Lab-bench perfusion setup for combined long-term multielectrode *in vitro* electrophysiology and time-lapse microscopy

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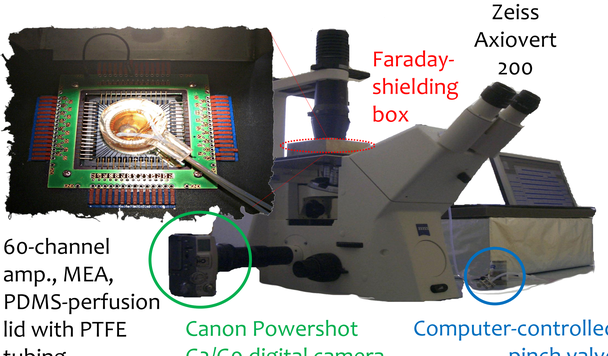
Abstract

To date, a majority *of in vitro* multielectrode array (MEA) electrophysiology studies on neural cell cultures are performed in short-term 'snap-shot' experiments lasting from minutes to hours. Acquisition time span is mainly limited by a drift in pH and osmolarity when experimenting outside of an CO_2 incubator, and activity may be biased by secondary handling artifacts (T- changes, mixing). Stabilizing the cell culture environment by a simple, gravity-driven, CO_2-independent perfusion concept allows the uninterrupted (minutes to months) and 'undisturbed' recording of neural activity from commercial MEAs on a lab bench at ambient conditions. It is based on a perfusion lid made of flexible, transparent and gas-permeable polydimethylsiloxane (PDMS) with particular internal shape for the expulsion of gas bubbles, embedded polytetrafluoroethylene (PTFE) tubing and self-sealing septa. The design neither obstructs the mounting of the MEA into the amplifier nor the microscopy optics for time-lapse imaging of network morphology on an inverted microscope. Only temperature (T-controller) and pH (chemical buffering) need to be controlled to provide appropriate physiological conditions. The lid creates a stabilized micro-environment that does not require any other incubation scheme. We present selected data on the evolution and fluctuation of network activity in differentiating, rat-derived (E18), cortico-hippocampal co-cultures continuously sampled over a period of up to two months. Recorded spike trains were analyzed by both their rate and spatio-temporal correlation. The stage-wise increase and spatial focalization of overall activity was not reflected in any obvious reorganization of the macroscopic network architecture.

Methods & Results

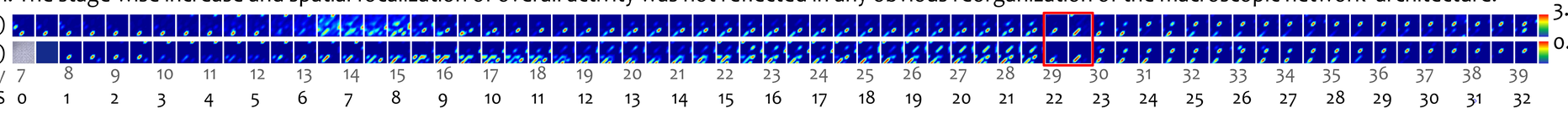


Setup

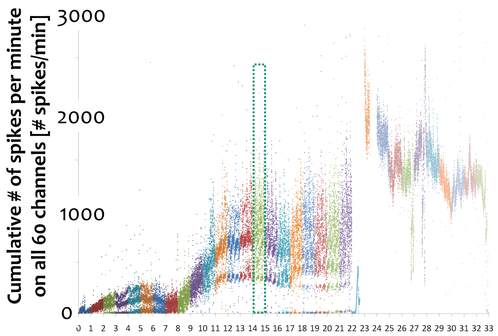
- inverted microscope (Zeiss Axiovert 200) with digital camera
- multielectrode array (MEA TiN, 30/200iR) with 60 channel amplifier and temperature controller (Multi Channel Systems, Reutlingen, Germany)
- Faraday shielding box
- poly(dimethylsiloxane) (PDMS) perfusion cap with poly(tetrafluor ethylene) (PTFE = Teflon™) tubing
- medium supply bottle (not shown) with buffered medium (NBM/B27/AlaGlu/PenStrep + HEPES, L-histidine, N-acetyl-histidine and/or citric acid buffer mixtures, all ≤ 10 mM)
- tubing (PTFE, silicone, Tygon™)
- computer-controlled pinch-valve (Velleman relay card K8056, Profilab)
- gravity-driven medium exchange (rel. positions with respect to tabletop: supply: ≤ 520 mm, MEA: 320 mm, valve: 10 mm, waste: ≥ -300 mm)

Culture, perfusion and recording parameters

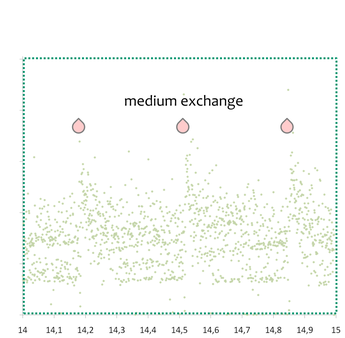
- hippocampal neurons (Sprague-Dawley rat ,E18, ~50,000 cells/dish)
- poly-D-lysine (0.1 mg/ml), laminin (5 µg/ml) as adhesion mediators
- cultures were kept in a standard 37°C/5%CO_2 incubator for the first 7 days; thereafter, they were transferred to the amplifier-embedded heating plate @ 36.5°C with an uncontrolled, but constant T-gradient through the culture dish
- timed perfusion every 8 hours (~200 µl exchange ; total medium volume: ~1.2 ml).
- time-lapse pictures every 5 minutes between day 7 and day 39 *in vitro*
- threshold spike extraction (5.5 StdDev from p-p noise, no spike sorting)



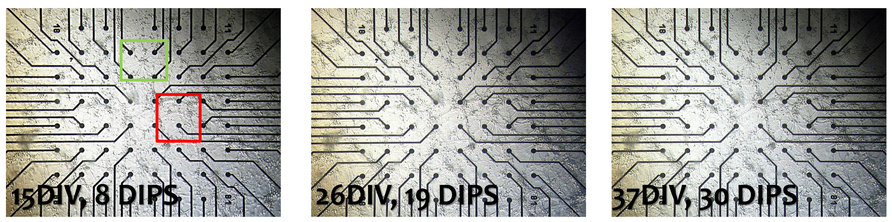
Color-coded cumulative firing and burst distribution on the 8x 8 MEA for 32 perfusion days (DIPS) in half-day steps. Activity focalization after 8 DIPS. The red box at day 22 indicates temporary power blackout (T-drift, no medium exchange) causing a notable change in activity distribution.



Activity evolution over 32 days on the lab bench after the first 7 days in a 5% CO2 incubator. Cumulative spiking activity on all 60 channels (1 min bins) is similar to the ASDR evolution reported by Wagenaar *et al.*, 2006. Gaps indicate temporary power blackout.

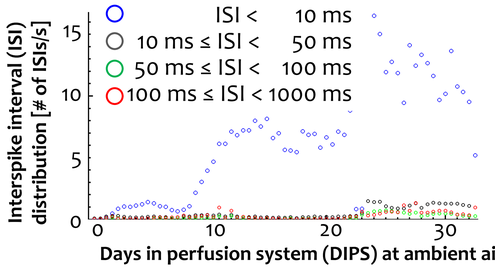


Zoom onto the activity on day 14: Demonstration of a) intra-day activity fluctuations and b) notable activity changes after each medium exchange (every 8 hours, ~1/6 of total culture container volume).



Comparative network morphology after 8 (15DIV), 19 (26DIV) and 30 (37DIV) days in the perfusion setup (DIPS). A slight interconnectivity rearrangement is visible during the first few days. The overall network morphology stays surprisingly stable after about 10 DIPS. This observation contrasts the dramatic changes and fluctuations in overall network activity (see graphs) and synaptic remodeling tendencies reported by Minerbi *et al.*, 2009. Rectangles: top green: exemplary constant region; central red: exemplary variable region. Electrode spacing: 200µm.

Temporal evolution of the array-wide interspike interval (ISI) distribution reflecting distinct developmental phases and a stepwise increase in interconnectivity density and/or entropy.



Summary

Automatization of cell culturing tasks outside of a standard incubator permit

- the **uninterrupted** (electro-)physiological and morphological **data collection** in long-term experiments and
- the **reduction of handling artifacts** (*e.g.*, temperature fluctuations, evaporation of medium, mechanical impact, drift in pH during culture transfer from the incubator to the experimental setup).

The intra-day activity of a hippocampal *in vitro* network is characterized by

- **drastic changes upon a medium exchange** and
- **basal activity fluctuations** even under constant environmental conditions.

The entire activity evolution is characterized by

- **day-to-day variations** with **local activity sinks,**
- **state changes** in activity that may reflect subsequent developmental maturity stages and be furthermore modulated by **external stimuli** reversibly (moderate nutrient flux) or irreversibly (*e.g.*, here the temporary system shutdown, more drastic change of chemical environment after delayed nutrient exchange),
- sparse but distributed **activity** in non-stimulated neural networks, which **tends to become spatially focalized and synchronized,**
- less active units that tend to disappear, while **more active units increase their activity even more.**

Outlook

- Finding the right culture conditions (pH buffer system) to start at 0 DIPS.
- Optimization of lid design for the more effective bubble-expulsion.
- Correlating electrophysiology to morphology at subcellular level.
- Comparing activity evolution between stimuli-deprived and –rich *in vitro* microenvironments.

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