ca^{2+}$ or $Na^+$ and added sucrose, or even a cocktail of antioxidants in order to preserve cellular viability. Prior intra-cardiac perfusion of the brain with cold aCSF is also in use in some labs. In our experience, good dissection technique, rapid removal of the brain, cold aCSF and high $MgCl_2$ are sufficient to produce high quality slices from a wide range of ages of rodents, including




**Schematic of the hippocampal slice preparation showing key neuronal populations and pathways. Created with BioRender.com**

in aged mice (12 – 16 months). The quality of slices can be gauged by visual inspection and intrinsic properties of hippocampal CA1 neurones in patch-clamp studies, and in the size and appearance in extracellular recordings of glutamatergic field excitatory postsynaptic potentials (fEPSPs). While the size of fEPSPs depends on the recording chamber (~ 1-1.5 mV for submerged slices; ~ 5 mV for interface slices), a good index of the quality of fEPSPs regardless of the chamber is the ratio of the presynaptic fibre volley (FV) to the fEPSP; the FV should be barely discernible, or very small in relation to the fEPSP. If the FV is a good proportion of the fEPSP (upwards of ~20 % of fEPSP amplitude), this suggests that either the slices have been prepared badly, or that the slice is hypoxic. The latter can be tested using an adenosine $A_1$ receptor ($A_1R$) antagonist, which will antagonise the hypoxia-induced adenosine release and $A_1R$-mediated suppression of the fEPSP. We would expect no more than a 10-15% increase in fEPSP amplitude in adequately perfused slices. Perfusion and oxygenation can be improved by: thinner slices, resting slices on a mesh so that aCSF travels underneath submerged slices; faster aCSF flow rate; vigorous pre-oxygenation of aCSF and the use of gas-impermeable tubing. Acutely-prepared and appropriately maintained hippocampal slices should be viable all day (8 – 10 hrs).

## Specific examples

We have used slices prepared in this manner to study the effects of environmental enrichment on synaptic transmission and plasticity. We observed that enrichment enhances both long-term potentiation (LTP) and long-term depression (LTD), thereby expanding the dynamic range of synapses, and likely contributing to the enhanced cognition seen in enriched WT mice (Privitera *et al*., 2020).

We have also shown that the reduced ATP levels associated with slice preparation can be restored to in vivo values through the provision of ribose and adenine (RibAde) (zur Nedden *et al*., 2011). RibAde increased the threshold for the induction of LTP through the greater release of adenosine and activation of $A_1$Rs (zur Nedden *et al*., 2011). This additional release of adenosine was also observed in response to oxygen/glucose deprivation (a model of stroke) (zur Nedden *et al*., 2014), and following slice seizure activity (Hall and Frenguelli, 2018), where the additional adenosine release exerted a greater inhibitory influence on synaptic transmission and epileptiform activity, respectively.

## References

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