Tracking the evolution of neural network activity in uninterrupted long-term MEA recordings

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Abstract

Most cell culture studies rely on taking representative, quasi-static data snapshots during limited time windows. To permit continuous experimentation, we devised an automated perfusion system based on microfluidic cell culture chambers for microelectrode arrays (MEAs). The design is based on a perfusion cap fabricated in replica-molding technology and on a hermetically shielded, gravity-driven perfusion mechanism. Network activity from neurons on MEAs was continuously recorded outside of a CO_2 incubator at ambient conditions over one month under stabilized temperature, osmolarity and pH conditions. Our analysis exemplarily focused on the evolution of paired spiking (PS) activity consisting of two spikes separated by 2 ms in spontaneously active hippocampal cultures. It started as early as 10 days *in vitro* (DIV) and triggered patterns of network-wide spreading activity that persisted until the end of the recording session lasting 32 days. We hypothesize that paired-spike activity is a general coding phenomenon that is conserved in spontaneously active *in vitro* networks. In lack of *in vivo*-like sensory stimuli, cultured neurons may use it to substitute for natural stimuli.

1 Introduction

The common approach of performing snapshot experiments bears several risks: each time, data is collected at different physiological cellular 'response states', which might furthermore be affected by non-reproducible variations in cell culture handling during their transfer from the incubator to the experimental setup. Furthermore, datasets are fragmented by lack of observation continuity. We therefore tested a simple gravity-driven perfusion concept to uninterruptedly record spike trains from neural cultures on MEAs over several weeks. The setup allowed us to investigate and compare the activity evolution in two separate cortical cultures over 32 days (hippocampal) and 59 days (cortical), respectively. We briefly discuss the evolution of paired-spike activity in the hippocampal culture.

2 Methods

2.1 Cell culture and perfusion setup

Hippocampal neurons (Sprague-Dawley rat, E17, ~50,000 cells/dish) were plated on Ti/TiN 30/200iR MEAs coated with poly-D-lysine/laminin (0.1 mg/ml; 5 µg/ml) and cultured in serum-free, pH-buffered medium (NBM, 2% B27, 2 mM AlaGlu, 100 U/ml PenStrep, 10 mM histidine/HEPES) following published protocols. They were kept 7 days in a humidified incubator before being transferred onto the 60 channel amplifier (Multi Channel Systems, Reutlingen, Germany) with T-control (36.5 °C). A polydimethylsiloxane (PDMS) perfusion cap based on a previously published design [1] featured ID 1.8 mm Teflon® tubing with in- and outlet silicone septa. Supply and waste Teflon tubing could be reversibly connected to these septa by OD 0.7 mm syringe needles. A pinch-valve (Velleman relay card K8056, Profilab) at the outlet tubing allowed timed gravity-driven medium exchange (~200 µl every 8 hours; relative positions with respect to tabletop: supply: 520 mm, MEA: 320 mm, valve: 10 mm, waste: - 300 mm; *Fig. 1*). Extracellularly recorded spikes were detected at 5.5 STDV from p-p noise and extracted (but not sorted) for time-stamp-based analysis.

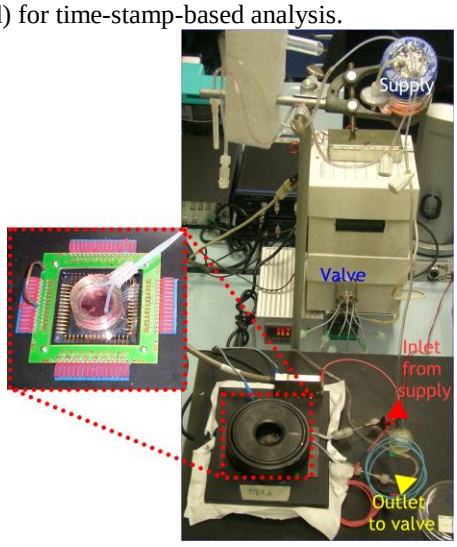

Fig. 1 *Perfusion setup and PDMS cap (inset).*

2.2 Paired spiking (PS) versus bursting activity at individual sites

We defined paired-spikes (PS) as the neural activity consisting of two spikes recorded from the same electrode that were separated by an interval of 2 ms followed by an inter-paired-spike interval (IPSI) larger than 50 ms. Bursting activity at individual sites was defined as events with more than 10 spikes separated by an interval lower than 100 ms followed by an interburst interval (IBI) larger than 200 ms.

3 Results and Discussion

In vivo, paired-spike (PS) enhancement plays a crucial role in information processing at different hierarchical stages within the nervous system [2, 3]. While spike pairing was very low at the early DIVs, it consistently increased after 3-4 weeks *in vitro* (WIV) (*Fig. 2*).

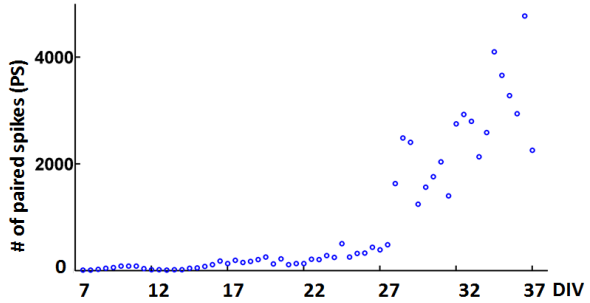

Fig. 2 *Network-wide number of paired spikes (PS) recorded at different developmental stages.*

Activity patterns consisting of PS separated by 2 ms were rarely encountered at the very beginning of the recordings. Instead, random isolated spikes rather than synchronized rapid firing dominated neural activity [4]. During the first 3 WIVs, the IPSI was very large (up to 170 s) and fluctuated highly with a low number of repetitions at individual sites, denoting that PS was not yet robust (*Fig. 3a & b*, first period).

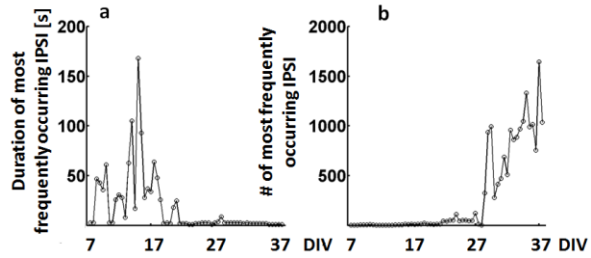

Fig. 3 a) *Plot of the duration of the most frequent IPSI in a certain 12-hours interval over DIVs: the distribution can be separated into two distinct periods with fluctuating high values during the first 3 WIVs and a second period with an almost constant value of 2-3 s.* ***b)*** *Inversely, the overall number of the most frequently occurring IPSI in 12 hours was low during the first period while it increased during the second period.*

After 3 WIVs, the IPSI dramatically decreased to 2-3 s and remained robust up to the end of the recording session. In the same period, the number of the most frequently occurring IPSI increased consistently. Furthermore, PS patterns that repeated at 2-3 s IPSI stayed associated to individual sites within the network as pointed out by the purple circles in *Fig. 4*. Moreover, in most cases, PS were recorded from those electrodes that also recorded bursting activity as shown in *Fig. 5a & b*. The network location with dominant PS and bursting activity could change over the days, though (*Fig. 5b*).

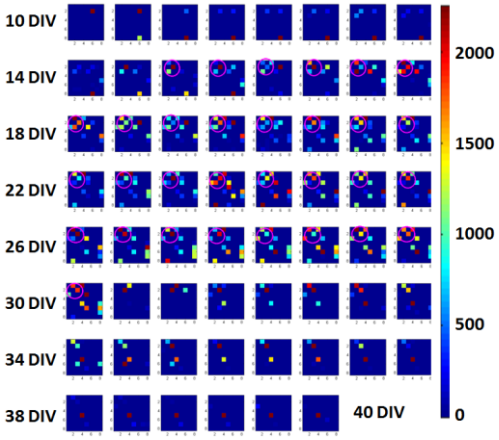

Fig. 4 *Cumulative number of PS and its distribution over an 8 x 8MEA at different DIVs in 12-hours intervals. The purple ellipse points out a robust PS pattern.*

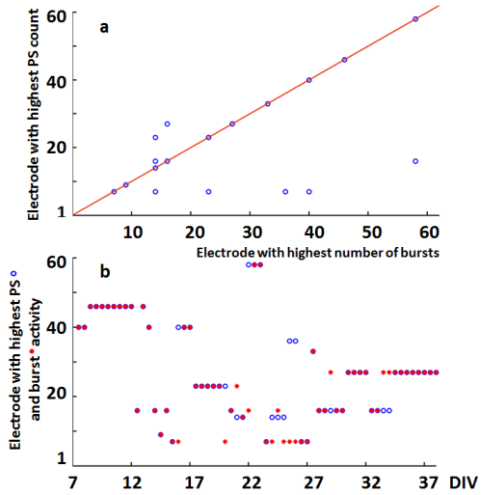

Fig. 5 a) *In most cases, the highest number of PS (y axis) were recorded from the same electrode from which the highest number of bursts (x axis) were recorded.* ***b)*** *The network location with highest PS (blue circle) and bursting (red dot) activity changed over time.*

4 Conclusions

We uninterruptedly recorded spontaneous activity from cultured neurons over 32 DIVs. Our findings show that PS activity becomes more robust after about 3 WIVs. PS might thus be used as an indicator for network maturity. The findings also suggest that neurons in culture use PS to replace the missing stimuli found in natural conditions within the nervous system. This hypothesis will be tested in future experiments.

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