Genetic fingerprinting of identified neocortical interneurons based on oligocellular samples

Sándor Lovas1, Márton Rózsa1, Ildikó Piszár1, Ágnes K. Kocsis1, Attila Patócs2, László G. Puskás4 and Gábor Tamás1

1Research Group for Cortical Microcircuits of the Hungarian Academy of Sciences, Department of Physiology, Anatomy and Neurosciences, University of Szeged, Középosztaf 52, Szeged, H-6726, Hungary; 2Laboratory of Functional Genomics, Department of Genetics, Biological Research Center, Hungarian Academy of Sciences, Temesvári 62, H-6726, Szeged, Hungary; 3Endocrine Genetics Laboratory, 2nd Department of Medicine, Semmelweis University, Szentei u. 46, Budapest, H-1088, Hungary; 4Avidin Ltd., Szeged, Középosztaf 52, Szeged, H-6726, Hungary

Abstract
Understanding the complexity of the central nervous system can be facilitated by the analysis of specific neuron types, but gene expression analyses typically require amounts of RNA that can only be isolated from thousands of cells. Separation techniques that are based on cellular morphology or fluorescence properties result in heterogeneous cell populations, and expression profiling of these samples cannot be considered as trustworthy representations of cell type-specific transcription patterns. Whole-cell patch-clamp recording enables the electrophysiological identification and cytoplasm collection from single neurons, and subsequent purification and amplification of RNA from less than 20 cells of the same type provide a solution for the problems outlined above. Gene chip analysis performed on identified GABAergic neurogliaform cells comparing hyper- and hypoglycemic conditions resulted in the identification of the “Type I Diabetes Mellitus Signaling” pathway with the highest significance level among pathways affected by hyperglycemia, and these results were validated by traditional single cell qRT-PCR and digital PCR.

Using this experimental arrangement we compared the gene expression profiles of fast spiking axo-axonic and basket cells and identified hundreds of differentially expressed genes indicating the suitability of our oligocellular approach for determining whole expression profiles of distinct neocortical neuron types.

(A) Typical responses of a neurogliaform cell (NGFC), fast spiking basket cell (BC), pyramidal cell (PC) and a fast spiking axo-axonic cell (AAC) to hyperpolarizing and depolarizing current pulses recorded before harvesting their cytoplasms. (B) Light microscopic reconstruction of a fast spiking axo-axonic cell (some dendrites, red, axon, black) and a neurogliaform cell (some dendrites, black, axon, red).

(B) The most significant network of genes whose expression were highly affected upon hyperglycemia in neurogliaform cells. This network contains molecules involved in immune response (cytokines: complement component 5, 6 and 7, thrombomodulin 13 and 19, tumor necrosis factor 11, and molecules involved in signal transduction: receptor RET, guanosine coupled receptor member 141, and amyloid polypeptide, etc.) (B) Analysis of the same gene set resulted in the identification of the “Type I Diabetes Mellitus Signaling” pathway with the highest significance level among pathways affected by hyperglycemia in neurogliaform cells. The organization of this network containing results and its interacting partners are presented in this figure. Up- and down-regulated molecules are shown in red and green, respectively.

(A) Single cell digital PCR results of rps18 housekeeping gene and ins2 under high (10 mM) or low (0.5 mM) extracellular glucose concentrations in neurogliaform (NGFC), pyramidal (PYR) and fast spiking (FS) cells. Results of negative controls for both genes (ins2 - PC + rps18 - FS) are also shown. (B) Single cell qRT-PCR results of the ins2 gene under high (10 mM, black font) or low (0.5 mM, red font) extracellular glucose concentrations in neurogliaform (NGFC), pyramidal (PYR), fast spiking (FS) cells. Results of negative controls are also shown.

Flowchart showing the steps for our experimental pipeline.