Connectivity profiling and single-cell RNA sequencing to study homeostatic plasticity in hippocampal neuronal networks in vitro

Taehoon Kim(takim@student.ethz.ch), Julian Bartram, Manuel Schröter
Andreas Hierlemann
ETH Zurich, Department of Biosystems Science and Engineering, Basel, Switzerland

Abstract

Homeostatic plasticity represents an important set of regulatory mechanism to help maintaining stable neuronal circuit function and to flexibly adapt firing rates to changes in external drive (Turrigiano and Nelson 2004; Turrigiano 2008). Moreover, deficits in the ability to homeostatically regulate neuronal activity have been linked to a series of neurological and neuropsychiatric diseases (Frere and Slutsky 2018; Roselli and Caroni 2015). Although the intrinsic and synaptic mechanisms underlying homeostatic responses have been an area of active research (Turrigiano 2012), only few studies, so far, have attempted to link the electrophysiological properties of individual neurons to their gene-expression profiles following up- or down regulation of activity (Schanzenbächer et al. 2018; Schanzenbächer et al. 2016; Schaukowitch et al. 2017).

In the present study, we sought to gain a more integrated understanding of how the homeostatic firing-rate regulation of single neurons correlates to their functional connectivity in the network and their transcriptomic changes, respectively. To track the development and spontaneous activity of primary rodent hippocampal networks in vitro, we used high-density microelectrode arrays (HD-MEAs) (Müller et al. 2015). HD-MEAs enable to study neuronal function both at the network and subcellular scale (Obien et al. 2014) and, futhermore, provide the means for tracking extracellular multi-channel footprints of single neurons over extended developmental periods (Gong et al. 2016). Finally, combined with simultaneous patch clamp recordings, HD-MEAs are a suitable tool to map the synaptic connectivity of individual neurons (Jäckel et al., 2017).

Here, we tracked the emerging spontaneous activity of hippocampal neuronal networks across development and inferred their functional connectivity. Once the network had reached a mature devlopmental stage, we applied bicuculline and followed the homeostatic response of the full network and a subset of pre-selected neurons at high resolution. In a second step, we applied the Patch-seq protocol (Cadwell et al. 2017) to extract cellular content from cells of interest and to combine the identification of the electrophysiological phenotype of neurons with post-hoc single-cell RNA sequencing. We demonstrate that the response dynamics of individual neurons to perturbation are heterogeneous, which is also reflected in the mRNA extracted from these neurons. We propose that a combined analysis of neuronal activity, synaptic connectivity and gene expression patterns is a promising approach to study the mechanisms underlying neuronal homeostasis.

Acknowledgments: Financial support through the ERC Advanced Grant 694829 “neuroXscales”.

Keywords: *MEA, Neuronal homeostasis, scRNA-seq, patch-seq, spike-sorting,*

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