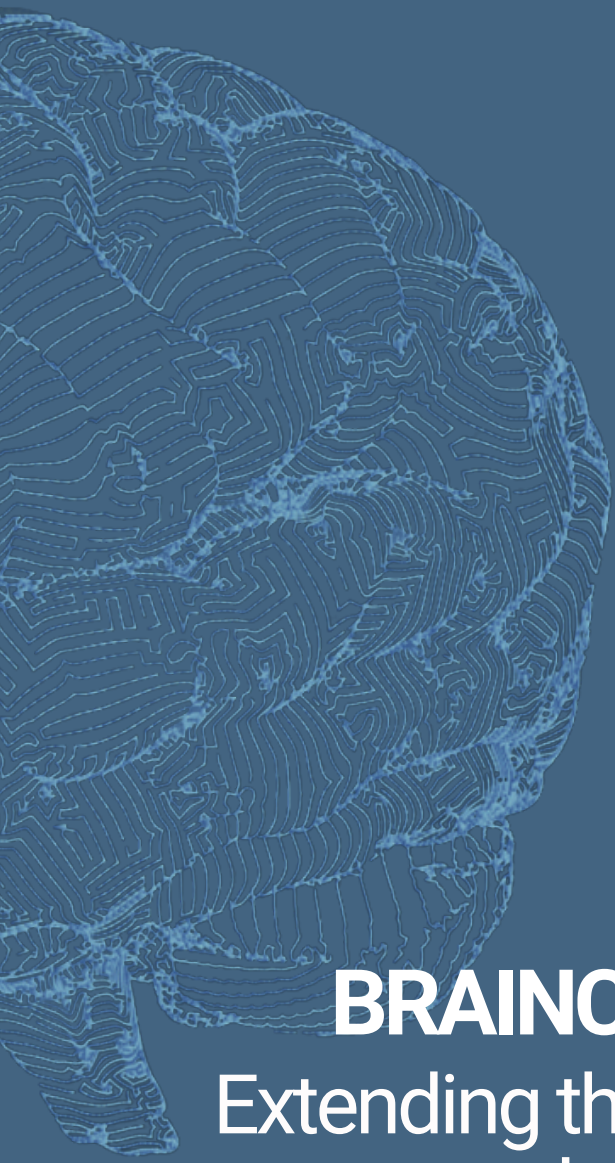
APPLICATION NOTE

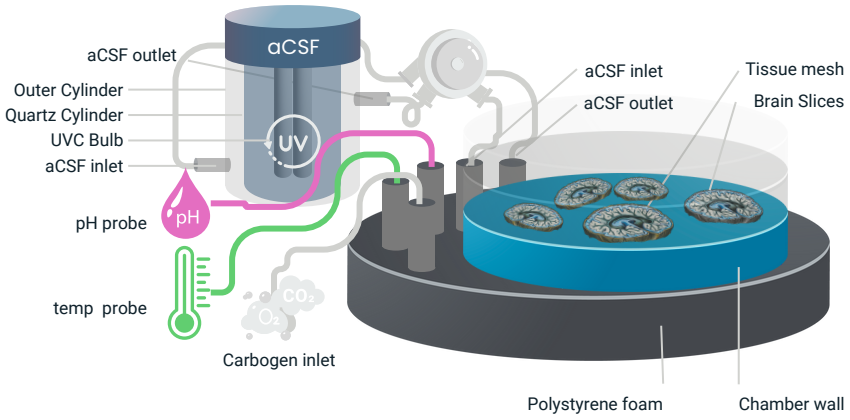


BRAINCUBATOR™

Extending the viability of acute brain slices

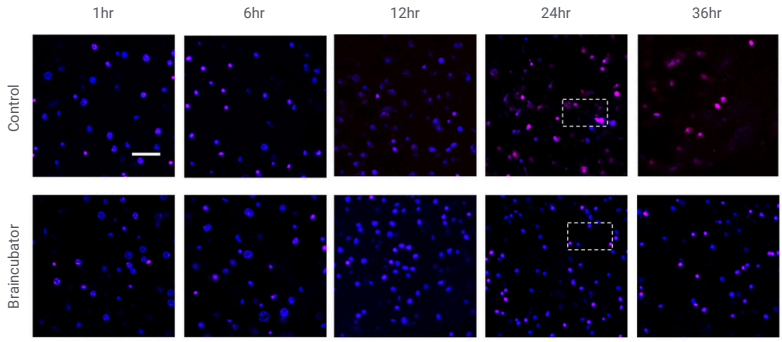
Yossi Buskila, Paul P. Breen, Jonathan Tapson, André van Schaik, Matthew Barton & John W. Morley

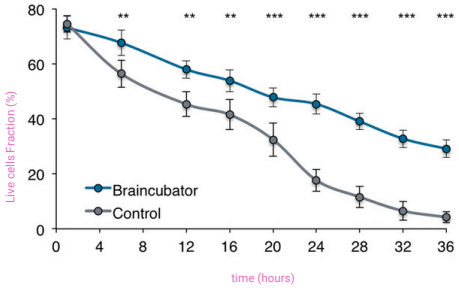
Fresh brain slices have a limited lifespan, leading to experimental inconsistencies and unnecessary animal use. This study examines how a **controlled storage environment (BrainCubator) can extend slice viability for neuron isolation and electrophysiology recording**. By maintaining artificial cerebrospinal fluid (aCSF) at 16°C and minimizing bacterial growth through UVC sterilization, **stable recordings of pyramidal neuron resting membrane potential (RM) and action potentials (APs) were achieved for up to 36 hours**. Through the use of the Braincubator, slices retained physiological activity for longer, extending the reliable recording window and allowing researchers to maximize data collection from each preparation. By reducing extracellular solution temperature and inhibiting bacterial growth, the Braincubator helped preserve neuronal function, ensuring that layer V pyramidal neurons maintained stable resting membrane potentials and synaptic activity. This **extended viability reduced the need for additional animals, improving both research efficiency and ethical standards**.

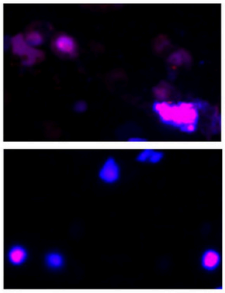


BRAINCUBATOR™ system - Schematic Diagram

SLICE VIABILITY – STANDARD CONTROL VS BRAINCUBATOR



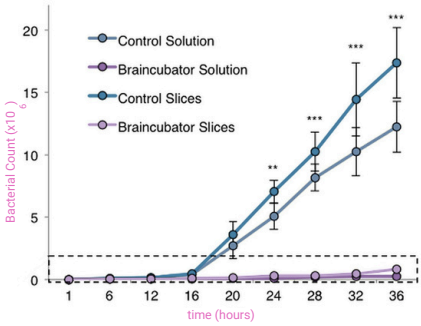




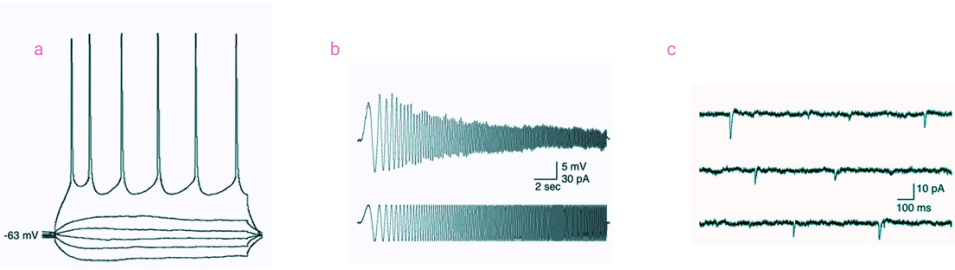
Brain slice viability was assessed using propidium iodide (PI) and DAPI markers. Viability was measured as the ratio of live cells (PI negative) to total visible cells (DAPI positive + PI positive) in the rat cerebral cortex. The experiment involved imaging slices maintained either in the Braincubator or control conditions (n=54) at various time points (1- 36 hour intervals indicated above) after being sectioned at the same time. At 1 hour, no significant difference was observed in cell viability between conditions. However, **after 6 hours, slices maintained in the Braincubator showed significantly higher cell viability, with a maximal difference of 28% at 24 hours (45% vs. 17%, p < 0.0001)**.

BACTERIAL CONTAMINATION

Bacterial numbers over time. Results display Pseudomonas and Stenotrophomonas maltophilia bacteria counts over time. These bacteria typically thrive in neutral pH and mesophilic conditions. In tIn the Braincubator solution and slices (15–16°C, UVC filtration), bacterial growth was minimal compared to control conditions (22°C, no UVC). Bacterial growth as not observed in BrainCubtor solution or slices until after 12 hours ($p < 0.0001$)



ELECTROPHYSIOLOGY - SLICE VIABILITY POST 31 HRS



Electrophysiological properties of layer V pyramidal neurons were recorded in slices following incubation in the Braincubator. (a) I-V traces. Increasing step currents of 500 ms were injected into the soma through the recording electrode to reveal the input resistance and firing properties. (b) The resonance frequency was measured by injecting a chirp stimulation of 60 pA (peak to peak). (c) **Sample traces of spontaneous synaptic activity recorded from a neuron after 31 hrs in the braincubator, indicating extended viability of acute brain slices.**

	Control < 4 hrs (n = 10)	1–3 hrs (n = 12)	5–8 hrs (n = 7)	15–24 hrs (n = 9)	26–41 hrs (n = 9)
Resting membrane potential (mV)	−63 ± 2	−65 ± 2	−64 ± 1	−64 ± 1	−63 ± 1
Input resistance (MΩ)	176 ± 29	165 ± 18	163 ± 27	179 ± 21	145 ± 19
Membrane time constant (ms)	25 ± 4	28 ± 3	24 ± 7	25 ± 2	23 ± 3
Resonance frequency (Hz)	2 ± 0.3	2 ± 0.2	1.7 ± 0.3	1.5 ± 0.3	1.6 ± 0.3
First spike Amplitude (mV)	97 ± 5	97 ± 2	94 ± 4	93 ± 3	92 ± 3
Half-width spike amplitude (ms)	2.4 ± 0.2	2.5 ± 0.2	2.3 ± 0.1	2.6 ± 0.2	2.2 ± 0.1
mEPSC's frequency (Hz)	2.2 ± 0.3	1.75 ± 0.3	2.3 ± 0.4	1.5 ± 0.2	2.2 ± 0.3
mEPSC's amplitude (pA)	−7.3 ± 0.5	−6.4 ± 0.5	−7.8 ± 0.5	−5.7 ± 0.4	−8 ± 0.7

Electrophysiological properties of layer V pyramidal neurons were stable across incubation times in the Braincubator, with no significant differences between groups (one-way ANOVA). The control group (<3 hours) was included for comparison.

Precisely regulates pH, temperature, and media

Tissue viability up to 36hrs

Halve the number of animals sacrificed

The Braincubator is an effective tool for significantly extending the viability of brain slices, **enabling neuron isolation and recording for up to 36 hours**. By combining reduced temperature (16°C) and UVC irradiation, it **minimizes bacterial growth and preserves slice integrity**, preventing neuronal damage typically caused by bacterial toxins. The use of the Braincubator enables **stable electrophysiological properties**, such as resting membrane potentials and functional synaptic activity, in neurons within the slice. This **controlled environment** not only **enhances the quality and duration of experimental data** but also **reduces the need for additional animal use**, making it a valuable tool in neuroscience research.

METHOD – STANDARD CONTROL VS BRAINCUBATOR

This study evaluated the effectiveness of the Braincubator™ system in maintaining acute brain slices over extended periods. Tissue viability, slice functionality, and bacterial contamination were assessed using fluorescence staining, electrophysiological recordings, and bacterial culturing. A standard interface chamber served as the control condition for comparison.

Animals
Male Wistar rats (2–5 weeks old) were housed in standard conditions with a 12:12 h light/dark cycle and ad libitum food and water. All procedures followed the University of Western Sydney Animal Research Committee guidelines (#A9452).

Slice Preparation and Incubation
Rats were anesthetized with 5% isoflurane, brains quickly removed and sectioned into 300 µm slices in ice-cold, carbogenated artificial cerebrospinal fluid (aCSF). Slices recovered for 30 minutes at 35°C in a submerged incubation chamber.
Experiment Setup: Slices were incubated in either the Braincubator™ (experimental) or a standard submerged chamber at room temperature (~22°C, control). with no active bacterial control or additional environmental regulation. Slices remained in their respective chambers for at least 30 minutes before further assessments.

Slice Viability and Electrophysiology
Slices were stained with propidium iodide (PI) and DAPI, and viability was assessed using confocal microscopy. Whole-cell patch-clamp recordings were conducted on layer 5 pyramidal neurons using a Multiclamp 700B amplifier. Resonance frequency was measured via impedance analysis using fast Fourier transform (FFT). Spontaneous miniature EPSCs were recorded in voltage-clamp mode at −70 mV in the presence of tetrodotoxin and picrotoxin.

Bacterial Detection
aCSF and brain slice samples were collected at multiple time points (1, 3, 6, 12, 24, and 36 h) from both incubation conditions and cultured on agar plates at 37°C. Colony-forming units (CFU) were counted, and bacterial identification was performed using MALDI-TOF mass spectrometry.

Braincubator™ System

T**he Braincubator™** – Recovery Incubation System
A custom incubation system was **designed to regulate pH, temperature, and bacterial contamination**. The system consisted of a main chamber housing brain slices and a separate UVC chamber for bacterial sterilization. The main chamber was insulated with polystyrene foam and monitored for pH and temperature. The UVC chamber contained a 5W Philips UVC lamp (254 nm), which operated intermittently (15–26 min every 15–30 min) to minimize heat buildup and prevent bacterial resistance. Solution was circulated between chambers via a peristaltic pump, and temperature was controlled using a Peltier thermoelectric cooler (TE Technology, MI).

Statistical Analysis
Data were analyzed using Student's t-tests or ANOVA, with results expressed as mean ± S.E.M. Statistical significance was set at $p < 0.05$.

Buskila, Y., Breen, P., Tapson, J. et al. Extending the viability of acute brain slices. Sci Rep4, 5309 (2014). https://doi.org/10.1038/srep05309

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Kekesi, O. et al (2019): **The differential impact of acute microglia activation on the excitability of cholinergic neurons in the mouse medial septum.** Brain Structure and Function, Volume 224, pp 2297–2309

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BRAINCUBATOR™

prolongs tissue slice lifespan, reduces animal usage, supports extended experimental time, and ensures more reliable experimental conditions

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