Mechanism and modulation of neuronal circuit function in human cerebral cortex

Ph.D. Thesis

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2012

Szeged

INTRODUCTION

The human cerebral cortex is considered to be the most elaborated living structure. Its most recent part in an evolutionary sense is the neocortex in which neurons are basically organized into six layers. Although one can discriminate multiple anatomically and functionally different areas of the neocortex, these areas can be grouped into three major cytoarchitectural divisions corresponding to sensory, motor and association areas. The prefrontal cortex a prominent part of the association cortex is considered to be essential in several higher order cognitive functions. The basic organization of the neocortex is common among rodents, carnivores and primates however the relative ratio of the prefrontal cortex tends to extent as we move from rodents toward primates and especially in human it is the biggest among primates. According to the Hebbian hypothesis a discrete, strongly interconnected group of active neurons, called the cell assembly, represents a distinct cognitive entity and serve as the neural basis of cognitive processes. However most of our knowledge about neuronal functions and interactions are derived from experiments carried out in rodents, carnivores and non-human primates. In the human cortical circuits, only single neurons were characterized, interactions between neurons were not studied.

The neocortex is built up basically by two types of neurons. The most abundant ones are the pyramidal cells which use glutamate as neurotransmitter and behave as excitatory neurons. Their sequential and coordinated activity is considered to be essential for the flow of information throughout the cortex. However excitatory synaptic connections among single pyramidal neurons are weak and it is considered that tens of simultaneously active, convergent pyramidal cells are required to excite their postsynaptic pyramidal neurons and sequential activation of synchronously active pyramidal neurons are required for effective signal propagation in the cortex.

The other type is the group of GABAergic interneurons which release gamma-amino-butyric acid (GABA) from their axons and with a few exceptions exert inhibition on their postsynaptic targets therefore regulate the activity of pyramidal neurons and thus the flow of information. GABAergic interneurons are various in morphology and function. The most abundant ones among them are basket cells, which innervate the soma and perisomatic region of pyramidal neurons and effectively coordinate their activity. Another quite rare type of GABAergic neurons is the axo-axonic cell. Axo-axonic cells innervate exclusively the axon initial segment of pyramidal neurons. Axo-axonic cells are specific among GABAergic

neurons because they can excite pyramidal cell activity due to a more depolarized GABA reversal potential in the axon than in the soma.

Proper function of cortical circuits is based on the coordinated activity of glutamatergic and GABAergic neurons, however several subcortical neurotransmitter system could affect their operation. Serotonergic neurons are located in the brainstem however innervate the whole cerebral cortex. They use serotonin as a neurotransmitter which they mostly release into the extracellular space, because of the minority of classical synaptic contacts between serotonergic axons and their postsynaptic targets. Serotonin thus reach glutamatergic and GABAergic cells of cortical network mainly by volume transmission. However its extracellular concentration is tightly regulated by the activity of serotonin transporters which constantly remove serotonin from the extracellular space keeping the extracellular concentration of serotonin relatively low.

Disfunction of the serotonergic system is implicated in several psychiatric disorders such as depression, anxiety, schizophrenia or Alzheimer disease. According to the monoaminergic hypothesis of depression, emotional and cognitive disfuntcions in depressed patients is caused by insufficient serotonergic function of the cortex. Several line of evidences showed that decreasing the brain serotonin content resulted in decreased cognitive performances. Selective serotonin reuptake inhibitors (SSRIs) elevate the extracellular level of serotonin by inhibiting the serotonin transporters which normally would remove serotonin from the extracellular space therefore SSRIs are considered to reinstate cognitive functions by increasing the extracellular level of serotonin. Although selective serotonin reuptake inhibitors are the most widely prescribed drugs targeting the central nervous system with their well documented effects in cognitive, emotional and behavioral processes, our knowledge about how they influence cortical network function in human is still missing.

AIMS

Electrophysiological recordings of synaptic connections between identified neurons and their correlated light and electronmicroscopic analysis in the human cortex is still missing. This thesis is focused on the mechanism and modulation of cortical circuit function in human. Our main questions were:

1. How single pyramidal cells affect the activity of cortical networks in human?

2. How physiologically relevant concentrations of serotonin modulate pyramidal cell triggered network activity in human?

3. How therapeutically relevant concentrations of the SSRI fluoxetine modulate pyramidal cell triggered network activity in human?

METHODS

All procedures were performed according to the Declaration of Helsinki with the approval of the University of Szeged Ethical Committee. Human slices were derived from material which had to be removed to gain access for the surgical treatment of deep brain tumors from prefrontal, temporal and parietal regions with written informed consent of the patients (aged 18-73 years) prior to surgery. Patients with pre-medication with SSRIs or other drugs related to the monoamine system were excluded from the study. Anesthesia was induced with intravenous midazolam and fentanyl (0.03 mg /kg, 1-2 μg/kg respectively). A bolus dose of propofol (1-2 mg/kg) was administered intravenously. To facilitate endotracheal intubation, the patient received 0.5 mg/kg rocuronium. After 120 seconds the trachea was intubated and the patient was ventilated with a mixture of O2 -N2O at a ratio of 1:2. Anesthesia was maintained with sevoflurane at MAC volume of 1.2-1.5. Blocks of tissue were immersed into ice cold solution containing (in mM) 130 NaCl, 3.5 KCl, 1 NaH2PO4, 24 NaHCO3, 1 CaCl2, 3 MgSO4, 10 d (+)-glucose, saturated with 95% O2 and 5% CO2 in the operating theatre, sliced at a thickness of 350 μm with a vibrating blade microtome (Microm HM 650 V) and were incubated at room temperature for 1 hour in the same solution. The solution used during recordings differed only in that it contained 2 mM CaCl2 and 1.5 mM MgSO4. Recordings were obtained at ~36 ˚C from up to four concomitantly recorded cells visualized in layer 2/3 by infrared differential interference contrast videomicroscopy at depths 60-130 μm from the surface of the slice. Access resistance was monitored with -10 mV voltage steps in between experimental epochs and data collection was terminated if access resistance exceeded 30 MΩ. Signals were filtered at 8 kHz, digitized at 16 kHz and analyzed with PULSE software. Micropipettes (5-7 MΩ) were filled with a low [Cl] solution for discriminating GABAergic and glutamatergic events containing (in mM) 126 K-gluconate, 4 KCl, 4 ATP-Mg, 0.3 GTP-NA2, 10 HEPES, 10 phosphocreatine and 8 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. Membrane properties of human neurons or polysynaptic events did not show significant changes for up to 20 hours after slicing, but recordings included in the analysis were arbitrarily terminated 15 hours after slice preparation. Traces shown are single sweeps or averages or of 20-100 consecutive episodes. The time window in which effects were quantified was 60 ms. Time points of polysynaptic events used for rasterplots were determined as the onset of polysynaptic potentials with automatized macros written in Origin software (Microcal, Northampton, MA). The effects during drug application were tested following a 5 min wash-in and >25 mins was-out periods. Data are given as mean±S.D., Wilcoxon signed-rank test and Mann-Whitney U-test was used to compare datasets using Statistica software (StatSoft Inc., Tulsa, OK), differences were accepted as significant if $p<0.05$. Visualization of biocytin and microscopy was performed as described earlier (Szabadics et al., 2006; Molnar et al., 2008).

RESULTS AND DISCUSSION

Complex events initiated by individual spikes in the human cerebral cortex

Our group recorded the first dataset on the synaptic effect of identified human pyramidal cells on various types of postsynaptic neurons. The first part of my thesis is focusing on these pyramidal cell triggered synaptic events. Using whole cell patch clamp recording single action potential was elicited in layer II/III pyramidal neurons and their postsynaptic effects were monitored in simultaneously recorded neurons in acute human prefrontal slices. In addition to monosynaptic postsynaptic potentials, individual action potentials in presynaptic pyramidal cells initiated long-lasting (37±17 ms) sequences of events in the network lasting an order of magnitude longer than detected previously in other species. These event series were composed of specifically alternating glutamatergic and GABAergic postsynaptic potentials. Moreover, to compare the incidence of polysynaptic versus monosynaptic events in human and rat we searched for our library of recordings performed in the somatosensory and prefrontal cortex of the rat and we found that the ratio of the occurrence of single pyramidal cell triggered polysynaptic and monosynaptic events are greater by two orders of magnitude in human than in rat.

Singe pyramidal cell triggered polysynaptic events required selective spike-to-spike coupling from pyramidal cells to GABAergic interneurons producing concomitant inhibitory as well as excitatory feed-forward action of GABA. Correlated light- and electron microscopy revealed that strong synaptic coupling between pyramidal cells and interneurons are not due to the elevated number of synapses and can be achieved by few synaptic contacts. Pyramidal cells were shown to elicit disynaptic inhibitory postsynaptic potentials (IPSPs) by single spikes in rodents through the activation of an inhibitory interneuron. Basket cells innervate the perisomatic region of pyramidal neurons providing fast and powerful inhibition. We also found direct spike-to-spike coupling in 20% of the pyramidal-basket cell connections. Thus basket cells are a plausible candidate mediating disynaptic inhibition of pyramidal neurons. Postsynaptic action potential was also elicited in 33% of monosynaptically coupled pyramidal-axo-axonic cell pairs. While basket cells hyperpolarize their postsynaptic pyramidal cells, axo-axonic cells were shown to be able to depolarize their postsynaptic neurons, which are exclusively pyramidal cells, due to a more positive chloride reversal potential in the axonal than in the somatic compartment. In our samples single spikes of human axo-axonic cells elicited disynaptic excitatory postsynaptic potentials (EPSPs) and trisynaptic IPSP thus recruitment of axo-axonic cell by single spike of a pyramidal neurons is a feasible mechanism that mediate pyramidal cell triggered polysynaptic excitation and inhibition.

Our results show that activity flows in the human microcircuit in a stereotyped way. First-order spike elicited in a pyramidal neuron triggers second-order spikes exclusively in GABAergic neurons. Activation of axo-axonic cell are required to elicit third-order spikes which occur exclusively in pyramidal neurons since these cells are the sole target of axo-axonic neurons. Since axo-axonic cells innervate multiple pyramidal cells they could recruit the firing of several postsynaptic pyramidal cells in synchrony, and thus they can contribute to pyramid–pyramid spike-to-spike coupling. This network mechanism in concordance with the selectively strengthened synaptic connections in the human cortex could underlie the formation and segregation of cell assemblies proposed to be the building block of higher order brain functions such as cognition.

Modulation of single cell initiated network events by serotonin and fluoxetine in the human cerebral cortex

Physiological concentrations of serotonin and therapeutic concentrations of fluoxetine effectively reduced the occurrence of single cell initiated polysynaptic postsynaptic potentials in slices of the human prefrontal cortex. Analysis of monosynaptic connections revealed that serotonin decrease the amplitude of excitatory transmission from pyramidal cells to its postsynaptic targets suppressing the recruitment of postsynaptic inhibitory interneurons. Additionally, we found that serotonin didn't influence inhibitory transmission from basket cells to pyramidal cells and excitatory transmission from axo-axonic cell to pyramidal cells, since axo-axonic cell triggered disynaptic EPSPs still occurred with high extracellular concentrations of serotonin. Finally, fluoxetine also decreased the amplitude of monosynaptic EPSPs.

Detailed analysis of monosynaptic excitatory connections revealed changes in failure rate of synaptic transmission, in paired pulse ratio and in the coefficient of variation of EPSP amplitude, suggesting a presynaptic effect of serotonin. The most abundant serotonin receptors in the prefrontal cortex are the 5-HT_{1A} and 5-HT_{2A} receptors. Activation of 5-HT_{1A} receptors hyperpolarize neurons through opening of K^+-channels. In agreement with previous studies achieved by extracellular stimulation in the rat entorhinal cortex, the 5-HT_{1A} agonist 8-OH-DPAT fully mimicked the effect of 5-HT. 5-HT_{1A} and 5-HT_{2A} receptors are considered to have opposite effects on the excitability of postsynaptic neurons. However in our experiments the 5-HT_{2A} agonist alpha-methyl-serotonin also fully mimicked the effect of serotonin. Suppression of glutamate release with serotonin mediated by both of these two receptor types was shown in the cerebellum however the mechanism mediates the 5-HT_{2A} evoked suppression of glutamate release is not understood.

Fluoxetine, as other SSRIs, exerts their antidepressant action only after several weeks of treatment. However temporary increase in anxiety and threat processing was observed in some patients early in acute SSRI treatment before anxiolytic actions were seen. During chronic stress or in response to an uncontrollable stressor extracellular serotonin level in the prefrontal cortex increase suppressing its activity and the ability of the animal to come over the stressor. Decrease in prefrontal glutamatergic transmission may also lead to learning deficits as may occur in severe stress.

Our results don't provide explanation for the mechanism by which chronic SSRIs treatment may improve cognitive functions, however, their early acute effects could also be important in the development of their subsequent chronic effects. Both fluoxetine and serotonin influence plasticity of neuronal networks. Thus changes in prefrontal serotonin level may alter not just the temporal recruitement of cell assemblies but also their plastic structural

organization on a longer timescale which could serve as a basis for the chronic effects of the SSRIs.

ORIGINAL PUBLICATIONS DIRECTLY RELATED TO THE THESIS

Gábor Molnár, Szabolcs Oláh, **Gergely Komlósi**, Mikós Füle, János Szabadics, Csaba Varga, Pál Barzó, Gábor Tamás; Complex Events Initiated by Individual Spikes in the Human Cerebral Cortex, PLOS Biology; 2008, Sept, Vol. 6., Iss.9.
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IF: 34,48

Cumulative impact factor: 55,095

CONFERENCE POSTERS

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LECTURES

Gergely Komlósi, Gábor Molnár, Szabolcs Oláh, Anna Simon, János Szabadics, Csaba Varga, Pál Barzó, and Gábor Tamás; Chemical and electrical connections between identified neurons in the human cerebral cortex, MÉT, Szeged, Hungary, 2006