Morphological, molecular and electrophysiological evidence for a human cortical specialized GABAergic cell type

Summary of Ph.D. Thesis

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INTRODUCTION

The human cerebral cortex is the most complex living structure. It is responsible for higher order cognitive functions such as perception, associative thinking, memory and consciousness. The cerebral cortex has two major cell types: neurons and glial cells. Cortical circuits consist of two major types of neurons: pyramidal cells and local interneurons. Cortical neurons integrate and process signals arriving from lower brain areas and other cortical structures within the framework of their connections. GABAergic interneurons play an important role in the synchronization of the activity of different cell-populations. While pyramidal cells mostly do not differ from each other in their morphological and physiological features, interneurons show diversity of great extent. It is possible to classify between them on the basis of their morphological, molecular and electrophysiological characteristics. Physiological classes could be correlated with certain morphological and molecular cell types.

Understanding the cellular and circuit organization of the neocortex, the substrate for much of higher cognitive function, has been intensely studied since Ramón y Cajal. However, conservation of cellular and circuit principles in human cortex is assumed but largely untested to date. Indeed, there is evidence for substantial neuronal differences between rodents and human; for example, distinct membrane and synaptic properties and dendritic complexity of human neurons might contribute to human-specific signal processing. With the mouse cortex as the dominant model for understanding human cognition, it is essential to establish whether the cellular architecture of the human brain is conserved or whether there are specialized cell types and system properties that cannot be modeled in rodents.

AIMS

To date, only a fraction of human neocortical cell types are described, thus, we set out to identify potentially novel cell types of human neocortex focusing on layer 1.

Our aim is a detailed

- morphological,
- molecular,
- electrophysiological and
- functional characterization of a neuron type that may have been identified.

METHODS

All procedures were performed according to the Declaration of Helsinki with the approval of the University of Szeged Ethical Committee.

Slice preparation

We used neocortical tissue surgically removed from patients (n=32, aged 47±16 years) over a course of 5 years as part of the treatment protocol for aneurysms and brain tumors. Tissue blocks were removed from prefrontal, temporal, and parietal areas. Blocks of tissue were immersed in ice-cold solution containing 130 mM NaCl, 3.5 mM KCl, 1 mM NaH_2PO_4, 24 mM $NaHCO_3$, 1 mM $CaCl_2$, 3 mM $MgSO_4$, 10 mM d(+)-glucose, saturated with 95% O_2 and 5% CO_2 in the operating theatre. Slices were cut perpendicular to cortical layers at a thickness of 350 μm with a vibrating blade microtome (Microm HM 650 V) and were incubated at room temperature for 1 h in the same solution.

Electrophysiological recordings

The solution used during recordings differed only in that it contained 2 mM $CaCl_2$ and 1.5 mM $MgSO_4$. Somatic whole-cell recordings were obtained at approximately 36°C from up to four concomitantly recorded cells visualized by infrared differential interference contrast videomicroscopy. Signals were filtered at 8 kHz, digitized at 16 kHz, and acquired with Patchmaster software. Micropipettes (5–7 MΩ) were filled with a low $[Cl]_i$ solution containing 126 mM potassium-gluconate, 4 mM KCl, 4 mM ATP-Mg, 0.3 mM GTP-NA_2, 10 mM HEPES, 10 mM phosphocreatine, and 8 mM biocytin (pH 7.20; 300 mOsm). Presynaptic cells were stimulated with brief (2–10 ms) suprathreshold pulses delivered at >7-s intervals, to minimize intertrial variability. For pharmacological experiments, 10 μM gabazine and 5 μM 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-1-piperidinyl-1H-pyrazole-3-carboxamide (AM251) were applied. Membrane properties of human neurons did not show significant changes for up to 20 h after slicing, but recordings included in the analysis were arbitrarily

terminated 15 h after slice preparation. Data were analyzed with Fitmaster and Origin 7.5. Data are given as mean ± s.d.. The Mann–Whitney U test was used to compare datasets; differences were considered significant if $P<0.05$.

Firing classification analysis

First, a set of n=200 electrophysiological features was calculated for each cell identified based on light-microscopic investigation of the axonal arbor. Then a wrapper-feature selection method using Support Vector Machine (SVM) was used on the cells (RCs: n=55; non-RCs: n=52) to find the best feature set separating the group of RCs from the group of other cells. The best feature set consisted of two features: the maximal s.d. of interspike intervals (ISI SD) and the amplitude of sag in response to hyperpolarization (–100 pA). Sweeps with <5 spikes were discarded for the calculation of ISI s.d. The sag value was calculated as the ratio of the voltages at the onset of the hyperpolarizing step to those during steady state.

Measurement of impedance profile

The impedance profile was determined by sinusoidal current injections using a standard exponential chirp pattern (0.2–200 Hz, 10 s duration) generated with Patchmaster (HEKA). Measurements (7–10 traces per cell) were made at resting membrane potential, and the peak-to-peak amplitude of the command current waveform was tuned between 40 and 100 pA to test subthreshold voltage responses. The impedance profile (Z) was determined for each trace by calculating the fast Fourier transform (FFT) of the voltage response and dividing by the FFT component of the corresponding command current, and then the impedance profiles were normalized to the value at 200 Hz. After anatomical identification of the recorded cells, the dataset was pooled on the basis of three defined cell types, and then the averaged impedance was plotted against input frequency. For statistical comparison of the impedance profiles, four parameters were considered: impedance at lowest frequency ($Z_{0.2Hz}$); resonance magnitude (Q, the impedance magnitude at the resonance peak, i.e., the maximal impedance value divided by the impedance magnitude at the lowest input frequency of 0.2 Hz); and the frequency at maximum impedance (f_{max}).

Two-photon calcium imaging

Structural labeling of RCs was based on 40 μM Alexa Fluor 594. We also applied 100 μM of Oregon Green 488 BAPTA-1 to measure intracellular Ca^{2+} dynamics of pyramidal cell dendrites in the intracellular solution (see above). Imaging with multiphoton excitation was performed using a modified Zeiss LSM7 MP (Oberkochen) two-photon laser scanning system and a FemtoRose 100 TUN (R&D Ultrafast Lasers, Hungary) titanium–sapphire laser with Finesse4 pumping laser (Laser Quantum) providing 100-fs pulses at 80 MHz at a wavelength of 820 nm. Fluorescence images were acquired through a 40× water-immersion objective (0.8 NA; Olympus).

Single-cell reverse-transcription and digital PCR

At the end of electrophysiological recordings, the intracellular content was aspirated into the recording pipettes by application of a gentle negative pressure while maintaining the tight seal. Pipettes were then delicately removed to allow outside-out patch formation, and the content of the pipettes (~1.5 μL) was expelled into a low-adsorption test tube containing 0.5 μL SingleCellProtect (Avidin Ltd.) solution to prevent nucleic acid degradation and to be compatible with the direct reverse-transcription reaction. Samples were snap-frozen in liquid nitrogen and stored or immediately used for reverse transcription. Reverse transcription of individual cells was carried out in two steps. The first step was performed for 5 min at 65°C in a total reaction volume of 7.5 μL containing the cell collected in 4 μL SingleCellProtect, 0.45 μL TaqMan Assays, 0.45 μL 10 mM dNTPs, 1.5 μL 5× first-strand buffer, 0.45 μL 0.1-mol/L DTT, 0.45 μL RNase inhibitor, and 100 U of reverse transcriptase (Superscript III). The second step of the reaction was carried out at 55°C for 1 h and then the reaction was stopped by heating at 75°C for 15 min. The reverse transcription reaction mix was stored at –20°C until PCR amplification. For digital PCR analysis, the reverse transcription reaction mixture (7.5 μL) was divided into two parts: 6 μL was used for amplification of the gene of interest and 1.5 μL cDNA was used for amplifying the housekeeping gene GAPDH. Template cDNA was supplemented with nuclease-free water to a final volume of 8 μL. We then mixed 2 μL TaqMan Assays, 10 μL OpenArray

Digital PCR Master Mix, and nuclease-free water (3 μL) to obtain a total volume of 20 μL, and the mixture was evenly distributed over 4 subarrays (256 nanocapillary holes) of an OpenArray plate using the OpenArray autoloader. Processing of the OpenArray slide, cycling in the OpenArray NT cycler, and data analysis were done as previously described (Faragó et al. 2013).

Histology and reconstruction

Following electrophysiological recordings, slices were immersed in a fixative containing 4% paraformaldehyde (for immunohistochemistry) or 4% paraformaldehyde, 15% (v/v) saturated picric acid, and 1.25% glutaraldehyde (for reconstructions) in 0.1 M phosphate buffer (PB; pH=7.4) at 4°C for at least 12 h. After several washes with 0.1 M PB, slices were frozen in liquid nitrogen then thawed in 0.1 M PB, embedded in 10% gelatin, and further sectioned into 60-μm slices. Sections were incubated in a solution of conjugated avidin-biotin horseradish peroxidase (ABC; 1:100) in Tris-buffered saline (TBS, pH=7.4) at 4°C overnight. The enzyme reaction was revealed by 3′3-diaminobenzidine tetra-hydrochloride (0.05%) as chromogen and 0.01% H_2O_2 as oxidant. Sections were postfixed with 1% OsO_4 in 0.1 M PB. After several washes in distilled water, sections were stained in 1% uranyl acetate and dehydrated in an ascending series of ethanol. Sections were infiltrated with epoxy resin overnight and embedded on glass slides. Three-dimensional light-microscopic reconstructions were carried out using a Neurolucida system (MicroBrightField) with a 100× objective. Reconstructed neurons were quantitatively analyzed with NeuroExplorer software.

Immunohistochemistry of biocytin-labeled cells

The recorded cells were first visualized with incubation in Cy3-conjugated streptavidin for 2 h, diluted at 1:400 in TBS. After examination by epifluorescence microscopy, the sections containing the soma of the labeled neurons were incubated in 20% normal horse serum in TBS to block nonspecific antibody-binding sites. Free-floating sections containing the soma were incubated in primary antibodies dissolved in TBS containing 0.05% NaN_3 for 72 h at room temperature (22°C). After several washes in TBS, the immunoreactions were visualized with A488- or Cy5-conjugated secondary

antibodies (1:500). The sections were mounted on slides in Vectashield. Images were taken by confocal laser scanning microscope (LSM 880, Zeiss) using a 40× oil-immersion objective (1.4 NA). After photography, the sections were demounted, washed in 0.1 M PB, and biocytin was visualized with the avidin-biotinylated horseradish peroxidase method described above. For quantification of positive and negative immunoreactions, we measured the mean fluorescence intensity of the immunostaining in the soma or in the axon terminals of the biocytin-filled rosehip cells and the fluorescence intensity of the background using the thresholding tool by ImageJ 1.48.

Electron microscopy

Axonal boutons of biocytin-filled rosehip cells and neurogliaform cells, identified based on distinctive electrophysiological properties and light microscopic investigation of the axonal arbor, were re-embedded and resectioned at 70-nm thickness. Digital images of serial EM sections were taken at magnifications ranging from 8,000× to 50,000× with a JEOL JEM-1400Plus electron microscope equipped with an 8-megapixel CCD camera (JEOL Ruby). Axon terminals were reconstructed in 3D and their volumes were measured using the Reconstruct software (http://synapses.clm.utexas.edu/). The areas of active zones of rosehip cells were measured at perpendicularly cut synapses, where the rigid apposition of the pre- and postsynaptic membranes was visible.

Statistics

No sample-size calculation was performed; all data were subject to statistical tests to decide whether parametric or nonparametric tests should be applied. All human specimens were controls (nonpathological) and were therefore allocated into the same experimental group. Randomization was not used. Data are presented as mean ± s.d.

RESULTS

Identification of a novel neuron type in the human cerebral cortex

We developed a dataset containing whole-cell-recorded, biocytin-filled interneurons in layer 1 of slices of nonpathological human samples of parietal, frontal, and temporal cortices. Unbiased recordings of layer 1 cell types yielded a set of interneurons with complete axo-somato-dendritic recovery. Light-microscopic examination of these cells identified neurons with previously described morphological features, for example, neurogliaform cells, as well as a previously undescribed group of interneurons with large, rosehip-shaped axonal boutons forming very compact, bushy arborizations. Due to the characteristic morphology, we named this cell type as rosehip cell. To our knowledge, interneurons with the phenotype of rosehip cells have not been identified previously in layer 1 of the cerebral cortex. Somata and dendrites of rosehip cells were confined to layer 1, with only distal dendrites occasionally penetrating layer 2. Proximal dendrites and somata of rosehip cells were decorated with stub-like spines. The axon of rosehip cells usually emerged from the basal part of the soma and gave rise to very compact, dense axonal trees predominantly arborizing in layer 1, with tortuous collaterals displaying spindle-shaped boutons with diameters not seen in other types of human layer 1 interneurons in our sample.

Quantitative morphological characterization of rosehip cells

Targeted recordings increased the number of rosehip cells in our database, and we quantitatively compared axodendritic parameters of randomly selected and three-dimensionally reconstructed rosehip cells to layer 1 neurogliaform and layer 2/3 basket cells. Bouton volume and the number of primary dendrites of rosehip cells were significantly different from those of neurogliaform cells. Maximal vertical extent of axon, total dendritic length, and dendritic node frequency of rosehip cells differed significantly from those of basket cells. Furthermore, interbouton interval, total axon length, and maximal horizontal extent of the axon were also significantly different.

Molecular phenotype of rosehip cells

To reveal the molecular profile of rosehip cells we performed immunohistochemistry on electrophysiologically recorded and anatomically recovered cells for known markers of GABAergic cell types. This identified that rosehip cells were immunpositive for cholecystokinin but negative for CB1 cannabinoid receptor, somatostatin and calretinin. Furthermore, rosehip cells were immunopositive for GABA and for chicken ovalbumin upstream promoter transcription factor II and negative for parvalbumin, neuronal nitric oxide synthase, neuropeptide Y, calbindin, and choline acetyltransferase. In parallel with the immunohistochemistry approach, researchers at the Allen Institute for Brain Science used single-nucleus RNA-sequencing to profile large numbers of nuclei from frozen postmortem brain specimens. Iterative clustering was used to group nuclei with similar transcriptional profiles, thereby identifying ten GABAergic interneuron subtypes in layer 1. The immunohistochemical profile of rosehip cells aligned closely with a single transcriptomic cell type, i5, which was similarly $GAD1^{+}$ CCK^{+} but $CNR1^{-}$ SST^{-} $CALB2^{-}$ $PVALB^{-}$. To more strenghten these results, we performed digital PCR for additional marker genes on cellular content extracted from individual rosehip neurons. As predicted by the transcriptome data, rosehip cells were positive for genes expressed, and low or absent for genes not expressed by cells in that cluster. These data strongly link the anatomically defined rosehip phenotype with a highly distinctive transcriptomic cell type signature that is found in human layer 1.

Intrinsic electrophysiological properties of rosehip cells

Anatomically identified rosehip cells responded to long suprathreshold current injections with stuttering or irregular spiking firing patterns when activated from resting membrane potential. Analysis of silent and suprathreshold periods during rheobasic firing of rosehip cells indicated that membrane oscillations and firing of rosehip cells were tuned to beta and gamma frequencies. The standard deviation of interspike intervals was higher in rosehip cells compared to neurogliaform or unclassified interneurons, indicating alternating silent and active periods during rheobasic stimulation. As described previously, human interneurons recorded in layer 1 had a

characteristic sag when responding to hyperpolarizing current pulses. However, the amplitude of the sag measured in rosehip cells exceeded that of neurogliaform cells or unclassified interneurons. Rosehip cells showed distinct impedance profiles relative to other layer 1 interneurons in response to current injections, with an exponential chirp. The resonance magnitude of rosehip cells was significantly higher compared to those of neurogliaform cells and unclassified interneurons. In addition, frequencies of maximal impedance in rosehip cells were significantly higher than in neurogliaform cells.

Function of rosehip cells in local microcircuits

To assess functional connectivity of rosehip cells in the local microcircuit, we established recordings from rosehip cells and then searched for potential pre- and postsynaptic partners without any cell-type preference. Rosehip cells receieve monosynaptic excitatory postsynaptic potential (EPSPs) from layer 2/3 pyramidal cells, and recive monosynaptic inhibitory postsynaptic potentials (IPSPs) from neurogliaform and other types of interneurons. None of the tested interneurons with somata in layer 2 were connected to rosehip cells. Rosehip cells rarely innervated postsynaptic interneurons and superficial layer 2 pyramidal cells. Rosehip cells outputs were predominantly directed toward layer 3 pyramidal cells. IPSPs elicited by rosehip cells were mediated by $GABA_A$ receptors, based on experiments showing blockade of IPSPs by application of the $GABA_A$-receptor antagonist gabazine. Rosehip cells in layer 1 might preferentially target pyramidal cells sending terminal branches of their apical dendrites to layer 1. Indeed, when randomly sampling the output formed by rosehip cells using serial electron microscopic sections, we found that axon terminals exclusively targeted dendritic shafts. Moreover, further ultrastructural analysis of postsynaptic dendrites suggested that these dendrites predominantly belonged to pyramidal cells. We found that rosehip cells were involved in single-cell-activated ensembles detected through disynaptic IPSPs triggered by layer 2 and layer 3 pyramidal cells and through polysynaptic EPSPs triggered by an axo-axonic cells. In addition rosehip cells also formed homologous electrical synapses between each other and established convergent heterologous electrical synapses with an unclassified layer 1 interneuron.

Preferential placement of output synapses on distal dendritic shafts of pyramidal cells reaching layer 1 suggest that rosehip cells might specialize in the control of dendritic signal processing. We found a correlation between the rise times of IPSPs arriving to the postsynaptic pyramidal cells and the distances of close axodendritic appositions from the somata. In dual recordings of synaptically connected rosehip cells to pyramidal cell pairs, we loaded rosehip cells with Alexa Fluor 594 to label presynaptic axons and filled the postsynaptic pyramidal cells with Oregon Green BAPTA 1 to structurally map the course of dendrites and to measure dendritic Ca^{2+} dynamics. Changes in Δ F/F in distal branches of the apical dendrites in layer 1 were consistently detected at multiple locations on the postsynaptic neurons, confirming action potential backpropagation into distal apical dendritic branches of human pyramidal cells. We triggered somatically evoked bursts in the pyramidal cells alone, for control, and together with bursts in the rosehip cell, in an alternating fashion. Rosehip inputs simultaneous with backpropagating pyramidal cell action potentials were effective in suppressing Ca^{2+} signals only at sites that were neighboring the putative synapses between the two cells. This suggests that rosehip cells specialize in providing tightly compartmentalized control of dendritic Ca^{2+} electrogenesis of human pyramidal cells, thereby enforcing inhibitory microdomains in dendritic computation.

DISCUSSION

Here we combine single-nucleus transcriptomics and slice physiology to study GABAergic neurons in layer 1 of human cortex and provide convergent lines of evidence for identification of a cell type with human-specialized features. Rosehip cells represent a type with a highly distinctive transcriptomic signature; a highly distinctive morphological, physiological, and connectional phenotype; and a strong correspondence between these properties. To our knowledge, a similar anatomical cell type has not been described in rodent. A complete comparison of all cortical cell types and assessment of relative similarities between cell types should be possible in the future as more comprehensive transcriptome data become available and linked to other cellular phenotypes in multiple species. Our study is based on a relatively limited number of multimodally characterized cells due to the scarcity of high-quality human samples, and further systematic analyses of human cell types in well-defined cytoarchitectonic areas using increased sample sizes are needed to substantiate further interpretations.

Addition of new human cell types, or specialization of existing types through major modification of cellular features, would be expected to alter circuit function and therefore cannot be studied in rodents. Dissimilarities of rosehip cells and other dendrite-targeting interneurons cannot be fully understood without further experiments testing these differences directly. Rosehip cells may be of particular importance in compartmental control of backpropagating action potentials and their pairing with incoming excitatory inputs. The sharp resonance in the theta-range detected in individual rosehip cells and its potential spread through gap junctions to a rosehip network could phase-selectively interact with long-range inputs similarly to mechanisms suggested (for example) in oscillation dependent memory consolidation. The function of neuron types specific to the human circuit could be important in understanding pathological alterations of network functions. For example, several highly selective markers for rosehip cells have been implicated as risk factors for neuropsychiatric disease. A better understanding of human cellular and circuit organization may help counteract the current lack of success in translating promising rodent results to effective treatment against human neuropsychiatric disorders.

PUBLICATIONS

Publication based on the dissertation:

TRANSCRIPTOMIC AND MORPHOPHYSIOLOGICAL EVIDENCE FOR A SPECIALIZED HUMAN CORTICAL GABAERGIC CELL TYPE

E. Boldog, T. E. Bakken, R. D. Hodge, M. Novotny, B. D. Aevermann, J. Baka, S. Bordé, J. L. Close, F. Diez-Fuertes, S.-L. Ding, N. Faragó, Á. K. Kocsis, B. Kovács, Z. Maltzer, J. M. McCorrison, J. A. Miller, G. Molnár, G. Oláh, A. Ozsvár, M. Rózsa, S. I. Shehata, K. A. Smith, S. M. Sunkin, D. N. Tran, P. Venepally, A. Wall, L. G. Puskás, P. Barzó, F. J. Steemers, N. J. Schork, R. H. Scheuermann, R. S. Lasken, E. S. Lein & G. Tamás

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IF: 21,126

Additional publications:

GABAERGIC NEUROGLIAFORM CELLS REPRESENT LOCAL SOURCES OF INSULIN IN THE CEREBRAL CORTEX

G. Molnár, N. Faragó, Á.K. Kocsis, M. Rózsa, S. Lovas, **E. Boldog**, R. Báldi, É. Csajbók, J. Gardi, L.G. Puskás & G. Tamás

The Journal of Neuroscience, 2014 Jan 22; 34(4):1133–1137

DOI:10.1523/JNEUROSCI.4082-13.2014, PMID: 24453306

IF: 6,747

DIGITAL PCR TO DETERMINE THE NUMBER OF TRANSCRIPTS FROM SINGLE NEURONS AFTER PATCH-CLAMP RECORDING

N. Faragó, Á.K. Kocsis, S. Lovas, G. Molnár, **E. Boldog**, M. Rózsa, L.I. Nagy, G. Tamás & L.G. Puskás

Biotechniques, 2013 Jun; 54(6):327-36

DOI: 10.2144/000114029, PMID: 23750542

IF: 2,948

Poster presentations:

ROSEHIP CELLS: AN EMERGING NEURON TYPE IN THE HUMAN CEREBRAL CORTEX; **E. Boldog**, G. Oláh, G. Molnár, M. Rózsa, A. Ozsvár, B. Kovács, J. Baka, S. Bordé, N. Faragó, Á. K. Kocsis, L. G. Puskás, P. Barzó, G. Tamás

ROSEHIP CELLS: A NOVEL NEURON TYPE IN THE HUMAN CEREBRAL CORTEX; **E. Boldog**, G. Molnár, G. Oláh, M. Rózsa, A. Ozsvár, B. Kovács, J. Baka, S. Bordé, N. Faragó, Á.K. Kocsis, P. Barzó, G. Tamás; FENS Regional Meeting, Pécs, 2017.

SYNTHESIS AND RELEASE OF INSULIN BY INTERNEURONS OF THE CEREBRAL CORTEX; G. Molnár, N. Faragó, Á.K. Kocsis, M. Rózsa, S. Lovas, **E. Boldog**, É. Csajbók, J. Gardi, A. Patócs, L.G. Puskás & Gábor Tamás; Inhibition in the CNS, Gordon Research Conference, Les Diablerets, 2013.

DIGITAL PCR TO DETERMINE THE NUMBER OF TRANSCRIPTS FROM SINGLE NEURONS AFTER PATCH-CLAMP RECORDING; N. Faragó, Á.K. Kocsis, S. Lovas, G. Molnár, **E. Boldog**, L.I. Nagy, G. Tamás and L.G. Puskás; qPCR and Digital PCR Congress, Lyon, 2013.

IDENTIFIED SOURCES OF INSULIN IN THE CEREBRAL CORTEX; G. Molnár, N. Faragó, Á.K. Kocsis, M. Rózsa, S. Lovas, **E. Boldog**, É. Csajbók, J. Gardi, G. Tamás; Society for Neuroscience, New Orleans, 2012.

IDENTIFIED SOURCES OF INSULIN IN THE CEREBRAL CORTEX; G. Molnár, N. Faragó, Á.K. Kocsis, M. Rózsa, S. Lovas, **E. Boldog**, É. Csajbók, J. Gardi, L.G. Puskás & G. Tamás; 8th FENS Forum of Neuroscience, Barcelona, 2012.

DIGITAL PCR TO DETERMINE THE NUMBER OF TRANSCRIPTS FROM SINGLE NEURONS AFTER PATCH-CLAMP RECORDING; Á.K. Kocsis, N. Faragó, S. Lovas, G. Molnár, **E. Boldog**, M. Rózsa, L.I. Nagy, L.G. Puskás & G. Tamás; 8th FENS Forum of Neuroscience, Barcelona, 2012.

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DIGITAL PCR TO DETERMINE THE NUMBER OF TRANSCRIPTS FROM SINGLE NEURONS AFTER PATCH-CLAMP RECORDING; N. Faragó, Á.K. Kocsis, S. Lovas, G. Molnár, **E. Boldog**, M. Rózsa, L.I. Nagy, G. Tamás & L.G. Puskás; IBRO - International Workshop, Szeged, 2012.

DIGITAL PCR TO DETERMINE THE NUMBER OF TRANSCRIPTS FROM SINGLE NEURONS AFTER PATCH-CLAMP RECORDING; N. Faragó, Á.K. Kocsis, S. Lovas, G. Molnár, **E. Boldog**, M. Rózsa, L.I. Nagy, G. Tamás and L.G. Puskás; 3rd RNAi Research & Therapeutics Conference, Boston, 2012.

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GLOBAL GENE EXPRESSION PROFILE OF IDENTIFIED NEUROGLIAFORM INTERNEURONS IN THE NEOCORTEX; **E. Boldog**, N. Faragó, M. Rózsa, E. Vámos, Sz. Oláh, V. Szemenyei, S. Lovas, L.G. Puskás & G. Tamás; Magyar Idegtudományi Társaság XIII. Konferencia, Budapest, 2011.

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