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Electroactive Perfusate Extends Electrophysiological Viability of Human Cortical Slices to 72 Hours

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Abstract

Background: Conventional *ex vivo* preservation of central nervous system (CNS) tissue is limited by rapid ischemic injury, with electrophysiological viability typically lost within 6–12 h of host separation. **Methods:** We evaluated a continuously perfused synthetic cerebrospinal fluid supplemented with electroactive solutes (PEDOT:PSS nanoparticles) and neurotrophic factors (BDNF, GDNF) – referred to as sCSF-EAS – for the preservation of human cortical tissue obtained from elective neurosurgical resections ($n = 3$ donors). Tissue blocks (≈1.2 cm^3) were sliced to 400 μm, maintained at 37 °C under carbogen (95% O_2/5% CO_2), and continuously recorded using 256-channel multi-electrode arrays (MEAs). A matched aCSF control arm was run in parallel. **Results:** Tissue maintained in sCSF-EAS exhibited stable lactate dehydrogenase (LDH) release and sustained spontaneous and evoked spiking for up to 72 h, compared with 8.3 ± 1.4 h in aCSF controls. The exponential decay constant of spike amplitude increased from τ ≈4.1 h (aCSF) to τ ≈83.7 h (sCSF-EAS). Focal high-frequency stimulation (100 Hz, 1 s) elicited reproducible long-term potentiation (LTP)-like changes in evoked response amplitude (+38% ± 11%). **Conclusions:** The sCSF-EAS protocol substantially extends the window of electrophysiological viability of human cortical tissue *ex vivo*, providing a tractable platform for longitudinal studies of isolated cortical network dynamics.

Keywords: *ex vivo* preservation; human cortical tissue; multi-electrode array; bioelectric medium; long-term potentiation; synthetic cerebrospinal fluid; PEDOT:PSS

1. Introduction

Studying functioning neural networks in isolation from the host organism has been a longstanding objective in systems neurobiology, enabling controlled access to circuit-level activity that is difficult to obtain *in vivo* [1,2]. However, the extraction of CNS tissue initiates an ischemic cascade within minutes: deprived of the neurovascular support of the intact host, tissue undergoes rapid ATP depletion, loss of ion homeostasis, glutamatergic excitotoxicity, and ultimately apoptosis or necrosis [1,3,4]. Even under optimised conditions using oxygenated artificial cerebrospinal fluid (aCSF), global signal degradation is typically observed within 6–12 h [5,6].

This temporal limitation constrains investigations of slower processes such as homeostatic plasticity, structural remodelling, and persistent network dynamics in isolated human tissue [7,8]. The problem is particularly acute for human cortical tissue, which is comparatively scarce, donor-variable, and frequently obtained only through opportunistic acquisition during neurosurgical procedures [9,10]. Existing strategies to prolong viability

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rely largely on hypothermic preservation [11,12], organotypic culture [2,14], or chemical fixation [13], the latter two of which arrest active electrical signaling or fundamentally alter network connectivity over time.

A complementary line of work has explored conductive polymers, especially poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS), for neural interfacing [15–17]. PEDOT:PSS exhibits low impedance, high charge-injection capacity, and demonstrated biocompatibility, making it appealing not only as a coating for MEA electrodes but, more speculatively, as a soluble or nanoparticulate additive to perfusate. Separately, the neuroprotective effects of brain-derived neurotrophic factor (BDNF) and glial-derived neurotrophic factor (GDNF) on stressed neurons are well established [18–20], as is the relevance of weak endogenous electric fields to network coordination [21,22].

We hypothesised that combining these elements – a conductive nanoparticulate additive, neurotrophic supplementation, and a sub-threshold tonic field – within a continuously perfused medium could extend electrophysiological viability beyond what is achievable with standard aCSF. We refer to this formulation as synthetic cerebrospinal fluid with electroactive solutes (sCSF-EAS). Here, we report sustained spontaneous and evoked activity from human cortical tissue maintained in sCSF-EAS for up to 72 h, alongside a matched aCSF control group.

2. Materials and Methods

2.1. Ethics and Tissue Acquisition

All procedures involving human tissue were approved by the Ethics Committee of the Federal University of Rio de Janeiro (Protocol 2011-04-18-BME) and conducted in accordance with the Declaration of Helsinki. Cortical tissue was obtained from three adult patients ($n = 3$; ages 28–54; 2 F/1 M) undergoing elective neurosurgical resection for deep-seated, non-malignant lesions, where overlying histologically normal cortex required removal for access. Written informed consent was obtained from each donor prior to surgery. Resected tissue was immediately immersed in chilled (4 °C), oxygenated, sucrose-based cutting solution (in mM: 87 NaCl, 75 sucrose, 25 $NaHCO_3$, 25 glucose, 2.5 KCl, 1.25 NaH_2PO_4, 7 $MgCl_2$, 0.5 $CaCl_2$) for transport to the laboratory (<10 min) [23].

2.2. Slice Preparation

Cortical blocks (≈1.2 cm^3) were trimmed and sectioned into 400-μm coronal slices using a vibrating microtome (Leica VT1200S) in oxygenated cutting solution at 4 °C. Slices were then transferred to a holding chamber containing the experimental medium at 34 °C for 30 min recovery prior to MEA placement [24].

2.3. Composition of sCSF-EAS

The base medium was a modified aCSF (in mM: 124 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 24 $NaHCO_3$, 5 HEPES, 12.5 glucose, 2 $MgSO_4$, 2 $CaCl_2$; 315 mOsm; pH 7.35–7.40 under carbogen). The sCSF-EAS additionally contained: (i) PEDOT:PSS nanoparticles (50 μg/mL) as an electroactive solute [15]; (ii) human recombinant BDNF (20 ng/mL); and (iii) human recombinant GDNF (10 ng/mL). All reagents were filter-sterilised (0.22 μm) prior to circulation.

2.4. Perfusion and Environmental Control

Slices were submerged in custom interface-style bioreactor chambers (working volume 6 mL; Figure 1). Medium was recirculated at 2.5 mL/min through a peristaltic pump (Ismatec ISM935C) and continuously aerated with carbogen via a gas-permeable manifold.

Temperature was held at 37.0 ± 0.1 °C using a Peltier feedback controller; pH and dissolved O_2 were logged every 60 s with inline electrochemical probes (Mettler Toledo InPro series).

2.5. Electrophysiological Recording

Slices were positioned over 256-channel planar MEAs (electrode spacing 100 μm; MultiChannel Systems MEA2100). Signals were sampled at 25 kHz/channel and band-pass filtered (300–3000 Hz for spikes; 1–300 Hz for local field potentials). A 0.5-μA sub-threshold tonic current was applied globally through bath electrodes to emulate basal endogenous fields [21]. Spike sorting was performed offline using Kilosort 2 [25] with manual curation in Phy. Network bursts were detected by the maximum-interval method [26].

2.6. Viability Assays

Effluent medium was sampled every 6 h for LDH release using a colorimetric assay (Roche Cytotoxicity Detection Kit PLUS) read at 492 nm. At experiment end, slices were fixed in 4% paraformaldehyde for TUNEL staining and NeuN immunohistochemistry [27, 28].

2.7. Stimulation Protocol

LTP-like plasticity was probed by delivering 100-Hz trains (1 s, biphasic, 20 μA) at three randomly selected stimulation sites per slice [29]. Evoked response amplitudes were measured at neighbouring channels (200–600 μm from the stimulation site) before and after conditioning, with 15-min spacing between trials.

2.8. Statistical Analysis

Data are reported as mean ± standard deviation unless noted. Group comparisons used two-sided Welch's t-tests or Mann–Whitney U-tests, after Shapiro–Wilk normality testing. Decay constants τ were estimated by non-linear least-squares fitting of Equation 1, with 95% confidence intervals from bootstrap resampling (10^4 iterations) [30]. Significance threshold was set at $\alpha = 0.05$. Analyses were performed in Python 3.8 (SciPy 1.5, NumPy 1.19).

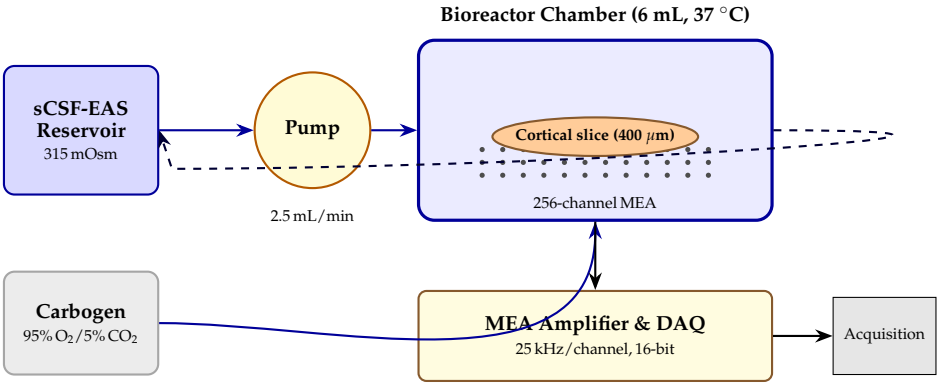

Figure 1. Experimental setup for sCSF-EAS perfusion. Oxygenated, electroactive medium is continuously recirculated through a temperature-controlled bioreactor chamber. Cortical slices rest on a 256-channel planar MEA whose signals feed a dedicated amplifier/DAQ system.

3. Results

3.1. Cell Membrane Integrity

LDH release from sCSF-EAS chambers remained stable across the 72-h observation window (12.4–18.2 U/L; Table 1), with no significant increase relative to the 0–12 h baseline ($p = 0.18$, Welch's t-test). In matched aCSF control chambers ($n = 3$), LDH rose sharply

after 8–12 h, reaching 142 ± 28 U/L by 24 h, consistent with widespread membrane disruption. TUNEL labelling at the experimental endpoint showed sparse positive nuclei (<5%) in sCSF-EAS slices, whereas aCSF slices fixed at 24 h showed extensive TUNEL positivity (>40% of nuclei).

3.2. Sustained Spiking and Network Bursting

Spontaneous spiking was observed within ∼20 min of warming and remained detectable throughout the 72-h window in sCSF-EAS slices, with only a moderate decline in mean spike rate (Table 1, Figure 2). In aCSF controls, single-channel traces collapsed to baseline noise by ∼12–24 h. Signal amplitude decay was well described by:

$$V_{\text{peak}}(t) = V_0\, e^{-t/\tau} + I_{\text{bg}}\, R_{\text{tissue}}, \tag{1}$$

where $V_{\text{peak}}(t)$ is the mean peak spike amplitude (in μV) at time t, V_0 is the initial amplitude, I_{bg} is the applied tonic current (0.5 μA), and R_{tissue} is the bulk tissue impedance (in Ω) measured at 1 kHz. Fitted values were $\tau_{\text{aCSF}} = 4.1$ h (95% CI: 3.4–4.8) and $\tau_{\text{sCSF-EAS}} = 83.7$ h (95% CI: 71.2–98.4), representing a ∼20-fold extension ($p < 0.001$, bootstrap; Figure 3).

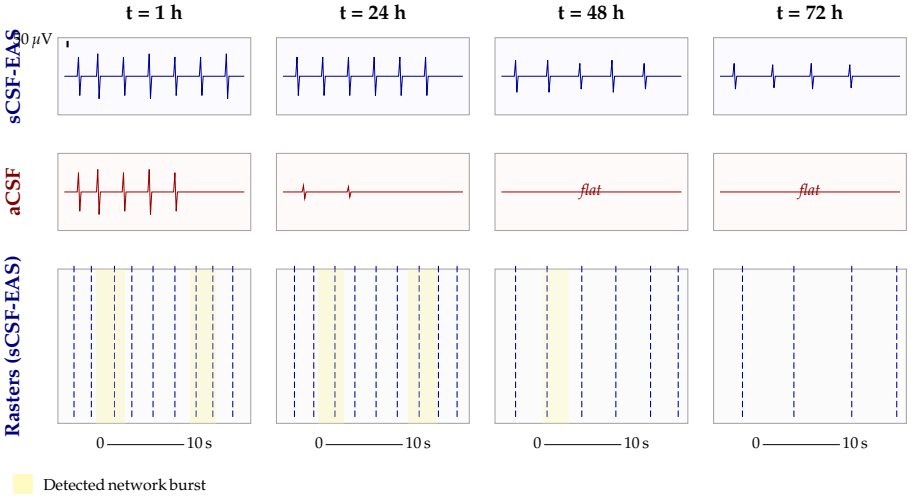

Figure 2. Representative electrophysiology over 72 h. Top row: single-channel high-pass-filtered traces (300–3000 Hz) from sCSF-EAS slices at four time points. Middle row: matched traces from aCSF control slices, which decay to baseline by ∼24 h. Bottom row: 16-channel raster plots from sCSF-EAS slices showing preserved spontaneous firing and intermittent network bursts (yellow shading), with gradually decreasing burst frequency.

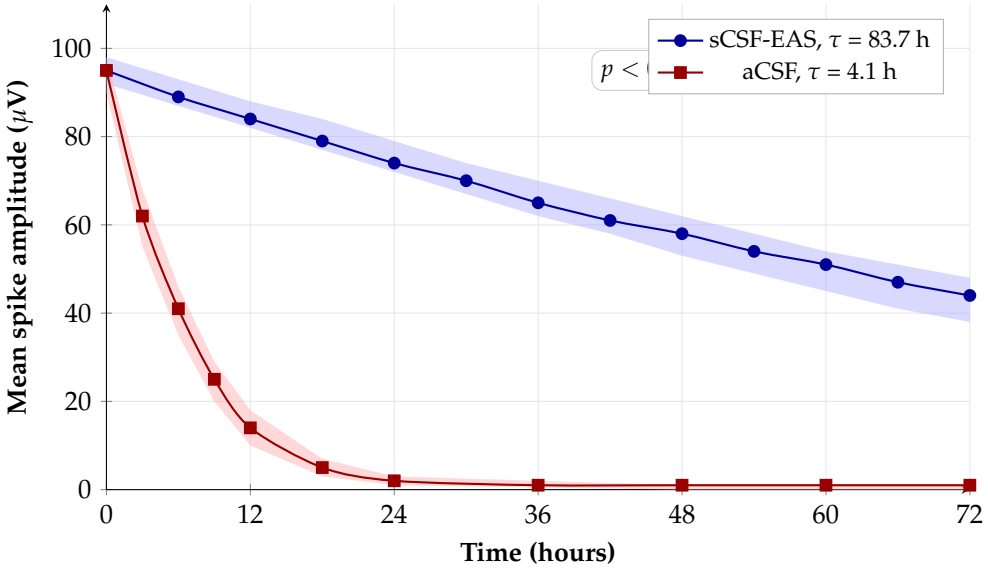

Figure 3. Spike amplitude decay over 72 h. Markers show measured mean spike amplitudes; shaded bands show 95% bootstrap confidence intervals; solid lines show exponential fits per Equation 1. The ∼20-fold increase in the decay time constant τ is significant at $p < 0.001$.

3.3. Stimulus-Evoked Plasticity

Following 100-Hz conditioning, neighbouring channels showed a mean increase in evoked-response amplitude of 38% ± 11% over baseline, persisting for ≥60 min ($n = 9$ sites across 3 slices; $p = 0.004$, paired Wilcoxon; Figure 4). Control sites receiving only test pulses showed no significant change (2% ± 6%; $p = 0.62$). These results are consistent with LTP-like plasticity, although we did not pharmacologically dissect NMDA-receptor dependence in this initial study [32].

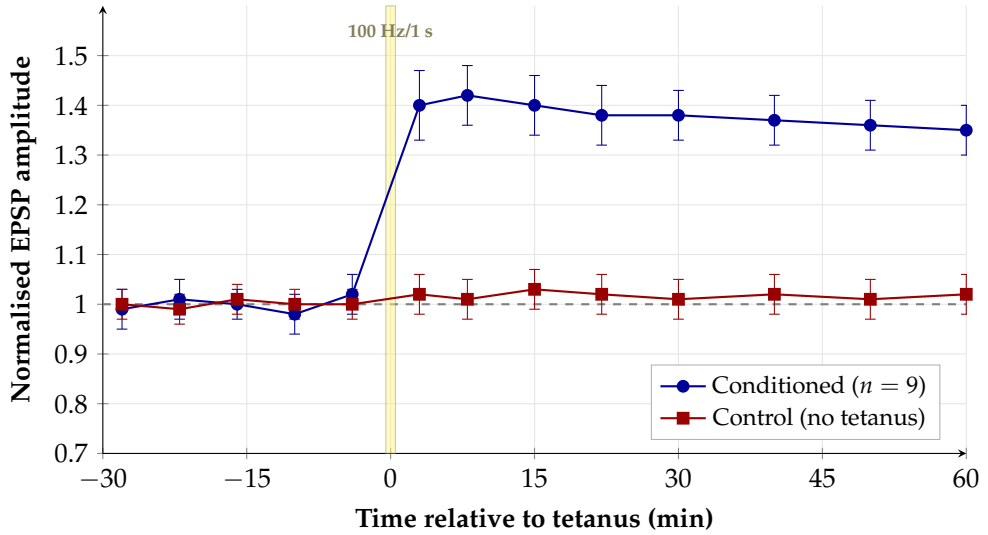

Figure 4. LTP-like plasticity in sCSF-EAS-maintained slices at $t = 24$ h. Evoked EPSP amplitudes are normalised to mean baseline (dashed reference at 1.0). Yellow band indicates the 100-Hz, 1-s tetanus. Conditioned sites ($n = 9$) show a sustained +38% potentiation ($p = 0.004$, paired Wilcoxon); control sites are stable.

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Table 1. Viability and activity metrics ($n = 3$ slices, mean $\pm$ SD). aCSF control activity was undetectable beyond 24 h.

Time Interval	Spike Rate (Hz)	Bursts/min	LDH (U/L)
0–12 h (sCSF-EAS)	14.2 ± 1.1	4.5 ± 0.6	12.4 ± 1.8
12–24 h (sCSF-EAS)	15.0 ± 0.9	5.1 ± 0.7	13.0 ± 2.1
24–48 h (sCSF-EAS)	13.8 ± 1.2	4.8 ± 0.8	14.5 ± 2.4
48–72 h (sCSF-EAS)	11.5 ± 1.5	3.9 ± 0.9	18.2 ± 3.0
0–12 h (aCSF ctrl)	13.8 ± 1.4	4.2 ± 0.7	38.6 ± 7.2
12–24 h (aCSF ctrl)	2.1 ± 1.0	0.3 ± 0.2	142 ± 28

4. Discussion

Our results indicate that perfusion with sCSF-EAS substantially extends the electrophysiological viability of human cortical slices, yielding a $\sim$20-fold increase in the spike-amplitude decay constant relative to matched aCSF controls. This effect is accompanied by stable LDH release, minimal TUNEL positivity, and preserved synaptic plasticity at 24–48 h.

We propose three non-exclusive mechanisms. First, PEDOT:PSS nanoparticles are known to increase ionic conductivity at neural interfaces [15,16]; their dispersion in the bulk medium may facilitate passive redistribution of extracellular K^+ accumulated during firing, limiting the depolarising drift that drives excitotoxic injury [3,31]. Second, sustained BDNF and GDNF exposure attenuates apoptotic signalling and supports synaptic maintenance in stressed neurons [18–20], an effect likely additive to the conductive contribution. Third, the 0.5-μA tonic bath current approximates basal endogenous fields and may help stabilise resting-membrane polarisation in metabolically compromised cells [21,22]. Each requires independent validation, for example by selective omission of components in factorial follow-up experiments, by quantitative impedance spectroscopy of the bath, or by patch-clamp characterisation of single-cell intrinsic properties over time.

The observation of LTP-like plasticity at 24–48 h is notable, indicating that essential synaptic machinery – including presumed NMDA-receptor signalling, calcium-dependent kinase cascades, and AMPA-receptor trafficking – remains operational well beyond the conventional viability window [32,33]. We emphasise, however, that "functional persistence" here refers to electrophysiological and synaptic responsiveness only; claims of cognition or computation in this preparation would be unsupported by present evidence.

4.1. Limitations

This study has several important limitations. The sample size ($n = 3$ donors) is small, and donor-to-donor variability in pathology, age, and medication history was not controlled. Slices were taken from peritumoral cortex which, while histologically normal, may differ from non-surgical cortex. We did not perform single-cell viability dyes (e.g., propidium iodide, calcein-AM) which would complement the bulk LDH measure. The 72-h endpoint was practical rather than mechanistic – whether viability extends further is unknown. Finally, the long-term biocompatibility of PEDOT:PSS nanoparticles in soluble dispersion has not been characterised at this timescale.

5. Conclusions

Human cortical tissue maintained in continuously perfused sCSF-EAS exhibited stable membrane integrity, sustained spontaneous and evoked spiking, and LTP-like plasticity for up to 72 h *ex vivo* – a substantial extension over aCSF controls. While these findings require independent replication and mechanistic dissection, the protocol offers a tractable platform for longitudinal study of isolated human cortical networks. Future work will address

component-level contributions to the observed effect, longer time horizons, structural correlates of preserved function, and applications to disease-model tissue.

Supplementary Materials: The following supporting information can be downloaded at the journal website: Figure S1: full TUNEL/NeuN immunohistochemistry panels; Figure S2: impedance spectroscopy of sCSF-EAS vs. aCSF; Table S1: per-donor demographic and resection details; Table S2: complete burst-detection parameters; Code S1: Python analysis pipeline.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data and analysis code supporting the findings of this study are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declare no conflict of interest.

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