Closing report Project K-128863:

Biophysical and ultrastructural properties of human cortical neurons

Cable properties of human neurons

We set out to directly measure the speed of signal propagation in neurons of individual human and rat L2/3 pyramidal cells and applied experiments-based models to identify cellular and subcellular properties involved in controlling neuron-to-neuron propagation delays. Our integrative experimental and modeling study provides new insights into the scaling rules that enable to preserve information processing speed albeit the much larger neurons in the human cortex. To investigate electrical properties of human dendrites action potentials we have done paired recordings from single neurons by patching soma and its dendrites simultaneously and triggered somatic and/or dendritic action potentials to measure the propagation velocity directly. We have collected data from pyramidal cells and interneurons from both rat and human samples. According to the cable theory increasing axon and dendrite diameter determine the conduction velocity. Wider cables have less internal electrical resistance and thus longer space constants, which effectively facilitates the electrotonic spread of the action potential. We performed several experiments to confirm influence of parameters on signal propagation in human dendrites: the membrane capacitance and membrane thickness, the influence of ion channels able to trigger active conductance, the dendritic microstructure. To strengthen our results, we recruited Idan Segev (Hebrew University, Jerusalem) and Attila Szűcs (ELTE) into a collaboration to generate models based on reconstructed morphologies of actual electrophysiological measurements and perform model-based measurements. Manuscript has been completed and submitted to Science Advances as part of the "BICCN Human and NHP single cell analysis" package also the manuscript has been published on biorxiv.org:

Gáspár Oláh*, Rajmund Lákovics*, Sapir Shapira*, Yoni Leibner, Attila Szűcs, Pál Barzó, Gábor Molnár*, Idan Segev*, Gábor Tamás*, 2022 Accelerated signal propagation speed in human neocortical microcircuits. www.bioRxiv.org

Author status: shared senior author

Plasticity in neural network of the human cortex

One of our goals was to estimate the change of quantal parameters during LTD however this task poses considerable technical challenge which I already detailed in my previous reports. To ensure effective progress of our project we decided to continue the examination of the properties of chemically induced LTD on high amplitude excitatory synaptic connections. Applying group I mGluR agonist (S)-3,5-dihydroxyphenylglycine (DHPG) we start to searched modifying effect in synaptic efficacy to mGluRs activation on excitatory inputs on the postsynaptic interneuron. Activation of group I metabotropic glutamate receptors (mGluR) in the brain can mediate changes in membrane potential of neurons, synaptic strength, and overall activity in cortical circuits. Influence of mGluRs on neural activity in the cortex has been linked to several types of human neurological disorders however studies showing their effect in the human cortex are scarce. Paired recordings from synaptically-connected human supragranular cortical neurons in human brain slices enabled us to study the effect of mGluR activation on excitatory synaptic connections.

First, we started to record the chemically induced plasticity by monitoring the so-called complex events. Pyramidal cells in the human cortex can initiate polysynaptic network events so called complex events that can last for tens of milliseconds with single spikes (Molnár et al 2008, Szegedi et al 2016, 2017, Campagnola et al. 2022). These events can be composed of series of excitatory and inhibitory events or can consist of either excitatory or inhibitory events alone. To determine the influence of group I mGluR on network events in human we used acute brain slices which were prepared from surgically resected non-pathological samples of human cortical tissue. We recorded synaptically connected neuron pairs from 9 patients where we monitored di- and polysynaptic events evoked by the action potentials elicited in the other recorded cell with current injection and applied the group I mGluR agonist (S)-3,5- Dihydroxyphenylglycine (DHPG, 50 µM). Network events were initiated from pyramidal cells (PC). We examined the frequency and onset time of single-spike-triggered di- or polysynaptic events within a 60 ms time window following the trigger spike before and during application of DHPG and found that it induced mixed types of responses. This was unexpected since based on the literature DHPG application cause a uniform depression in hippocampal connections. In most of the recorded neuron pairs (46% of experiments) we could not detect any modification in the frequency or jitter of complex events following DHPG application. The remaining experiments showed two types of modulation: (i) depression of dIP responses detected in frequency decrease (18% of experiments) and (ii) facilitation of dIP occurence (36% of experiments) manifested in the shortening of the latency onset or decrease in onset jitter.

Next, we tested if glutamatergic monosynaptic connections arriving to basket cells and axo-axonic cells are sensitive to group I mGluR activation we performed paired recordings of pyramidal cells and interneurons (IN). With paired pulse stimulus we induced action potentials in the pyramidal cell and recorded evoked postsynaptic excitatory currents (EPSCs). Both presynaptic PCs and postsynaptic INs were whole-cell recorded with biocytin-containing intracellular solutions, allowing post hoc identification of the recorded cell types. In our recordings we grouped our recordings based on the identified postsynaptic cells. Identification could be done based on electrophysiological and morphological analysis of the postsynaptic cells, but due to technical difficulties could not strengthened with immunohistochemistry method. This way we grouped our experiments into 3 groups in which the postsynaptic cell was (i) fast spiking basket cell, (ii) axo-axonic cell and (iii) accomodating cells. In the first group for postsynaptic partner basket cells were targeted, which were chosen based on their intrinsic membrane and firing pattern properties: the characteristic non-adapting, sometimes intermittent firing pattern, low or moderate sag ratio, low or moderate action potential adaptation and narrow spike half width. Each recorded cell was addressed to morphological examination if it was fully recovered (n=5 out of 10) after a *post hoc* staining method which enabled us to detect axonal branches enveloping pyramidal cell bodies or terminating on perisomatic dendrites. To determine if group I mGlu receptor activation induces change in excitatory synaptic currents we applied the group I mGluR agonist (S)-3,5- Dihydroxyphenylglycine (DHPG, 50 µM) after recording control session repeating paired pulse protocol with 10 seconds intervals. We found that DHPG application in most of the experiments (n=7 out of 15) led to a significant increase (ctrl: 53.69±25.65 pA vs. DHPG: 77.26±38.56 pA,) in the amplitude of EPSCs without significantly changing the holding current of the postsynaptic cells (P=0.87). In our previous paper (Szegedi et al., 2018) we showed that type I mGluR activation cause the depression of high amplitude synaptic excitation. Accordingly, in n=2 cases in which the baseline amplitudes were the highest we found a significant decrease of the amplitude. In the rest of the experiments (n=6 out of 15) DHPG did not cause

significant change. We analyzed the paired-pulse ratio (PPR) of the evoked EPSCs in experiments with DHPG potentiation. Mean PPRs of the 6 experiments were not significantly changed after DHPG application (1.07±0.26 vs. 1.07±0.19). To confirm the involvement of group I mGluRs in the evoked synaptic strength changes we bath applied MPEP (25 µM) a potent selective mGluR5 antagonist and LY367385 (10 µM) an antagonist for mGluR1 in a separate set of experiments using the same protocol. We found that the application of the antagonists prevented the enhancing action of DHPG on evoked EPSC amplitudes (n=3, 20.82 ± 13.41 pA vs. DHPG: 17.45 ± 8.86 pA). In some of the experiments with DHPG induced EPSC amplitude increase we were able to carry out a washout session (n=4) with extracellular recording solution for at least 15 minutes and found that the increase in EPSC amplitudes by DHPG was reversible, and the amplitudes reduced back on average.

In some cases, we found cell pairs in which the postsynaptic interneurons were identified as axo-axonic cells (AAC, n=2) based on the fully recovered morphology of axon arborization and the presence of their characteristic axonal cartridges. Upon DHPG application both axo-axonic cell showed amplitude potentiation increasing amplitudes by 28% and 62%.

From our recorded pyramidal cell interneuron cell pairs, in some cases (n=4) we found postsynaptic cells with characteristic accommodating firing pattern (classical-accommodating cells, Gupta et al., 2000) without much action potential adaptation during high frequency firing. Morphological analysis of the single fully recovered cell revealed multipolar anatomy with spherically spreading axons and few sparse thin dendrites. Based on membrane and firing properties we could separate accommodating cells from basket and axo-axonic cell groups. Among the electrophysiological parameters the interspike interval adaptation ratio was the most distinct parameter between basket and accommodating cells. Then we examined whether excitatory inputs arriving to accommodating cells are affected by group I mGluR activation with observing paired pulse protocol induced postsynaptic EPSCs following DHPG bath application. We found significant depression of EPSCs in most of the experiments (n=3) and significant potentiation in one case.

To test whether group I mGlu receptors mediate synaptic strength in the rodent brain we repeated the same experimental protocol in Wistar rat model on induced excitation arriving to layer 2/3 fast spiking cells in the somatosensory cortex. Based an anatomical analysis we identified the postsynaptic interneurons with full recovery (7 out of 19 cells) as axo-axonic (n=1) and basket cells (n=6). Since separation of basket and axo-axonic cells based on membrane and firing properties only is difficult we merged the two fast spiking cell types in our analysis. We found significant potentiation of synaptic excitatory current after DHPG application in n=8 cases and no significant change in n=11 experiments.

At this point we plan further experiments to support our dataset: we want to increase the complex event related experiments and perform more pyramidal cell – basket cell monosynaptic pairs to test with DHPG with cannabinoid receptor antagonist to reveal more mechanistic insight.

We recently introduced our data in the FENS meetings (Forum of European Neuroscience, the Federation of European Neuroscience Societies - FENS, 2022 July 9-13, Paris, poster title: Joanna Sandle*, Gábor Molnár*, Martin Tóth, Katalin Ágnes Kocsis, Pál Barzó, Karri Lamsa, Gábor Tamás, Group I metabotropic glutamate receptor-mediated modulation of excitatory synaptic transmission shows interneuron specificity in the human neocortex, Author status: shared first author). With this research topic our student Joanna Sandle won a 1st price at the scientific student conference hold at

the University of Szeged (neuroscience section, April 27th, 2022). We are on to publish our results and we are preparing a manuscript.

We reported additional results linked to our proposal elaborating on functional aspect of cortical microstructure:

Microglia functional imaging

Microglia are the main immune cells in the brain and have roles in brain homeostasis and neurological diseases. Mechanisms underlying microglia–neuron communication remain elusive. In our collaborative work with Ádám Dénes's group we identified a structural and functional interaction between neuronal cell bodies and microglial processes in mouse and human brain. We aimed to test whether microglial process recruitment to somatic junctions was functionally linked with the activity of mitochondria in neurons. To study the functional relationship between microglial junction formation and activity of neuronal mitochondria, we assessed intracellular changes of the metabolic electron carrier nicotinamide adenine dinucleotide (NADH) in coronal slices of visual and somatosensory cortices from CX3CR1^{+}/GFP microglia reporter mice. To this end, we developed a technique for detecting two photon fluorescence illumination simultaneously with NADH autofluorescence in long term time-lapse recording. We found that recruited microglial processes came into close apposition with neuronal mitochondria. These processes stayed in the vicinity of neuronal mitochondria for couple minutes. Intracellular NADH fluorescence showed a granular pattern, indicating a mitochondrial NADH source which was corroborated with mitochondria-targeted CAG-Mito-R-Geco1 reporter construct. To search for somatic junction formation, we performed 2P imaging, which allowed us to track the movement of microglial processes and monitor cytosolic NADH in viable layer 2/3 neurons simultaneously. We detected apparent increases in NADH intrinsic fluorescence in parallel with the formation of somatic microglial junctions. By contrast, we found no changes in the mean intrinsic NADH fluorescence detected at neuronal somata contacted by microglial processes in P2Y12 receptor$^{-/-}$ tissue. Thus, microglial process recruitment to somatic junctions is linked to the metabolic activity of neuronal mitochondria through a P2Y12 receptor–dependent mechanism. These data confirmed that somatic microglia–neuron junctions have a specialized nanoarchitecture optimized for purinergic signaling.

These results have been published in the following paper: Csaba Cserép, Balázs Pósfai, Nikolett Lénárt , Rebeka Fekete, Zsófia I. László, Zsolt Lele, Barbara Orsolits, Gábor Molnár, Steffanie Heindl, Anett D. Schwarcz, Katinka Ujvári, Zsuzsanna Környei, Krisztina Tóth, Eszter Szabadits, Beáta Sperlágh, Mária Baranyi, László Csiba, Tibor Hortobágyi, Zsófia Maglóczky, Bernadett Martinecz, Gábor Szabó, Ferenc Erdélyi, Róbert Szipocs, Michael M. Tamkun, Benno Gesierich, Marco Duering, István Katona, Arthur Liesz, Gábor Tamás , Ádám Dénes: Microglia monitor and protect neuronal function through specialized somatic purinergic junctions, Science 367, 528–537, 2020

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Structural and functional properties of human cortical basket cell autapses.

Inhibitory autapses are self-innervating synaptic connections in GABAergic interneurons in the brain. We investigated GABAergic parvalbumin-expressing basket cells in layer 2/3 (L2/3) in human neocortical tissue resected in deep-brain surgery, and in mice as control. Most basket cell showed robust $GABA_A$R-mediated self-innervation in both species, but autapses were rare in non fast-spiking GABAergic interneurons. Light- and electron microscopy analyses revealed basket cell axons innervating their own soma and proximal dendrites. GABAergic self-inhibition conductance was similar in human and mouse basket cells and comparable to that of synapses from basket cells to other L2/3 neurons. Autaptic conductance prolonged somatic inhibition in basket cells after a spike and inhibited repetitive firing. Perisomatic autaptic inhibition is common in both human and mouse basket neurons of supragranular neocortex, where they efficiently control firing of the basket cells and therefore it is likely that autaptic GABAergic contacts undergo activity-dependent plasticity which regulates their strength.

These results have been published in the following paper: Viktor Szegedi, Melinda Paizs, Judith Baka, Pal Barzo, Gabor Molnar, Gabor Tamas, Karri Lamsa: Robust perisomatic GABAergic selfinnervation inhibits basket cells in the human and mouse supragranular neocortex, eLife, 2020

Author status: co-author

Summation properties of metabotropic postsynaptic potentials.

We have revealed ultrastructural phenomenon of dendritic GABA receptor distribution which forms different summation of ionotropic and metabotropic inhibitory responses. We characterized the combined ionotropic and metabotropic output of neocortical neurogliaform cells (NGFCs) using electrophysiological and anatomical methods in the rat cerebral cortex. Subpopulations of GABAergic neurons contribute to network mechanisms at different temporal windows and synchronized cells of particular interneuron types appear to fire in a stereotyped fashion. In general, this frequently results in coactivation of similar GABAergic inputs arriving to target neurons, which leads to postsynaptic summation of GABAergic responses synchronously activated by presynaptic cells of the same type. Our recent experiments confirmed coactivation of putative NGFCs in superficial cortical layers in vivo and revealed that GABA receptors are activated outside release sites. Triple recordings from presynaptic NGFCs converging to a postsynaptic neuron revealed sublinear summation of coactive ionotropic $GABA_A$ responses and linear summation of coactive metabotropic $GABA_B$ responses. Using simulation environment based on a 3D reconstruction of a postsynaptic dendritic segment we determined that summation properties were influenced by the relative location of $GABA_B$ receptors and GIRK channels when several presynaptic inputs converge. Our ultrastructural model design corroborates previous suggestions that the effect of $GABA_B$ receptors is more prominent on dendritic spines compared to dendritic shafts, having approximately twice the number of activated GIRK channels per $GABA_B$ receptor on spines versus shafts.
These results have been published in the following paper: Attila Ozsvár, Gergely Komlósi, Gáspár Oláh, Judith Baka, Gábor Molnár, Gábor Tamás, Predominantly linear summation of metabotropic postsynaptic potentials follows coactivation of neurogliaform interneurons, eLife, Jul 26, 2021

Author status: co-author

Automatic deep learning-driven label-free image-guided patch clamp system

Another project we succeeded is the automatization of patch clamp electrophysiology technique. Patch clamp recording of neurons is a labor-intensive and time-consuming procedure. We developed a tool that fully automatically performs electrophysiological recordings in label-free tissue slices in human and rodent brain slices. The automation covers the detection of cells in label-free images, calibration of the micropipette movement, approach to the cell with the pipette, formation of the whole-cell configuration, and recording. The cell detection is based on deep learning. The model is trained on a new image database of neurons in unlabeled brain tissue slices. The pipette tip detection and approaching phase use image analysis techniques for precise movements. High-quality measurements are performed on hundreds of human and rodent neurons. We also demonstrate that further molecular and anatomical analysis can be performed on the recorded cells. The software has a diary module that automatically logs patch clamp events. Our tool can multiply the number of daily measurements to help brain research. We published our results and continue the development of the system to increase the number of recording pipettes.

These results have been published in the following paper: Krisztian Koos, Gáspár Oláh, Tamas Balassa, Norbert Mihut, Márton Rózsa, Attila Ozsvár, Ervin Tasnadi, Pál Barzó, Nóra Faragó, László Puskás, Gábor Molnár, József Molnár, Gábor Tamás & Peter Horvath, 2021, Automatic deep learning-driven label-free image-guided patch clamp system, Nature Communications volume 12

Author status: co-author