User interface refinements and quality assurance assessment in an updated popular tissue vibratome

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Introduction

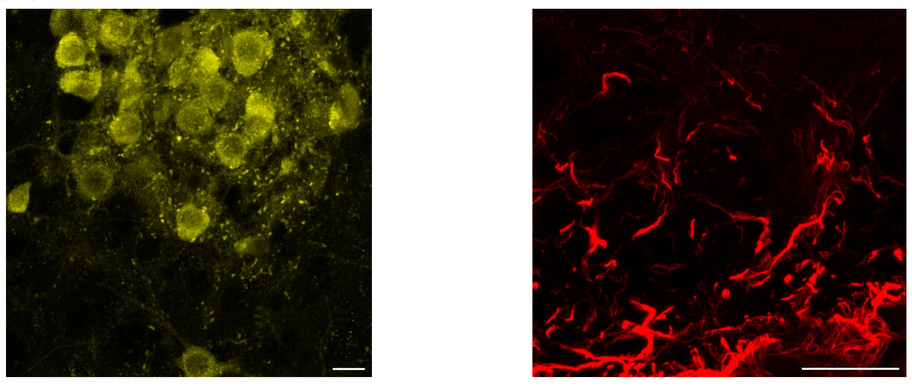
Vibrating microtomes (vibratomes) are indispensable tools in neuroscience research, enabling precise sectioning of delicate biological tissues for histological and electrophysiological studies. Significant technological advances have been made in vibratome design and performance over recent years. Enhanced vibration frequency control, blade oscillation stability, and precision cutting technology have improved tissue section quality. Equally, ease of use plays an important factor in the efficient and ergonomic use of vibratomes, which can be for extended periods of time. Here we report refinements of the graphical user interface of a popular vibratome, the Campden Instruments 7000smz (Loughborough, UK) and initial field trials of brain slice preparation in several independent neuroscience laboratories.

Methods

- Sectioning the brainstem (50 µm) of 6-8 month-old male and female C57BL/6 mice with kainic acid-induced epilepsy was followed by immunohistochemical staining for choline acetyltransferase (ChAT) and astrocytes (GFAP).
- Hippocampal slices from 3 week-old C57BL/6J mice were prepared with the standard reusable stainless steel blades (60 Hz; 1.0 mm amplitude; 0.08 mm/s) for immunohistochemical staining of microglia (Iba1; 200 µm) exposed to LPS, and horizontal hippocampal slices (400 µm) for extracellular recordings of the effects of tau on carbachol-induced oscillatory activity in area CA3.
- Using a ceramic blade, (50 Hz; 1.0 mm amplitude; 0.05 mm/s) sagittal and horizontal male and female Wistar rat brain slices (p21-p300) were prepared for patch-clamp recordings of hippocampal and cortical cells (350 µm), and LFP recordings (450 µm) of theta and gamma oscillations in the hippocampus.

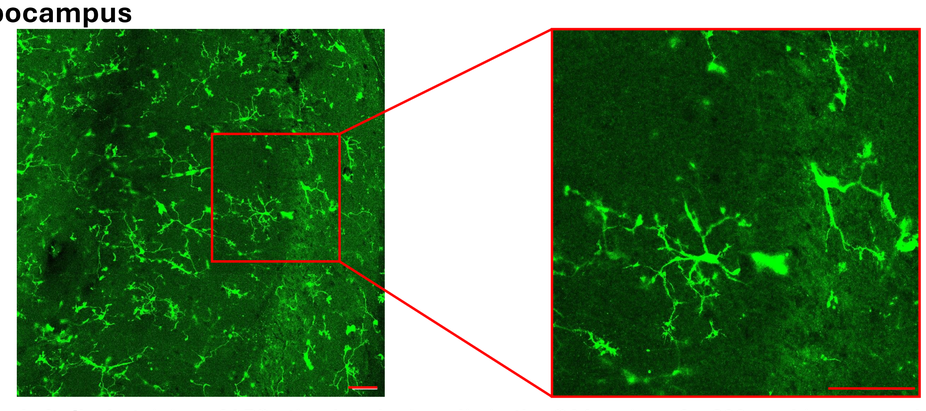
Immunohistochemistry

Brainstem



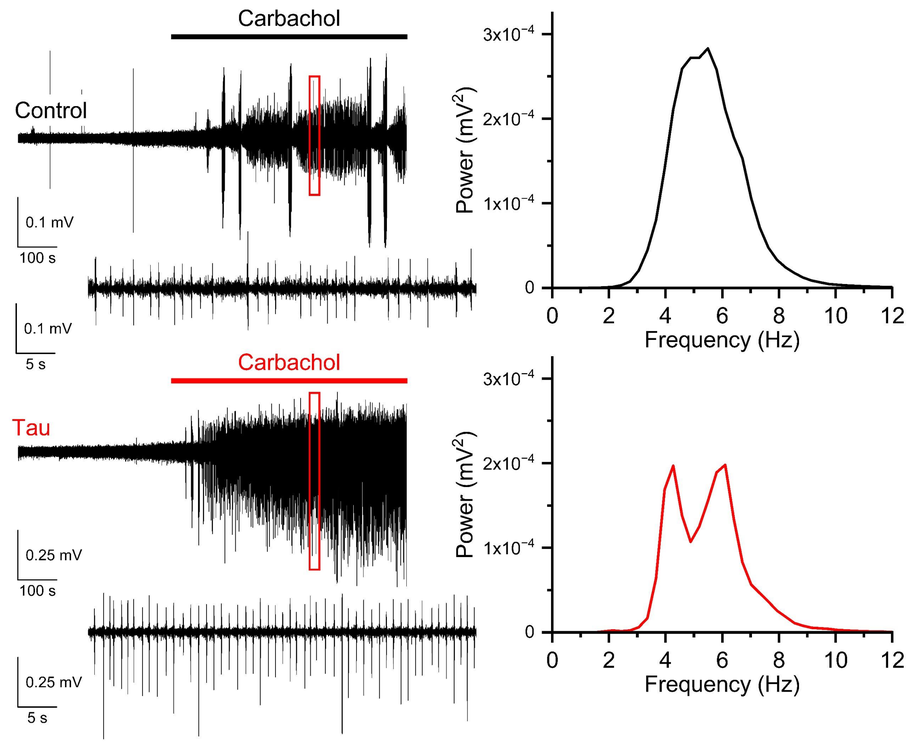
Maximum intensity projections of confocal images showing choline acetyltransferase $(ChAT)^+$ neurons (left, yellow) and glial fibrillary acidic protein $(GFAP)^+$ astroglia (right, red) in 40 µm thick mouse brainstem sections. *Scale bars = 20 µm*

Hippocampus



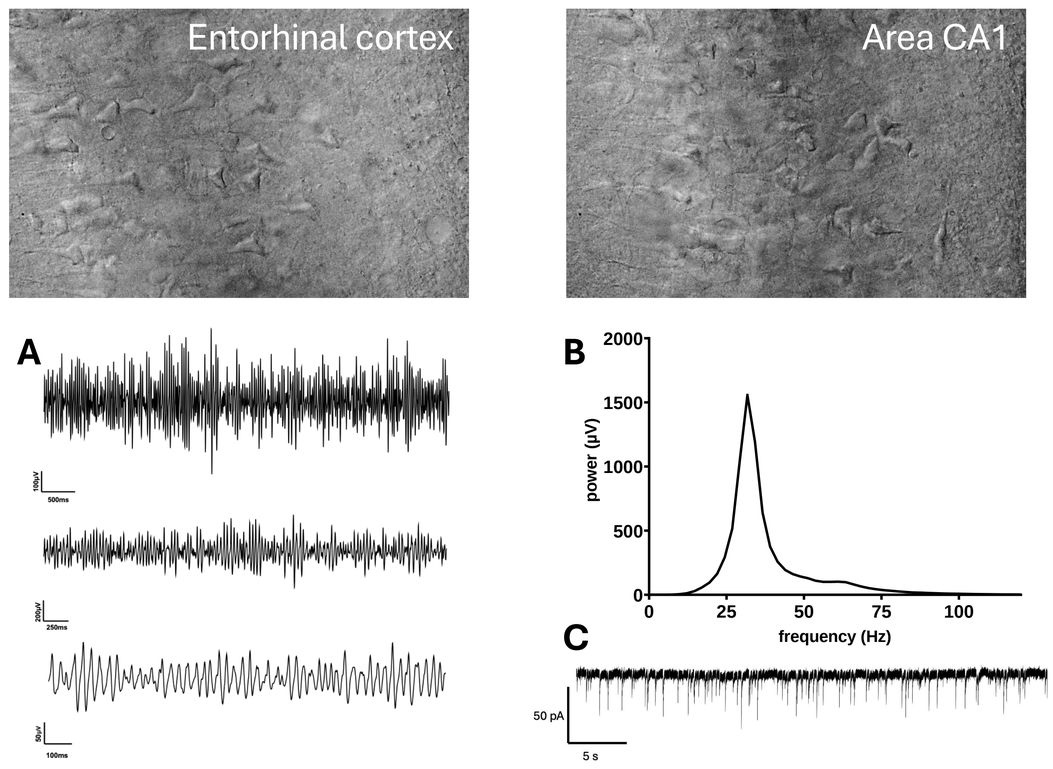
Left: Iba1 staining of LPS-stimulated microglia in the CA1 region of a 200 µm hippocampal slice. Right: highlighted region of interest. *Scale bars = 50 µm*

Oscillations in slices from 3 week-old mice



Neuronal oscillations in the theta frequency range (4 – 8 Hz) induced by the acetylcholine muscarinic receptor agonist carbachol (50 µM) in hippocampal area CA3 from 3 week-old C57BL/6J mice. Slices were either incubated in control aCSF or in aCSF supplemented with sonicated recombinant human tau preformed fibrils (133 nM; rPeptide; CF-1001-1). Left panels: raw electrophysiological traces showing induction of oscillations after carbachol, and 25 s of activity (red boxed region; lower traces). Right panels: spectrograms of theta activity induced by carbachol in both conditions.

Oscillations in slices from 9 month-old rats



Improved microtome design allows neuronal preservation and physiological oscillations in older rats (9 months). Above: DIC images from entorhinal cortex and area CA1. A) Raw electrophysiological data at increasing magnification demonstrating characteristic spontaneous sawtooth gamma oscillations in LFP recordings (450 µm slice). B) Panel shows example FFT from LFP recording in hippocampus with no added drugs, with high power in gamma range and excellent coherence. C) Whole-cell patch-clamp recording from hippocampal cell with regular EPSCs and stable baseline (350 µm slice).

Conclusions

- Overall, the interface of the new Campden 9000smz is an improvement on the previous GUI and will likely lead to more rapid learning, more efficient slice cutting and to exploring the instrument's additional sectioning capabilities.
- Coupled with zero-Z deflection compensation and ceramic blades, the 9000smz represents a versatile vibratome for the preparation of a variety of high quality fresh and fixed brain tissue.
- Studies conducted so far indicate that slice quality is at least on a par, if not better with older animals, than those cut with the earlier version.

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