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 osmolality when experimenting outside of an CO2 incubator, and activity may be biased by secondary handling artifacts (T-changes, mixing). Stabilizing the cell culture environment by a simple, gravity-driven, CO2-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

Firing (Hz)

Zeiss Axiovert 200

DIV 7 DIPS

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-minute, no medium exchange) causing a notable change in activity distribution.

Days in perfusion system (DIPS) at ambient air

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.

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 Mineri et al., 2009. Rectangles: top green: exemplary constant region; central red: exemplary variable region. Electrode spacing: 200μm.

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 in vivo 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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