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Karolína Hrůzová

Dysfunctions of interneurons in schizophrenia

Dysfunkce interneuronů u schizofrenie

Bachelor´s thesis

Supervisor: prof. RNDr. Aleš Stuchlík, Ph.D., Dr.Sc.

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Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu

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Podpis

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Abstract

Schizophrenia is a serious neuropsychiatric disorder characterised by abnormal behaviours, perception and thoughts. It is a neurodevelopmental disease of two types of factors – genetic predispositions and environmental factors. The exact cause of schizophrenia remains, however, elusive. Interneurons are types of neurons, mostly exerting inhibitory action and their dysfunctions are associated with pathogenesis of schizophrenia. They are essential in the generation of neuronal oscillations, which play an important role in cognitive functions. Disruption of these oscillations (especially gamma band) could be paralleled by negative, positive or cognitive symptoms of schizophrenia. These interactions could be possible discerned with an innovative technique called optogenetics. Optogenetics is a combination of genetic and optical approaches to controlling activity of specific targeted neurons. With this method we can study animal models of schizophrenia with great insight, which could give us an explanation of abnormalities in behaviour caused by neuronal disruption.

Keywords: schizophrenia; interneurons; animal models; neuronal oscillations; optogenetics

Abstrakt

Schizofrenie je celosvětové rozšířené závažné neuropsychiatrické onemocnění, které se projevuje abnormálním chováním, percepcí a myšlenkami. Schizofrenie je považována za neurovývojové onemocnění, které je patrně vyvoláno jak genetickými predispozicemi, tak environmentálními faktory. Přesné příčina schizofrenie však dodnes zůstává neznámá. Interneurony jsou typy neuronů, které zřejmě hrají důležitou roli v mechanismech této nemoci, jelikož se podílejí na generování a udržování neuronálních oscilacích, které jsou prokázané jako nepostradatelné pro kognitivní funkce. Tuto problematiku nám můžou nastínit inovativní metody, např. optogenetika. Tato technika kombinuje genetické a optické přístupy za účelem ovládání specifických populací neuronů. Díky této metodě můžeme studovat animální modely schizofrenie s hlubokým vhledem, a mohou nám poskytnout vysvětlení abnormálního chování způsobeného neuronálním narušením.

Klíčová slova: schizofrenie; interneurony; animální modely; neuronální oscilace; optogenetika

List of abbreviations

AIS: axon initial segments

AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

CA: *Cornu Ammonis*

CCK: cholecystokinin

ChR2: Channelrhodopsin-2

CNS: central nervous system

DA: dopamine

EEG: electroencephalogram

GABA: γ-aminobutyric acid

GAD65: Glutamate decarboxylase, isoform 65 (protein)

GAD67: Glutamate decarboxylase, isoform 67 (protein)

ING: interneuron network gamma model

IPSP: inhibitory postsynaptic potential

LTD: long-term depression

LTP: long-term potentiation

NO: nitric oxide

NMDA: N-methyl D-aspartate

NMDA R: N-methyl D-aspartate receptor

MK-801: (+)-5-methyl-10,11-dihydro-5H-dibenzocycloheptene-5,10-imine maleate

PING: pyramidal interneuron network gamma model

PV: parvalbumin

SST: somatostatin

VIP: Vasoactive intestinal peptide

Contents

General Introduction

Schizophrenia is an incurable mental disorder affecting approximately 1% of the human population. This neuropsychiatric disorder is manifested by negative (as loss of functions), positive (as hallucinations or delusions) and cognitive symptoms. This thesis provides a simple explanation of the main theories of the induction of the disorder, such as the dopamine theory, the glutamate theory and the neurodevelopmental theory- all of them are dependent on genetic and environmental factors. Then I will describe, in general, two main structures involved in pathophysiology of schizophrenia – hippocampus and neocortex.

There are many possibilities how to characterise specific types of neurons - by their structure (anatomy) and function. Although one single classification cannot include both types of view (functional and structural) there is tendency to describe them together. One of the possibilities is to divide neurons into principal cells and interneurons. Principal neurons are mostly excitatory, and interneurons have mostly inhibitory influence on their synchronicity in firing. This synchronicity can be visible in the form of neuronal oscillations, whose types are characterised by their frequency as infra-slow, slow, delta, theta, alpha, beta and gamma brainwaves. Disruption of interneurons an this oscillatory activity are probably mechanisms causing abnormalities in schizophrenia. These deficits can be simulated using optogenetic method.

Schizophrenia

Schizophrenia is a mental disorder characterized by abnormal perception, thoughts and behaviours and problems with comprehending reality. It is a typical thought disorder that afflicts about 1% of the world's population. It seems, that disease affects men more frequently than women with a ratio 1.4 : 1[1] Although schizophrenia is not a fatal or neurodegenerative disease and does not kill patients by itself, morbidity is still high. Between 2 and 13% schizophrenic patients kill themselves (this proportion is twelve times higher than in the general population) and approximately 55% of patients attempt to commit a suicide in a lifetime scale [23].

The first description of disease (wrongly as a neurodegenerative) was put forward by Emil Kraepelin. He coined the name *dementia praecox* and classified it as a form of presenile dementia.
Schizophrenia literally means "split mind" and because of this it is often wrongly associated with dissociative identity disorder. The term schizophrenia was first used by Eugen Bleuler in 1908 however the "splitting" has been used to describe a rupture in thinking and emotions in this case. [4]

Symptoms of schizophrenia

Abnormalities in behaviour and thinking of people affected by schizophrenia are highly heterogenous. We can categorise these abnormalities into three groups – negative and positive symptoms and last, but not least, cognitive deficits. The initial diagnosis of schizophrenia is usually based on positive symptoms. These distinctions are "something added to behaviour that normal people do not have" – this includes hallucinations, delusions, formal thought disorder or bizarre behaviours. These symptoms respond well on neuroleptics, there is no intellectual impairment inherent to them and they are associated with elevated mesolimbic dopaminergic activity [5,6]

The second class of symptoms is characterised by the loss of functions - this class is called negative symptoms. These symptoms used to be considered secondary with respect to positive symptoms or consequences of the illness. Typical negative symptoms include poverty of speech, loss of drive, flattened affect, anhedonia, asociality, apathy, attentional impairment etc. These impairments do not respond well to medication, they are accompanied by structural changes in the brain and intellectual disability [6]. In contrast to positive symptoms we can easily illustrate these abnormalities using animal models. [5,7] Third class could insist of cognitive symptoms such as disruption of working memory and inability to organise effectively life. They are present early in the disease and persist throughout lifes.[8]

In the past, first and second group of symptoms were even considered types of schizophrenia. They included positive, negative and mixed schizophrenia.[9] But in fact positive and negative symptoms are interdependent and they exist in parallel. [10]

Schizophrenia and genetic predispositions

Schizophrenia appears to be at least partly heritable[11], but genetics is not the only factor in the development of this disease. Other external variables need to be taken into consideration.
The risk ratio for relatives is higher with increased proportion of shared genes, but the statistical determination of risk is just approximate. Monozygotic twins are genetically identical and if a phenotype of disease is dependent just on a genetic factor, then the concordance would be 100% and for dizygotic twins it would be 50%, but according to Kendler studies is 53% concordance for

monozygotic twins and 15% for dizygotic twins[11], with average heritability at 68%.[12] But Finnish twin study reported 83%. [13] There was not found any sex difference in the magnitude of heritability. [13]

Genetic mechanisms that produce schizophrenia are unknown, but multiple theories offer an explanation. One of these is that the disease has a homogenous pathogenic genotype with pleiotropic effects. Pleiotropy means when one gene has influence on two or more apparently unrelated phenotypic traits. Another explanation is that schizophrenia could be a phenotype resulting from the additive effect of multiple genes and environmental factors (in this interpretation there is no manifestation of clinical effect unless the threshold level is reached). A different theory is that schizophrenia could arise from heterogenous genetic effects (one type of damage can be caused by many types of different mutations). But it also can be multiple aetiologies causing a multiple phenotypes or multiple phenotypes with same etiology [14]

There are also sporadic (nonfamiliar) cases of schizophrenia, these are characterised, for inane, by de novo copy mutation[15], or sporadic mutations caused by paternal age [16], drug abuse, psychosocial causes, schizophrenia-like psychosis caused by trauma or epilepsy, complications during pregnancy and environmental factors such as prenatal and postnatal infections, maternal malnutrition or head injuries (see page 9).

Main theories

As mentioned earlier, there are multiple theories for explaining mechanisms and the development of schizophrenia, but in this part, I will describe three main theories.

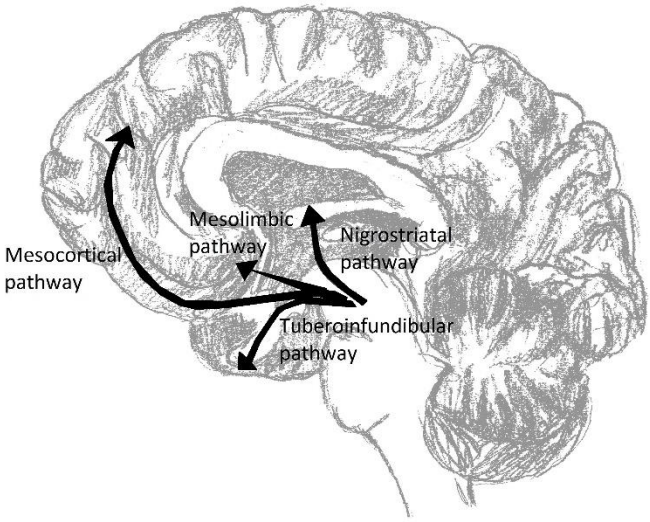
Dopamine theory

The dopamine hypothesis suggests that positive symptoms of schizophrenia are caused by overactivity of dopamine synapses and it was first explanation of the neurochemical principles of the disease [17]. This theory is based on pharmacological evidences – drugs that decrease dopamine activity (like phenothiazines) can reduce positive symptoms of schizophrenia and otherwise drugs promoting dopamine (DA) activity (like amphetamine) may produce or exacerbate these symptoms.[18]

The classical dopamine hypothesis postulates a hyperactivity of dopaminergic transmission at the D2 receptor, because symptoms respond better to D2 receptor blockade and all antipsychotic drugs are antagonists at the D2 receptor. [19]

First-generation antipsychotics (also known as typical antipsychotics) were developed in 1950. The first drug was chlorpromazine; later perphenazine and haloperidol were synthetized. These alleviate positive distinctions successfully, but they are not effective in treatment of negative symptoms.[20]. Unfortunately, when receptors are chronically blocked they become supersensitive, so after long-term exposure Typical antipsychotics have serious side-effects like tardive dyskinesia, tremor or

dystonia [18]. Second group of antipsychotics are second-generation antipsychotics (atypical antipsychotics). This group includes clozapine, risperidone and ziprasidone and others. These replaced typical antipsychotics, because their side effects (like weight gain or sexual dysfunction) are not so serious and long-term exposure side-effects are not known yet. This group of antipsychotics reduce both positive and negative symptoms but they are still not effective enough. [20]



Img. 1: Dopamine pathways (© Karolína Hrůzová)

Glutamate theory

Glutamate is a primary excitatory neurotransmitter in the mammalian brain. [21] Kim et al. found that the level of glutamate in cerebrospinal fluid is about half the normal value in patients with schizophrenia. [22] Also, in patients with schizophrenia there are structural changes in cortical and limbic regions of the brain. Changes in these regions are suggested to be characteristic of the disorder. [23] NMDA (N- methyl D-aspartate) antagonists (both competitive and non-competitive) causing positive symptoms-like psychosis in humans, can lead to similar behavioral changes in rodents. [24] Moreover, higher doses of these drugs were shown to induces degenerative changes in selected structures such as retrosplenial cortex or anterior cingulate (i.e. Olney´s lesions[25])

Animal models of transgenic manipulation of NMDA receptor subunits or pharmacological models of NMDA antagonists indicate a role of NMDA receptors in a wide spectrum of behaviour resembling schizophrenia. [26] Kim et al. [22] have offered an explanation – the glutamate hypofunction hypothesis. Hypofunction of the NMDA receptor can clarify major features of the disorder - negative and positive symptoms, structural changes and cognitive deterioration.

The advantage of this theory is the availability of animal models (the disease seems to be a by-product of the hominization process so it is probably impossible to create a completely equivalent model using laboratory animals [27]) Although the dopamine theory remains valuable, many aspects cannot be explained just by this theory, based on dopaminergic dysfunctions. Combination of dysfunction dopaminergic and glutamatergic system can explain better fundamental features of schizophrenia. The glutamate theory is a new promising target for research. [24] It is possible that D1 and D2 can regulate glutamate release from corticostriatal and corticolimbic circuits by tuning the output of glutamate neurons . [28]

Neurodevelopmental theory

Schizophrenia is certainly developmental disease, with typical onset from puberty to early adolescence. It is manifested by disruption in cognition and symptoms mentioned earlier. The disease is caused by combination of genetic and environmental factors. Illness manifests in adolescence or in adult age, but the origin can be tracked to neurodevelopment.

Keshavan et al. [29] described development of the disease in two hits – early brain development (prenatal and perinatal) and adolescence. Early developmental insults lead to dysfunctions of specific neural networks and may be the reason of premorbid symptoms observed in "pre-schizophrenic" patients. In puberty loss of plasticity and elimination of synapses can account for the emergence of symptoms. [30]

Many of epidemiological studies find relationship between onset of schizophrenia and obstetric complications. We can categorise them in three groups complications in pregnancy (pre-eclampsia, diabetes, bleeding), abnormal foetal development (small head, low weigh, congenital malformations) and complications during delivery (Caesar section, uterine atony, asphyxia).[31] These events can have an impact on development of schizophrenia combined with the genetic predisposition.

Another environmental factor can be viral infections and it can also have influence on development. [32]Significantly more schizophrenic patients were born during late winter and spring and there was found positive association between prenatal influenza infection (during the second trimester of pregnancy)[33]

Schizophrenia has neurodegenerative effect on the brain. Abnormalities we found in this regard, but due to the heterogeneity of the illness it is quite controversial to generalize it. Meta-analytic studies found regional volume decreases in hippocampus[34], thalamus[35] and frontal lobes[36].There were increased volume of lateral ventricles and decreased grey and white matter volume (between 2 and

3%) [37] [32].Post mortem imaging suggests that compromised structures of white matter could be a core feature of this disorder and should be targeted by direct pharmacological interventions. [38]

Main brain structures involved in schizophrenia

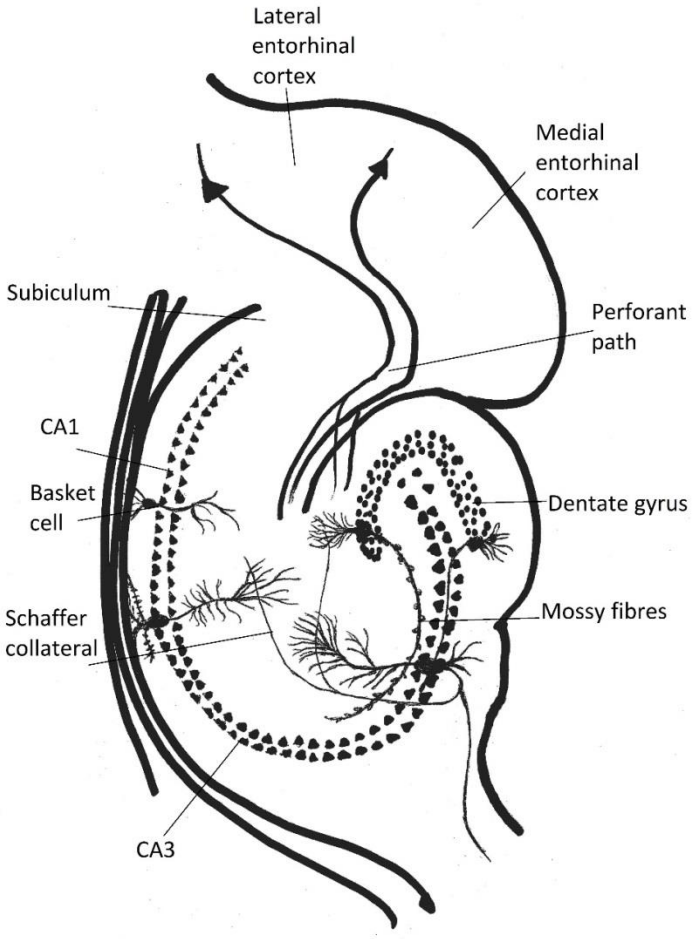
Hippocampus

The hippocampus is a major component of the vertebrate brains. The term “hippocampus” literally means a “seahorse” due to the resemblance in shape (in the human brain). It is a complex brain structure embedded in the temporal lobe (it is an extension of the temporal part of the cerebral cortex, archicortex), with major role in learning and memory.

Anatomy

The *hippocampus proper* has two major parts: Cornu ammonis (CA1-4; also Ammon´s horn) and the dentate gyrus, which are separated by the hippocampal sulcus and they are curving into each other. Below the sulcus is the subiculum. The entire hippocampal formation is composed of the CA, dentate, subiculum and the entorhinal cortex. [39] The hippocampus is a unique region due to the fact, that there is an ongoing neurogenesis in throughout the whole life in the dentate gyrus. [40,41]

CA fields are, in contrast to six layers of the cortex, composed of only three layers. The principal cell layer of Ammon's horn is the pyramidal cell layer. The subregions of the hippocampus are interconnected by two main neural circuits: the monosynaptic circuit. and the trisynaptic loop. The trisynaptic loop is composed from three synapses – 1. the first synapse is connection from perforant pathway from the entorhinal cortex onto dentate granule cells, 2. the second one is between mossy fibres from the granule cells of the dentate gyrus and the CA3 pyramidal cells, 3. and the third one is connection from the CA3 cells to CA1 pyramidal cells via Schaffer collaterals. The monosynaptic input is from the dentate gyrus and CA3 and from the entorhinal cortex to CA1.[42]



Img 2: ***Schematic of the hilppocampal formation in mammalian brain****. The perforant pathway leads from entorhinal cortex to dentate gyrus. Here, the granular neurons produce mossy fibers to pyramidal cells in CA3-area. From CA3, the Shaffer collateral leads to CA1. Last, but not least, there are commissural pathways from pyramidal cells of CA1 area leading to subiculum. © Karolína Hrůzová*

Pyramidal cells constitute a major subset of excitatory neurons in the hippocampus (about 90%), other excitatory cells include granule cells and mossy fibres - these neurons are the principal information processing structures in the hippocampus. The remaining 10% of cells are interneurons, primarily inhibitory, which are classified according to their morphological, physiological, molecular and synaptic characteristics into many sub-classes [43]. Basket cells are inhibitory GABAergic interneurons, which can also be found in CA3, where they receive excitatory outputs from pyramidal cells. There are numerous connections between pyramidal cells and interneurons, like basket cells, organised to local microcircuits and each microcircuit function as an individual piece of machinery, receiving, processing and transmitting information. Adapted from [44]

Function of the hippocampal formation

Hippocampus is essential for the formation of new memories and recalling episodic memories. Hippocampus encodes an emotional context signals coming from the amygdala. The amygdala is connected to the sensory-processing cortical regions, responding to emotional stimuli from the environment. Hence, when recalling episodic memory, memories of emotional events are prioritised. Moreover, in the process of consolidation of hippocampal-dependent memories, the amygdala has an influence on storing emotional memories – they are more important for survival, therefore they need to be consolidated.[45] Hippocampus, amygdala, fornix, mammillary bodies, medial septum and hypothalamus form one large complex called the limbic system, which is responsible for memory, behaviour and emotions.

Hippocampus and schizophrenia

Several studies show that the hippocampus is central to the pathophysiology of schizophrenia. Magnetic resonance imaging studies show a reduction of hippocampal size in schizophrenic patients[37]. This involvement is not secondary in the disorder nor caused by treatment [46]. Symptoms (like cognitive functions disability) in schizophrenia also indicate damage in the hippocampus. A significant reduction was found in of grey matter volume in the hippocampus, mostly in CA1 (where psychosis-related hippocampal CA1 hypermetabolism is observed too) and subiculum region[47]. The volume loss may also secondarily impact other brain structures which receive input from the hippocampus; this includes the temporal gyrus, insular cortex or parahippocampal gyrus, where reduction of grey matter has been also found. This abnormality could explain clinical manifestations such as mood and memory impairments in schizophrenics. [48] Experimental studies using animal models based on the neonatally-induced ventral hippocampal lesions showed comparable behavioural deficits in accordance with this theory [49]. These lesions lead to structural changes in prefrontal circuits that are accompanied by schizophrenia-like impairments which emerge only after puberty. Hippocampus-dorsolateral prefrontal cortex coupling is important for working memory processing. [50]

Neocortex

The neocortex (also known as isocortex or neopallium) is a complex, highly organized, six-layered surface structure of the mammalian brain. Neocortex means “new barn” and it is a relatively young part of the brain in evolutionary perspective. It contains many different neuronal cell types, responsible for cognitive functions, sensory perceptions and consciousness.[51]

In the human brain, neocortex is the largest part of the cerebral cortex (it accounts for almost 80 % of whole cortex[52]). Comparing to the rodents, primates neocortex is highly folded (gyrencephalic) with deep grooves and ridges, whereas rodent neocortex is smooth(lissencephalic) [53]. Most neocortical neurons (around 80%) are excitatory pyramidal (principal) neurons with relatively stereotypical anatomical, physiological and molecular properties. The remaining 20% of neurons here are interneurons, mostly inhibitory, with very diverse morphological, functional and molecular characteristics. [52]

As mentioned before, neocortex consists of six layers. The first layer is found most superficially, while layer VI is located most deeply. Embryonic development of the neocortex is a highly complex process. Neurons of the central nervous system (CNS) are generated in ventricular zone - a region near the lumen of the neural tube. Cortical neurons migrate to their final location near the pial surface, through the subplate and previous neuronal layers and stop under the marginal zone. The oldest neurons of the preplate are in layer I and in deep portions of layer VI. The remaining cortical layers are like "inside-out" with respect to their age, so layer II contains the youngest cells. [54]

Neocortex and schizophrenia

Neocortex is believed to be strongly involved in schizophrenia, especially heteromodal association neocortex, which consists of the prefrontal cortex, superior temporal gyrus and inferior parietal lobe (and other areas). These structures integrate information from multiple sensory modalities. It is involved in higher functions like working memory, executive functions, attention, language, etc., which are also disrupted in schizophrenia[55]. Below are some examples of neocortex functions which are disrupted in patients with schizophrenia.

The dorsolateral prefrontal cortex is involved in executive tasks, motivation, memory, planning and socialisation. Disruptions in this area can manifest in negative symptoms like asociality or emotional poverty[56]. Broca´s area is responsible for functions linked to speech production. Malfunctions can be manifested as auditory hallucinations[57]. Problems with paying attention can be caused by deficits in the inferior parietal lobe, which is the main structure involved in attention[58].

Types of neurons

Neurons are electrically excitable cells, which receive, processes and transmits information through chemical and electrical signals. They are structural and functional units of the nervous system. We can find this type of cells in all dipoblasts, in many shapes and varieties, according to their specialisation. A typical neuron is divided into three parts: soma, dendrites and axon. Soma (the cell body) contains a nucleus, dendrites are transmitting incoming electrical impulses and axon is

conducting action potential away from the nerve. Neurons are connected by synapses, junctions between the terminal buttons of the presynaptic cell and of the postsynaptic cell membrane. There are several main criteria, by which neurons can be classifies, In this thesis, I will focus on classifications by firing and division of neurons to principal cells and interneurons. Other classification systems can be found in textbooks and other resources

Pyramidal neurons

Pyramidal neurons, also known as pyramidal cells, are multipolar neurons found in the cerebral cortex. They are often found structures associated with higher cognitive functions. These cells are common in neocortex as well as in subcortical structures like the hippocampus or amygdala. They are characterized by their distinct apical and basal dendritic trees and the pyramidal shape of their soma. The body of these neurons are covered by thousands of dendritic spines, which integrate with other neurons mostly by excitatory glutamatergic synapses. Different dendritic locations seem to be specialized to perform different functions. They are projection neurons – it means that they often send their axons to long distances[59]. Most of pyramidal neurons are inhibited by GABAergic interneurons. [60] Notably, decreased density of dendritic spines on prefrontal cortical pyramidal cells is one of specific pathophysiological characteristics in schizophrenia. [61]

Granule cells

Granule cells are large group of neurons with small cell bodies. They are found in many structures, including the cerebellum, the olfactory bulb, cortex and hippocampus. In the hippocampus they are located in the gyrus dentate (principal cells of this region). These cells are highly arborized, and their dendrites are spread through the entire molecular layer and their bodies are visible in granular layer of the dentate gyrus. A unique feature of these cells is that they are generated from neural progenitor cells even in the adulthood. In rodent brain, neural progenitor stem cells are continuously dividing in the subgranular zone of the dentate gyrus. Granule cells migrate from there to the granular cell layer where they fully differentiate and begin to express their characteristic marker proteins[62].

Interneurons

Interneurons are types of neurons with axons and dendrites targeting a single area, this feature separates them from other principal neurons. While principal cells are mostly excitatory, interneurons have inhibitory function. Although interneurons in the spinal cord can inhibit principal cells by glycine, interneurons are, generally, most often inhibitory neurons that interact with their targets by GABA (gamma ammino-butyric acid, an inhibitory neurotransmitter) through the

hyperpolarization of a target cell. For addition to GABA, interneurons in the basal ganglia or the cortical areas may also release various neuropeptides such as cholecystokinin, somatostatin, enkephalins, vasoactive intestinal peptide (VIP, neuropeptide Y, etc. The major functions of interneurons are regulating firing activity and neuronal coordination and generation of rhythmic activity and control of excitatory inputs of principal cells.[63]

There are three main characteristics that distinguish interneurons from other neurons (e.g., pyramidal cells). 1. Interneurons can receive both excitatory and inhibitory synapses onto their bodies, 2. most mature inhibitory interneurons have aspiny dendrites and 3. axons of inhibitory interneurons arborize within the cortical column to project across the column but not into white matter (they do not contact distant regions of brain).[52].

Classification of interneurons

Interneurons are the most diverse cells within the forebrain. They have various morphological and physiological properties. In the past, classification of interneurons was mainly descriptive. Even though their diversity is limited by their developmental and functional criteria and there are many commonalities at a genetic, circuit or functional level, the classification of interneurons, nonetheless, remains complicated. [64]

Interneurons can be classified by morphological properties (such as shape of somas, arborization and polarity of dendrites or type of connections), by molecular features (transcription factors, neurotransmitters, calcium binding proteins, ion channels or cell surface markers) and by physiological characteristics (action potential measurements, firing patterns or post synaptic responses)[65]. For the purpose of this thesis I will stick to the molecular markers and classification by a type of inhibition.

Molecular markers of interneurons

Calcium-binding proteins

Calcium-binding proteins participate in calcium-signalling pathways in cells by binding Ca^{2+}. There are about 70 different proteins, that has been reported in various cell types and they appears to be rather specific [66]. Parvalbumin (PV), calbindin and calretinin are the main calcium binding proteins found in inhibitory interneurons and some pyramidal cells in mammalian neocortex. Calbindin- and calretinin-immunoreactive neurons are predominant in layers II and III, whereas parvalbumin immunoreactive neurons are in middle and lower cortical levels. [67] Apparently, they are important in

neurodevelopment, because calcium ions have been postulated to control cell division, cell movement and process outgrowth. [68] Regarding methodological aspects, calcium-binding proteins are brilliant neuronal markers due to the fact that antibodies against these proteins do not cross-react with each other. [68]

Somatostatin-expressing interneurons

Somatostatin is a growth-inhibiting peptide hormone. 30% of interneurons express this peptide. Martinotti cells, bitufted cells and regular-spiking non-pyramidal cells are examples of somatostatin-(SST-) expressing interneurons. All the SST-expressing interneurons are GABA inhibitory interneurons, mainly in the neocortex and hippocampus[69]. Activation of somatostatin receptor leads to suppression of firing, other words it has inhibitory effect. SST neurons are participating during decision making and learning. They probably facilitate a synaptic plasticity (see in appendix) by enhancing excitatory transmission).[70]

Perisomatic and dendritic inhibition

According to this classification, we can divide interneurons into two groups, depending on which area of target cell they innervate, dendritic interneurons and perisomatic interneurons. Dendritic inhibition is thought to control the efficacy and plasticity of excitatory synaptic inputs of principal cells and perisomatic inhibition is likely to control output and synchronise the action potential firing of larger groups of principal cells. [71] Perisomatic inhibitory cells target cell bodies, proximal dendrites and axon initial segments of principal cells. Chandelier or axo-axonic cells are connected to the axon initial segments (AIS) of principal cells and they form multiple contacts with each AIS target. They only contain parvalbumin (as marker). By synaptic connection with pyramidal cells they evoke a large-amplitude inhibitory postsynaptic potentials (IPSPs, indistinguishable from basket cells IPSPs)[72]. Chandelier cells share another feature one more thing in common with basket cells and it is firing our-of-phase with pyramidal cells during theta activity. [73]

Basket cells are another group of perisomatic inhibitory interneurons with multiple synaptic contacts. One basket cell carries, in average, 1500-2000 synapses with principal cells [63]. There are two groups of basket cells, differing by molecular markers they express and their neurochemical differences. The first group contains parvalbumin and the second group contains vasoactive intestinal polypeptide (VIP) or cholecystokinin (CCK) (but this type can contain both VIP and CCK). Morphological features for both types are similar (they all have a bitufted dendritic tree spreading through all layers). The difference is in spiking: PV basket cells are fast- spiking , whereas most CCK containing cells are regular spiking (their maximum firing rate is between 40 and 50 Hz)[74]

Dendritic inhibitory cells is a large group of interneurons and the most diverse group both morphologically and functionally, present in many different parts of the nervous system such as the cerebellum, olfactory bulbs and the cerebral cortex. Martinotti cells are interneurons specialised in projecting their axons toward layer I, where they inhibit tuft dendrites of pyramidal cells. They contain the neuropeptide somatostatin.[52] Bipolar cells are small cells, which can be excitatory by realising VIP or inhibitory by releasing GABA. They do not come into contact with as many principal neurons as other interneurons, only a few and mainly on basal dendrites of pyramidal cells[52].

There are also many other types of dendritic inhibitory neurons including double bouquet cells, neurogliaform cells, Schaffer collateral-associated cells, interneuron-specific cells, etc. In general, the functions of many dendritic interneurons are not know yet.[43]

Neural oscillations

Neural oscillations, also called brainwaves, are rhythmic activities of the central nervous system.
In general, oscillation is a repetitive movement between two points. Oscillations are characterised by a certain polarity, amplitude and a latency and oscillatory period. Neural oscillations can be generated by individual neurons or result from communication between neuronal ensembles (via synchronisation of their firing patterns). Synchronised activity of large group of neurons can turn results in macroscopic oscillations, detectable by electroencephalograms (EEG). Oscillation relates to a variety of neural processes, from plasticity, binding and consolidation to complex processes such as cognitive functions, regulation of emotions, memory and attention. Specific functions depend on where these oscillations are generated and their types.[75] Brainwaves with frequencies from approximately 0.05 to 500 Hz occurs in the mammalian forebrain.[76]

Infra slow waves (lower than 0.3 Hz)

Infra slow waves or slow cortical potentials seem to represent a basic cortical rhythm underlying higher brain functions. It is difficult, however, to detect and measure these waves. Consequently their real function is not known yet. [77] Vanhatalo et al. proposed a theory that infra-slow waves play a role in modulation of cortical excitability and provide a putative mechanism for aggravation of epileptic activity during sleep. [78]

Slow oscillations (0.3-1Hz)

This type of oscillation can be observed during slow-wave sleep and anaesthesia. It is a process of global depolarisation with excitation, hyperpolarisation and neuronal silence – these oscillations can also be described as "up and down" states of neuronal activity with low frequency. Slow oscillations arising from the prefrontal cortex during sleep are associated with consolidation of memory[79]

Delta oscillations (1-4Hz)

Delta waves are slower brainwaves with low frequency and rather high amplitude. They are associated with slow-wave sleep - mostly with the third stage of non-rapid-eye-movement sleep. Delta waves also play an important role in cognitive functions. It has been proposed that delta response is related to signal detection and decision making[80]. They might also be essential for attention and concentration, as well as motivation[81]. Knyazev et al. suggested a correlation between the putative side of delta generation and the cortical terminal field of the mesotelencephalic dopamine system.[82] Delta oscillations correlate with theta oscillations and can transfer to them.

Theta oscillations (4-7Hz)

Theta waves are usually present during rapid-eye-movement sleep (REM). Theta oscillations can be found in many areas of the cortex (mainly the limbic system, but also hypothalamus and neocortex), but the most studied theta waves occur in the hippocampus. Theta is a dominant rhythm in the hippocampus of lower mammals. The frequency in lower mammals is higher than humans (the range there is about 3 to 12 Hz)[83].Theta oscillations in the hippocampus are generated by medial septal nuclei (lesion to this area abolishes theta activity). In research of theta oscillations in cognition and memory, it is vital to acknowledge that sometimes, alpha and theta exhibit antagonistic behaviour. In some cases, when an increase in theta activity is required, alpha activity becomes desynchronised. For example, this occurs when the subject closes his or her eyes[84]. There is a link between theta and emotional states (emotions are also associated with memory; see above) - rhythmically synchronised theta activity between amygdala and CA1 can be observed during emotional arousal or fear conditioning[85] There are two main types of theta rhythm in the hippocampus- atropine resistant and atropine non-resistant. Theta activity sensitive to atropine occurs spontaneously even during anaesthesia, and theta resistant to atropine is associated with certain movements like walking, struggling and rearing.[86]

Alpha oscillations (7-12,5 Hz)

Alpha is the dominant frequency in the human scalp electroencephalography (EEG), except for irregular activity in delta range. Alpha frequency is positively correlated with cognitive performance and increases from childhood to adulthood but then decreases with old age. Alpha wave frequency is significantly higher in subjects with better memory and lowered in patients with dementia[83].

Alpha waves may stem from rhythmic fluctuations of inhibitory neurons, this could be the reason why the activity is desynchronised or supressed by processing of semantic information or visual stimuli – alpha activity has been suggested to play important role in attention control function.[87]

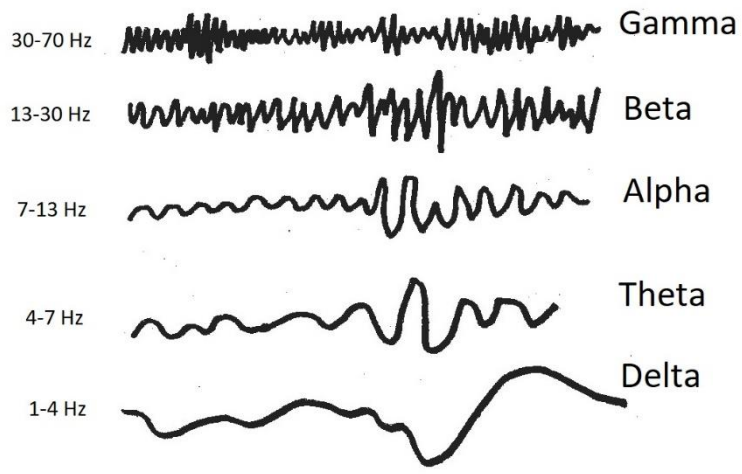
Beta oscillations (13-30 Hz)

These brainwaves can be recorded mainly in the somatomotor cortex, basal ganglia and cerebellar system. As we can infer from their location, they play role in motor activity regulation. Function of beta activity in the motor system is still unknown. [88]Beta waves have been observed during movement preparation and initiation. Beta oscillations significantly decrease during Parkinson's disease resulting from the reduction of dopaminergic control in the basal ganglia (this leads to instability of beta waves). Beta oscillations decrease during memory formation in the inferior frontal gyrus. This may help local circuits enhance information-coding capacity. Moreover, synchronisation in the beta frequency range occurs during episodic memory encoding[89]. Neurons in the prefrontal cortex dynamically synchronise and desynchronise during executive functions. Beta coherence is suggested to play a role in responsiveness to self- generated speech, that can be expressed as an auditory hallucinations[90].

Gamma oscillations (30-70 Hz)

Gamma waves are usually observed during wakefulness and sleep in many regions of the brain (mostly cortex). These oscillations are transient and typically stem from coordinated interaction of inhibitory and excitatory activity and commonly occur with irregular firing of single neurons. The frequency of oscillation and its magnitude is modulated by slower rhythms. Gamma rhythmogenesis is related to perisomatic inhibition.[91] There are two models of networks which have essential function in gamma waves. The first (older) model is I-I, oscillations are supposedly regulated by a neuronal network that consists of GABA inhibitory interneurons. Interneurons with stochastic inputs fire irregularly and in contrast to when signals are tonic, where interneurons fire with periodicity [92] The second model E-I is based on a reciprocal connection between excitatory pyramidal cells and inhibitory interneurons [91]. Several studies support the view of fast- spiking basket cells play a critical role in gamma oscillations[93]. Perisomatic basket cells induce both theta and gamma activity and due

to this fact researchers hypothesised that these cells are responsible for cross-frequency coupling. It has been well-documented that gamma oscillations are coupled with other slower waves within brain regions.[91]



Img. 3: Oscillations (© Karolína Hrůzová, adapted from Basar et al. [94])

Oscillations and schizophrenia

Despite mechanisms and functions of brain oscillations are not clearly understood, there is strong evidence suggesting brainwaves participate in cognitive processes. Many differences in the synchronicity or anomalies in schizophrenic patients have been investigated, during cognitive tasks, which might someday explain the symptoms and deficits caused by the disorder. These atypical waves and differences could also arise as consequences of anatomical deficits like widespread reduction of grey matter or abnormalities in neurotransmitter systems[90].

Dysfunction of neural oscillations and synchronicity is observed at many frequencies, but scientific research focusses primarily on gamma waves, because it could possibly explain behavioural disruptions and might clarify the role of GABA interneurons in the schizophrenia.

Schizophrenic patients show significant decrease in post-stimulus gamma response in frontal regions and the left hemisphere and an increase in right hemisphere signalling after auditory stimuli. [95] During a working memory task (working memory is strongly disrupted in schizophrenic patients) controls had increased gamma amplitude values depending on difficulty of working memory task, but schizophrenics had same the same increase of amplitude regardless on the task difficulty [96]. Hermann suggested, that negative symptoms correlate with decrease of gamma activity, whereas increase of that activity could correlate with positive symptoms[97]. A relationship between hallucinations and

occipital-response locked oscillations is apparent. Patients with hallucinations show increased activity in high frequency oscillations. [98]

Abnormalities in theta oscillations (during a working memory task) and beta oscillations may be associated with cross frequency coupling in gamma oscillations[99].

GABA interneurons and schizophrenia

As shown above, GABAergic interneurons support cortical circuits in functionality and maintain oscillations. They regulate excitation/inhibition ratio within neuronal circuits. They also seem to be essential during neurodevelopment – specifically, for the proper maturation of neural circuitry during postnatal development. Proper GABAergic inhibition during cortical maturation is necessary for the refinement of cortical circuity. Malfunction of these circuits caused by incorrect maturation of interneurons can lead to psychiatric disorders[100]. According to experimental and clinical evidences, dysfunction of proper GABAergic inhibition in the cerebral cortex can be manifest in pathophysiological states like epilepsy[101], mood disorders[102], autism spectrum disorders[103] and, importantly in the context of this thesis, schizophrenia[104].

Major physiological features of schizophrenia are disturbances in gamma frequency neuronal synchrony. Activation of fast spiking PV interneurons seems critical for generation of the gamma oscillation activity that may organise functional neuronal ensembles[105]. Optogenetic studies (addressed later) have shown that activation of fast spiking interneurons by light stimulus can selectively amplify gamma oscillations and in contrast pyramidal neuron activation amplifies only lower frequency oscillations[106].

A large amount of evidence supports the glutamate theory of schizophrenia. Many symptoms of the disease (both positive and negative) can be induced by non-competitive antagonists of the NMDA receptor (such as ketamine or MK-801)[107]. This finding corroborates the hypothesis of NMDA receptor hypofunction. Cortical GABAergic interneurons may paradoxically be crucial for NMDA receptor hypofunction. This hypothesis is supported by many findings. For instance, GABAergic interneurons are significantly more sensitive to NMDAR antagonists than pyramidal neurons[108]. Homayoun´s experiment with MK-801 on rats showed that NMDA receptor inhibition, in the prefrontal cortex, decreases the activity of putative GABA interneurons but increases the firing rate of the majority pyramidal neurons[109] .

GABA synaptic inhibition is a process whereby an inhibitory interneuron fires an action potential and triggers a release of GABA to the synaptic cleft from presynaptic vesicles. The neurotransmitter binds to a receptor on the postsynaptic cell and causes IPSP. This hyperpolarisation inhibits cell firing on the attached cell. Shortly after the end of inhibition, there is a bigger chance of firing spikes by the postsynaptic cell. This process is the reason for highly synchronous rhythmic action on pyramidal cells induced by interneurons (or reciprocal inhibition in an interneuronal network). An axon of each interneuron is linked to multiple synapses on the individual postsynaptic neurons. GABA-A receptor mediated inhibition is sufficient to generate network oscillations. This process, though seemingly simple, is complicated and it is highly possible that it is a key for understanding the molecular origins of this disease.[110]

GABA neurons produce an enzyme called glutamate decarboxylase (GAD). GAD catalyses the decarboxylation of glutamate to GABA and carbon dioxide. In mammalian brain, there are two isoforms of GAD: GAD67 and GAD65 (differentiated by their molecular weight). In mice, deletion of the gene encoding GAD67 results in a 90% reduction in GABA in the brain - such a big reduction is lethal[111]. On other hand, deletion of the GAD65 encoding gene has not got such a dramatic consequence. The reduction of GABA level is about 20% and animals with this deficit can survive[112]. Levels of mRNA coding GAD67 are significantly lower in the prefrontal cortex (but also in other regions of cortex) in the post-mortem studies of schizophrenic brains [113]. Expression of GAD65 seems normal or only slightly different in patients with schizophrenia [114].

Decreased levels of GAD67 in schizophrenia are variable across individuals; this phenomenon itself can reflect reduced cortical activity, which is secondary to other factors relevant to disorder with regard to its origin. Decreased level of GAD67 does not necessary support a glutamate theory, because expression is downregulated in response to reduction of GABA metabolism. GAD67 mRNA decreases in schizophrenia are mainly studied in interneurons. In approximately 50 % of parvalbumin interneurons, the GAD67 mRNA in not detectable and GAD67, itself, is markedly reduced in patients compared to health controls. The other types of interneurons like calretinin-containing interneurons do not appear to be afflicted in schizophrenia[115].

As I mentioned before there are two types of PV interneurons: chandelier cells and basket cells. Gamma oscillations require a fast decay of inhibitory activity of interneurons and chandelier cells appear to be too slow to drive a circuit in gamma frequency and they even do not seems to be coupled with gamma oscillations and GABAergic inputs to the axon initial segment appear to be

excitatory[116]. But they have an influence on theta oscillations and working memory, which is also disrupted in schizophrenia[117].

Basket cells inputs are fast-spiking inhibitory interneurons, which are highly sensitive to the pharmacological compounds that diminish gamma oscillations. There are two models of circuits explaining the role of basket cells rhythmic activity during gamma oscillations. Firstly, the interneuron network gamma model (ING) postulates that oscillation depends on mutual inhibition between interneurons. Secondly, the pyramidal interneuron network gamma model (PING) suggest that oscillations are dependent on the relationship between a pyramidal cell (as phasic excitatory output) and interneuron (as a source of feedback induced inhibition)[110]. These two mechanisms stabilize gamma activity in the brain via a resonance-induced gamma mechanism[118].

Despite myelination occurs mainly in long-distance white tracks, PV interneurons are also often myelinated. This myelination facilitates and accelerates conduction of action potential. Myelination abnormalities of these neurons have been observed both *in vivo* and *post mortem* in the brain of patients with schizophrenia. These abnormalities are also observed in drug-naïve patients in the first episode of the disorder[119]. In late adolescent age maturation and myelination of prefrontal cortex takes a place. Reductions of oligodendrocytes in hippocampus have also been observed[120]. Myelination abnormalities can result from disturbances of neuronal signalling. Fast-spiking basket cells are most sensitive to this phenomenon[121]. This abnormality can have an influence on disruption of the generation of gamma oscillations[122]. Also, pathophysiological reduction of PV interneurons has been observed in the prefrontal cortex, which is crucial for gamma oscillation activity[123].

Optogenetics

Optogenetics is a powerful innovative method used in neuronal systems both *in vivo* and *in vitro*. It combines genetic and optical methods to achieve starting or stopping of defined events in targeted cells (neurons, cardiac cells, stem cells). By this method we can control specific populations of neurons or even individual cells to induce excitation (depolarisation, resulting in firing) or inhibition (hyperpolarisation, resulting in cessation of firing). In contrast to electric stimulation by electrodes, one can genetically target a specific type of neuron (for example PV interneurons) or even individual neurons[124] and control the activity by light stimulation via photosensitive rhodopsins [125].

Bacteriorhodopsin is a microbial single-component light-activated ion pump. This protein is the evolutionary product of the bacterium *Halobacterium salinarum* (Archaea), which is living in the highly saline environments, which using the light as an energy source. Bacteriorhodopsin is a retinal

molecule, converting light energy to a proton gradient by photoisomeration and pumping chloride ions inside a membrane[126].

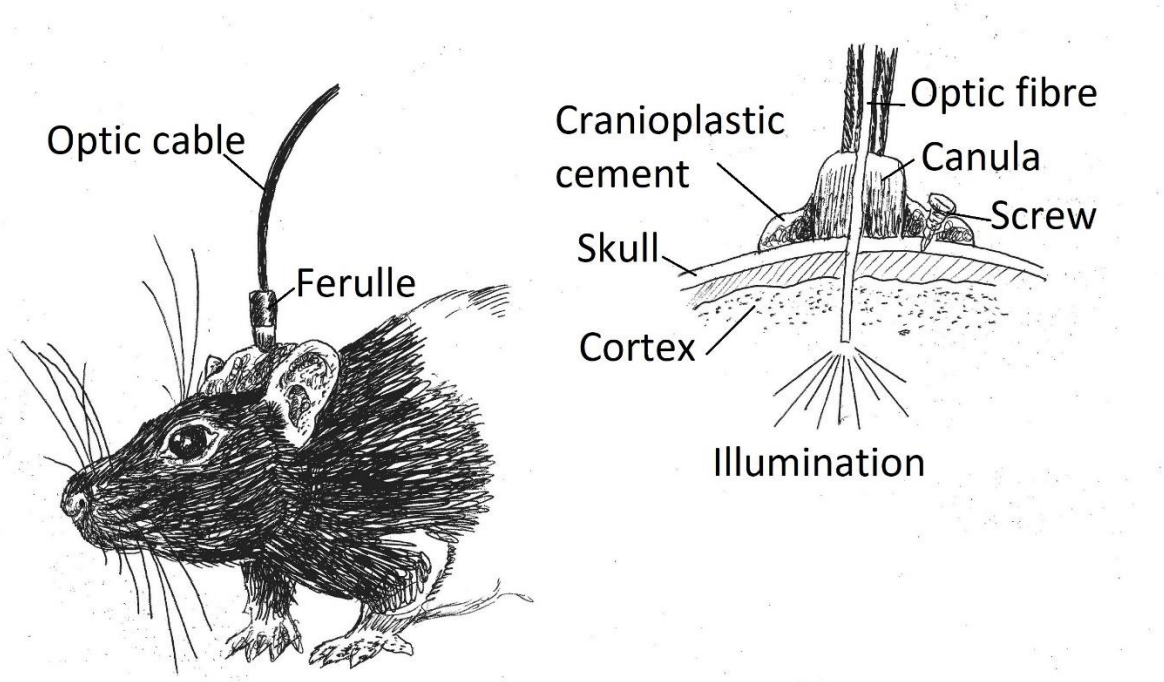
Many organisms developed a mechanism to sense electromagnetic radiation in their environment. Therefore, opsins can be divided into two groups – microbial opsins (type I) and animal opsins (type II). All opsins require a vitamin A-related organic cofactor called retinal. Microbial opsins can be used to add a photosensitisation property to non-light sensitive cells. Thanks to genetic engineering, there are a plenty of possibilities to use microbial opsins that respond to various wavelengths, and to incorporate them into a genome of targeted cells. The most often used opsins are channelrhodopsin (for depolarisation) and halorhodopsin (for hyperpolarisation).[127]

One of the major steps in this method is to deliver opsin-encoding plasmids into a cell. Generally, adeno-associated viruses or lentiviruses are used for this as vectors. The gene is integrated into a neuron, subsequently the cell expresses it. The problem with widespread use of the virus is the need for a high expression level to activate the neurons. Even with *in utero* electroporation or recombinant viruses, the number of channels is not sufficient[128]. For this reason, new genetic studies use Cre-expressing transgenic lines. Inserting virus into Cre recombinase-expressing cells by viral transfection activates a robust expression of these channels[129]. Another advantage of animals carrying Cre is that the virus only spreads in the target region of the brain, where stimulation of the neurons by light is required. PV-Cre transgenic mice or rats develop normally and do not exhibit any anatomical abnormalities.

Light stimulus is conducted to a targeted region by an optic fibre and an optic cable. The optic cable consists of optical glass fibre (one or more) and a protective tube. Conduction works based on the total internal reflection phenomenon. The optic implant (fibre) is connected to the cable by an optic ferulle. Illumination depends on opsin characteristics, for example channelrhodopsin-2 is activated by blue light (470 nm), whereas halorhodopsin is activated by orange light (~597 – 622 nm). Neurons are controlled by light pulsing, usually pulse is used in the range of 1 to 500 ms and frequency is used in the range of 1 to 100 Hz[130]. Higher intensity of illumination, or continuous light can damage a tissue by heat[131].

Another way combining optical control of cells and registering activity is an optrode. Optrode is a combination of optogenetic stimulation and electrophysiological recording. It is used to validate excitatory and inhibitory effects on targeted neurons. This is a new method in electrophysiology for recording and identification of specific neurons by testing their responsiveness to light. After this identification, activity of these neurons can be tracked to determine their behavioural correlates[132].

To avoid damage to the tissue, the fibre should be placed more than 200 µm from the recording electrodes[133].

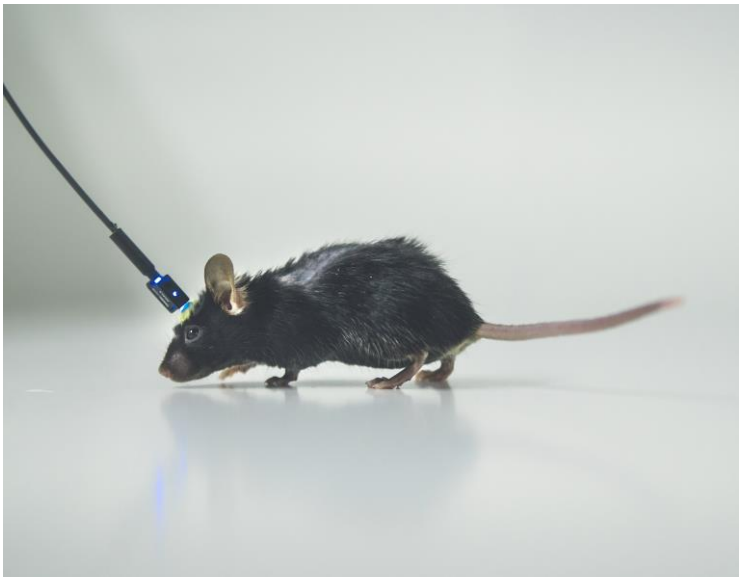


Optogenetic implant and basic setup (© Karolína Hrůzová, inspired by Pama et al. [134])

Optogenetics currently offers new possibilities in neuroscientific research. It can clarify anatomical projections of neurons as well as explain functions of structures in behavioural and cognitive processes. This innovative technique can identify interneuron types based on their molecular patterns and can define their specific roles in neuronal circuits by stimulation in freely moving animals. As mentioned above stimulation of PV interneurons in neocortex is amplifies gamma oscillations, enhancing signal transmissions and coordinating the timing of sensory inputs relative to gamma cycles[135]. Furthermore activation of PV interneurons in neocortex (as in hippocampus) produce theta resonance of pyramidal cells nearby the targeted interneuron and their rebound spiking[136].

Manipulation of behaviour in animals is a powerful tool for understanding neuronal mechanisms of various processes. For example, a fear conditioning task is a model for associative learning dependent on basolateral amygdala. Increase an activity of PV interneurons in basolateral amygdala during conditioned stimulus correlates with enhanced learning and with stronger auditory response, but their inhibition during aversive unconditioned stimulus also enhance learning. Results demonstrate that associative learning is regulated by activation specific to stimuli on various disinhibitory microcircuits via interaction between distinct types of local interneurons[137]. By reactivation of neurons that has been active during learning of fear conditioning task freezing

behaviour can be induced[138]. These neuron-specific in vivo manipulations during tasks where behaviour is investigated make this uniquely useful, due to its potential to elucidate functions of distinct areas in the brain. These findings can then be generalised.



A transgenic PV-ChR2 mouse with implanted optic fibre and stimulation by blue light (© Kristýna Malenínská)

Optogenetics is becomes widely used in research of animal models of schizophrenia and schizophrenia-like symptoms in rodents. Optogenetic activation of fast-spiking PV interneurons enhances gamma oscillations in the neocortex. NMDA antagonists increase the power of gamma oscillations in rodents over the cerebral cortex and the disruption of NMDA receptors especially on fast spiking PV interneurons is postulated by some as the reason of neural network dysfunctions that can manifest as the symptoms of schizophrenia. Transgenic mice lacking NMDA receptor neurotransmission in PV interneurons show decreased rates of gamma oscillations in cortex (disruption in ability to induce gamma waves) after optogenetic stimulation and sensitivity to disruption of gamma brainwaves by NMDA antagonists is also reduced [139].

The NMDA receptor dysfunction hypothesis and the hypothesis regarding disturbances in brain rhythms can be tested by optogenetics via investigation of cognitive functions of rodents. Combination of NMDA antagonist MK-801 (known also as dizocilpine) and optogenetic stimulation can clarify the role of interneurons in cognitive disability. Carlén et al. at Karolinska Institute in Sweden tested PV-Cre/NR1f mice (lacking NMDA receptors on PV interneurons) as a model for these two hypotheses. There were no differences between the open-field behaviour of control mice and PV-Cre/NR1f mice. The PV-Cre/NR1f mice, however, showed a markedly reduced sensitivity to MK801 (0,3mg/kg). PV interneurons are a target for the non-competitive NMDA receptor antagonist.

Results from different tasks (water maze task, T- maze task, fear conditioning task) suggest that mice lacking NMDA show spontaneous and evocated abnormalities like those observed in mice exposed to NMDA receptor non-competitive antagonists. Light pulses in the gamma range on controls (with ChR2) amplify local field potential in gamma, whereas the pulses on mice lacking NMDA receptor lead to a specific disruption in the ability to induce gamma waves and reduced activity upon light pulsing targeted at fast spiking interneurons[139]

Discussion, conclusions and outlook

Schizophrenia is a devastating disorder which should not be underestimated; therefore, further research of this disease is of a great importance. The exact cause of schizophrenia is unknown, which prevents causal treatment. Not only traditional techniques but also innovative methods offering great levels of insight should be used in the research of schizophrenia. While neuroimaging and high-resolution quantitative EEG provided some clues regarding this disease in humans, much greater levels of details are available in animal models.

Despite classification of neuronal cells is not very straightforward, there are many possible ways distinguishing types of neurons- based on functional or anatomical characteristics. Interneurons and their relationship to neuronal oscillation seem to be essential for understanding the disease and are readily accessible in animal models. Animal models are obviously limited since human behaviour is more complex and is not possible to reflect all disruptions in behaviour, as result of the disorder, in evolutionarily simpler rodents. Despite these models serve as a powerful tool that can clarify several processes in the brain upon their modification and observing their impact on behaviour. Additionally, they provide a way how to test innovative ways of treatment

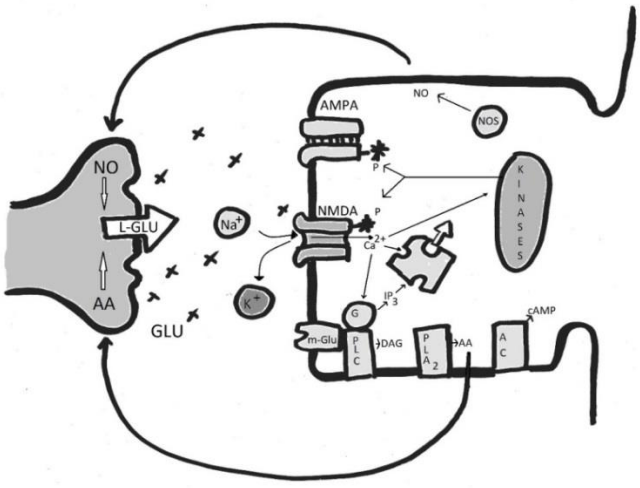
Optogenetics is one of many methods that can expand our knowledge in neuroscience. A huge advantage of this innovative technique is the possibility to observe induced change on neuronal systems *in vivo* and the specificity which cells are targeted. Combination of an NMDA non-competitive antagonist (as MK-801) and optogenetic stimulation of PV neurons in hippocampus or prefrontal cortex could be a very useful model for better understanding of the relationships between interneurons, the hypothesis of NMDA receptor dysfunction and neuronal oscillations. In my work in laboratory I aim at establishing this approach with my colleagues and my supervisor. We hope to be able to test hypothesis that disruption of interneurons function is related to cognitive coordination and flexibility deficit in animal model of psychosis induced by MK-801, a non-competitive NMDA receptor antagonist.

Appendix

Short overview of synaptic plasticity

Synaptic plasticity is a complicated process of biochemical and structural changes in synapses as a result of their activation. In 1949, Donald Hebb postulated a theory of learning, which suggests that synapse strengthen, when pre- synaptic and post synaptic neurons are activated simultaneously. (Hebb, 1949). A few years later Tim Bliss and Terje Lømo at the laboratory of Per Andersen discovered that intense electrical stimulation of axons from the entorhinal cortex to dentate gyrus caused a long- term increase of magnitude of excitatory potentials in the post-synaptic neurons. He named this phenomenon "long-lasting potentiation". Long-term potentiation (LTP) can also be induced in other regions of brain and can last for several months. [140]. If we stimulate two synapses on a single neuron, whereby one is stimulated more strongly than the other, the weaker one becomes strengthened. This process is called associative long-term potentiation. [141]

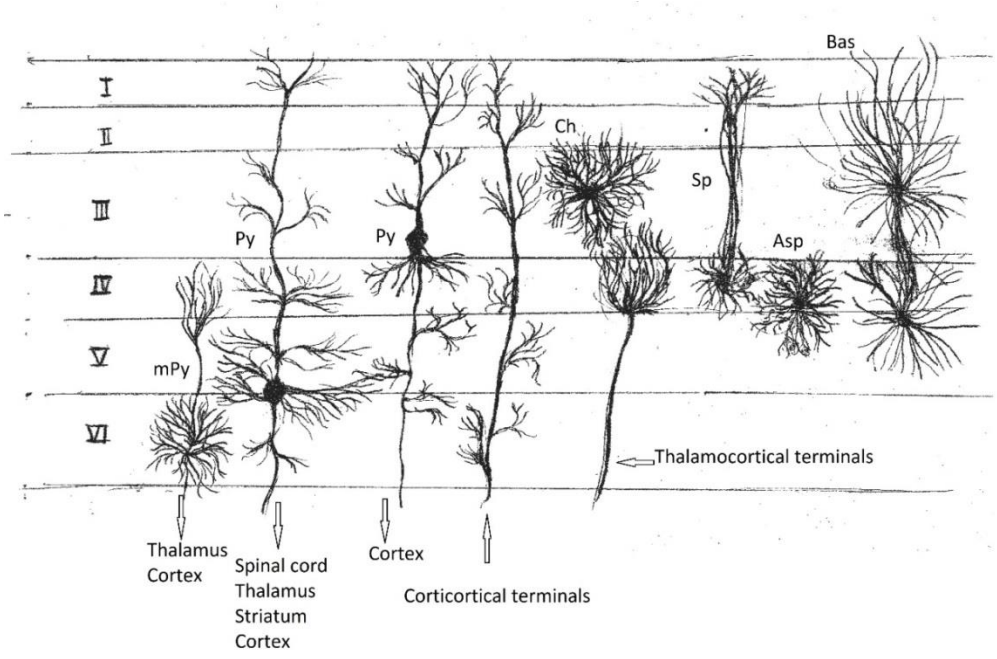
LTP is characterised by three basic properties. Cooperativity, implying an intensity threshold for induction and possible cooperative action of many small inputs, associativity, dexcribed above above and input-specificity - inputs that are not active at the time of stimulation, do not contribute to potentiation induced in the stimulated pathway. [141] LTP is usually dependent on NMDA and AMPA subtypes of receptors and it is characterised by an increased number of AMPA receptors after induction. NMDA receptors are sensitive to glutamate and they contain calcium channels, which can be open only upon depolarisation of the membrane. A combination of depolarisation and activation of NMDA receptors causes the influx of calcium ions in to the cell. An increased level of calcium activates calcium dependent enzymes, which in turn, activate translocation of AMPA receptors into a dendritic spine [142]. However, this process is more complicated and, yet, not well understood. Many components and biochemical pathways are involved, for instance nitric oxide (NO), which plays important role on presynaptic cell by facilitation of the release of glutamate. [143], or involvement of kainate and metabotropic glutamate receptors. Low frequency stimulation can lead to the opposite phenomenon called long-term depression (LTD) is characterised by decreased number of the AMPA receptors in the spine. Both processes LTP and LTD play important role in learning.



A simplified mechanism of LTP
NMDA (NMDA channels), AMPA (AMPA channels), G (G-proteins), PLC (phospoinositide specific phospholipase C), PLA2 (phospholipase A2), AC (adenylate cyclase). DAG (diacylglycerol), AA (arachidonic acid), NOS (nitric oxide synthase), NO (nitric oxide), GLU (glutamate) [141]

Layers of the neocortex

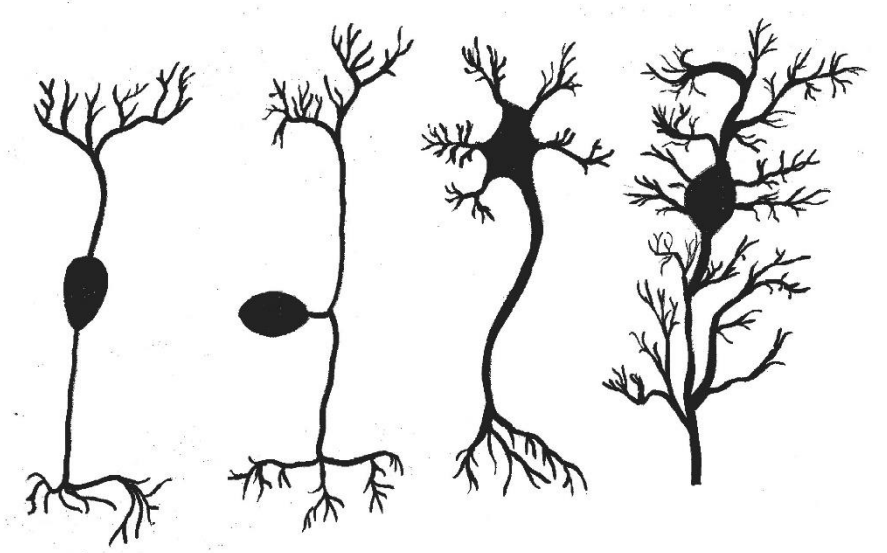
Layer I - Molecular layer (*Lamina molecularis* in Latin) – this is the most superficial layer. This layer contains very few neuron cell bodies and consists mainly of horizontally oriented axons and extensions of apical dendritic parts of pyramidal neurons. Layer II - External granular layer (*Lamina granularis externa*) – this layer is relatively thin, and it consists of a small neurons like granule cells and pyramidal cells or their dendrites. Layer III - Pyramidal layer or external pyramidal layer (*Lamina pyramidalis externa*) – Small to medium sized pyramidal cells are found here, as well as other non-pyramidal cells like chandelier cells, spiny stellate neurons or basket cells. Layer IV- Inner granular layer (*Lamina granularis interna*) - Aspine stellate neurons and spine stellate neurons are found in this layer. These types of neurons are sometimes categorised as granule cells. Layer V - Ganglionic or inner pyramidal layer (*Lamina* pyramidalis interna) – This layer contains large pyramidal cells. Layer IV - Multiform layer (*Lamina multiformis*) - This is the deepest layer of cortex, consisting of pyramidal and fusiform cell bodies.



Layers of neocortex. *(mPy- modified pyramidal cells, Py – pyramidal cells, Ch- chandelier cells, Asp- aspine stellate neurons, Sp- spine stellate neurons, Bas- Basket cells*) © Karolína Hrůzová, https://clinicalgate.com/the-cerebral-cortex-2/

Classification of neurons by polarity and anatomy

Anaxonic - axon cannot be differentiated from dendrites. These neurons are located in the brain and in some sensory organs. Their function is poorly understood, but it seems, they are non- spiking and have been suggested to have synergic effects on the stimulation of sensory or motor response. **Unipolar** - one neurite (neuronal process) extends from the cell body. These types are common in insects. **Pseudounipolar** - The have an axon that splits into two branches. They are sensory neurons in peripheral nervous system, so one branch goes to periphery and other runs to the spinal cord. Their axons may extend one meter or more, longest are those carrying sensation from tips of toes to spinal cord. **Bipolar** - this type of neurons has two distinct processes, one dendritic and one axon on opposite side of soma. Bipolar cells are quite rare, they are located in special sense organs, where they relay information about events in the environment. They are often smaller than unipolar or bipolar neurons. **Multipolar** - this is the most common type of neuron in the central nervous system. These neurons have one single axon and many dendrites. They integrate information from other neurons.



By direction of nerve pulse

Afferent neurons - carry information from tissue to the central nervous system. (ex. sensory neuron). **Efferent** neurons - transmit signals from the central nervous system to an effector. (ex. motor neurons). **Interneurons** - connect other neurons.

Classification by function

By electrophysiological characteristics

Regular spiking neurons are constantly active.

Bursting neurons fire in bursts, there is a rapid action potential followed by G0 phase.

Fast spiking neurons have high firing rates and they are spiking with high frequency.

By neurotransmitter production

Neurons can be classified by the neurotransmitters they release. Neurotransmitters have two general effects - they cause a depolarisation or hyperpolarisation of the membrane on a postsynaptic neuron, in other words inhibitory or excitatory effects. The group of neurotransmitters is really sizeable. According to this classification we have many groups for example cholinergic, GABAergic, Glutamatergic, Dopaminergic, Serotonergic neurons etc. Problem of this classification is fact, that one neuron can release more than one neurotransmitter.

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