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Cortical microcircuits and synaptic transmission in epilepsy

Kortikální mikrookruhy a synaptický přenos v epilepsii

Bachelor's thesis

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Prohlášení

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Poděkování

Abstrakt

Epilepsie je trvalá predispozice mozku generovat epileptické záchvaty. Toto onemocnění je ve společnosti značně rozšířené a pacientům výrazně komplikuje život. Jelikož se jedná o onemocnění definované pouze společnými příznaky, výzkum je náročný kvůli množství příčin, které k epilepsii vedou. Přestože existuje mnoho forem terapie cílících na různé etiologie této nemoci, často je náročné nasadit pacientům správnou léčbu a asi třetina z nich stále trpí záchvaty refrakterními vůči jakékoli léčbě. Nicméně bylo identifikováno množství proteinů, které se podílejí na synaptickém přenosu, a popsáno, jaké důsledky mohou mít změny v jejich funkci. Změna funkce jednotlivých proteinů ovlivňuje aktivitu synapsí a také to, jak se jednotlivé synapse zapojí v rámci neuronálních mikrookruhů. Mikrookruhy jsou motivy zapojení excitačních a inhibičních neuronů, důležité pro přenos a zpracování informací v mozku. Mikrookruhy mohou být dočasně, nebo i dlouhodobě pozměněny patogenními ději na úrovni molekulárních mechanismů, které mohou zodpovídat za vznik a propagaci epileptických záchvatů.

Klíčová slova

epilepsie, iktogeneze, mikrookruhy, synaptický přenos

Abstract

Epilepsy is a permanent predisposition of the brain to generate epileptic seizures. This widespread disease significantly impairs patients' life. Since epilepsy is defined only by its common symptoms, the research is complicated due to the variety of possible causes that can result in this disorder. Although many forms of therapy targeting different etiologies of this disorder exist, it is often challenging to select the right treatment and about one third of the patients still suffer from refractory seizures. Nevertheless, several proteins that participate in synaptic transmission and may undergo specific changes in their function have been identified. These changes in function affect both the activity of synapse and the involvement of synaptic connections in neuronal microcircuits. Microcircuits are common motifs of connections amidst excitatory and inhibitory neurons that are necessary for the transmission and processing of information in the brain. Microcircuits can be temporarily or permanently impaired by pathogenic processes on the molecular level, which may be responsible for the origination and propagation of seizures.

Keywords

Abbreviations

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPAR(s)	AMPA receptor(s)
ASM(s)	anti-seizure medication(s)
E/I	excitation/inhibition
FCD(s)	focal cortical dysplasia(s)
GABA	γ-aminobutyric acid
GABA$_A$R(s)	GABA type A receptor(s)
HME	hemimegalencephaly
KA	kainate (kainic acid)
KAR(s)	kainate receptor(s)
KO	knockout
MCD(s)	malformation(s) of cortical development
MFS	mossy fiber sprouting
mTOR	mammalian target of rapamycin
NMDA	N-Methyl-D-aspartic acid
NMDAR(s)	NMDA receptor(s)
PMG	polymicrogyria
SNAP25	synaptosomal-associated protein 25
SNARE(s)	soluble N-ethylmaleimide-sensitive factor attachment proteins receptor(s)
Stx1B	syntaxin 1B
StxBP1	syntaxin-binding protein 1
SV(s)	synaptic vesicle(s)
Syb2	synaptobrevin 2
TSC	tuberous sclerosis complex

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1. Introduction

Epilepsy is one of the most prevalent neurological disorders in the human population (Feigin et al., 2019), that manifests itself by predisposition for, or presence of two or more recurrent, unprovoked seizures (Fisher et al., 2014). The greatest complication in search for a proper treatment of epilepsy is the fact that it is not just one disease with the exact same origin, but a wide range of different pathologies with varying causes, that only manifest in similar way (Berg and Millichap, 2013). While therapies to treat epilepsy are present, around 30 % of patients are refractory to anti-seizure medication (ASM) (Mohanraj and Brodie, 2005), and even though many new ASMs have been introduced in recent years, the proportion of refractory epilepsies is not decreasing (Löscher and Schmidt, 2011). And patients that use ASMs with success still have to cope with adverse effects, some of which may be impairing their quality of life (Marson et al., 2005). Surgical intervention is an option for treatment of refractory epilepsies, although it is not always viable since the regions of the brain responsible for eliciting seizures might host critical functions. Therefore, it is imperative for researchers to study cellular and molecular mechanisms underlying epilepsy to find possible new treatments for patients using novel approaches, such as gene therapy (Zhang and Wang, 2021).

In this thesis, I am going to focus on specific proteins regarding synaptic transmission and their role in epilepsy. Later I am going to review some of the cortical microcircuit motifs and their alterations in the brain and assess how said alterations may result in epileptic seizures.

2. Focal cortical dysplasia

Epileptogenesis is a long-lasting process that is induced genetically (genetic epilepsy) or by traumatic event (acquired epilepsy). It alters the brain in a way that it becomes more susceptible to seizures over time and proceeds long after the first seizure appears (Williams et al., 2009). While epilepsy may affect any part of the brain, in this thesis, I am going to focus primarily on the cortex. There, one of the most frequent causes of epileptogenesis is a range of disorders known as malformations of cortical development (MCD) (Barkovich et al., 1996).

Focal cortical dysplasia (FCD) is one of the MCDs that is in many cases responsible for refractory epilepsy (Taylor et al., 1971; Desbiens et al., 1993). FCD is a result of somatic mutation in the genes for proteins involved in the N-glycosylation pathway (SLC35A2) and in the mammalian target of rapamycin (mTOR) pathway (Mirzaa et al., 2016). MTOR is a serine/threonine kinase that participates in the regulation of cell cycle, metabolism and other cellular processes (Sarbassov et al., 2005). The mutation occurs early in the development in

very few brain cell precursors, which leads to mosaicism in the cortex. Affected cells show abnormal migration, shape, growth, function and connectivity (Spreafico et al., 1998).

2.1. Types of FCD

FCDs divide into 3 subtypes with characteristic histological images. FCD type I is described by lesions with dyslamination of cortical architecture. FCD type II features immature and dysmorphic neurons (type IIa), balloon-like cells (type IIb) and delaminated lesions. Type III is similar to type one, however it has to be located adjacent to other lesions, like hippocampal sclerosis or brain tumor (Najm et al., 2018). FCD Type II is often investigated in epilepsy research, since the dysmorphic neurons are suspected to be the one of the initiators of the epileptic activity in the brain (Chvojka et al., 2024) and the delamination in cortex may further contribute to propagation of abnormal signaling and recruitment of local neurons into seizures.

2.2. Other MCDs manifesting with epilepsy

A plethora of malformations exist other than the FCD, that often result in refractory epilepsy. MTORopathies are a group of MCDs, that are caused by altered signaling in the mTOR pathway, similarly to FCD. These include tuberous sclerosis complex (TSC) (Huang et al., 2008) and hemimegalencephaly (HME) (Lee et al., 2012). TSC is a result of mutation in TSC1 and TSC2 proteins. These two proteins participate in regulation of the mTOR signaling pathway (Huang et al., 2008). TSC causes small, benign tubers to appear all over the human body including the brain, which are in most cases accompanied by epilepsy (Henske et al., 2016). In HME, the AKT3 enzyme, which is a part of the mTOR pathway, shows increased activity. This leads to one hemisphere of the brain being enlarged, which coincides with the emergence of epilepsy (Poduri et al., 2012). Polymicrogyria (PMG) is a MCD that causes delamination in the cortex and formation of excessive amounts of small gyri with impaired synaptic connections on brain surface (Stutterd et al., 1993; Sarnat and Flores-Sarnat, 2021). Multiple causes of PMG have been identified such as chromosomal aberrations (Kobow et al., 2020; Stutterd et al., 2020) or variety of mutations, for example mutations in the proteins regarding the mTOR pathway (Stutterd et al., 2018, 2021) or subunit of NMDA channel (Fry et al., 2018).

3. Ictogenesis

Ictogenesis is a set of complex processes that induce transition from inter-ictal state to seizure. Many different causes of ictogenesis have been described so far (Blauwblomme et al.,

2014). However, one abnormality is characteristic for ictogenesis in any epileptic tissue, this being the local imbalance between excitatory and inhibitory output (E/I) of specific neuronal population (Lau et al., 2000; Yu et al., 2006). Researchers suggest that one of the reasons for E/I imbalance may be the presence of abnormally connected clusters of neurons (Bragin et al., 2000; Bikson et al., 2003).

While ASMs targeting E/I imbalance might be able to restore it and avert ictogenesis, they come with the price of affecting the whole brain and causing adverse effects. That's why future epilepsy therapies need to target specific cells or proteins, so the patient's quality of life is affected in the least way possible.
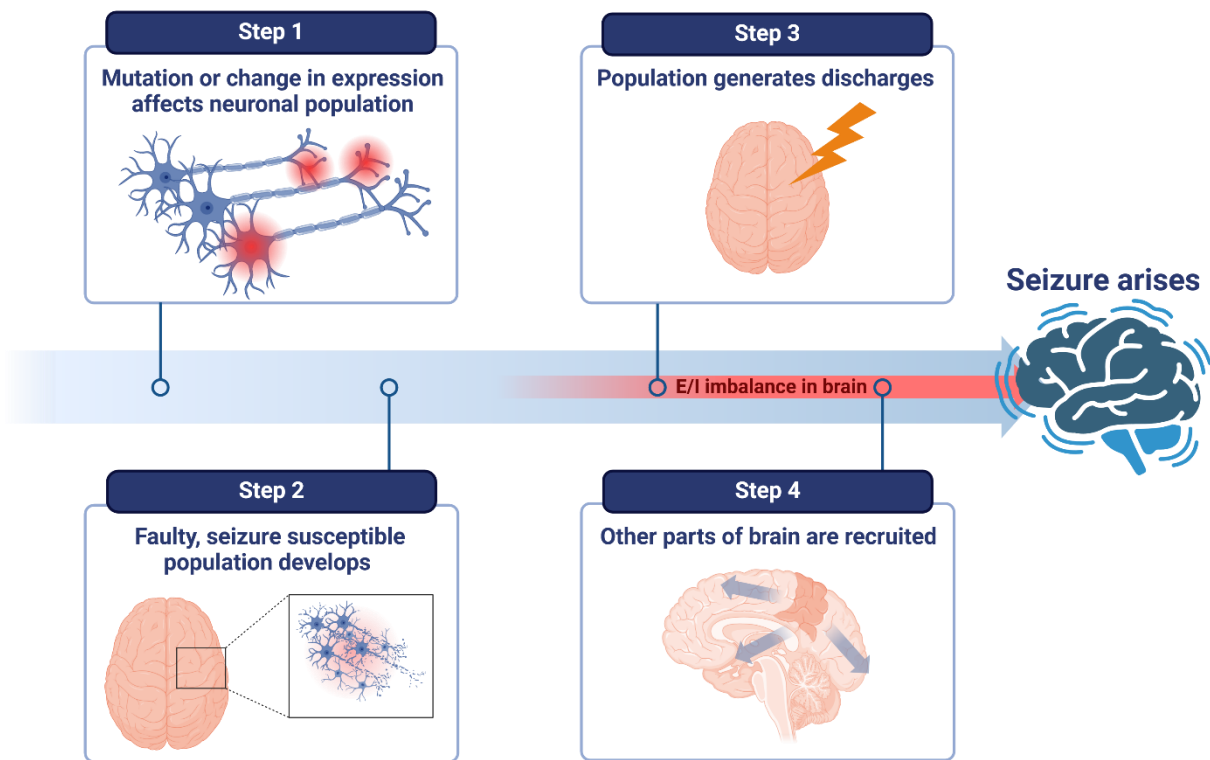
Figure 1: Step-by-step scheme of epilepsy onset and seizure generation. Steps 1 and 2 describe events leading to seizure susceptibility (epileptogenesis), while steps 3 and 4 describe events that happen preceding any seizure (ictogenesis) (author's illustration).

4. Synaptic transmission

Synaptic transmission allows one neuron's action potential to induce a change of membrane potential on the following neuron through release of neurotransmitter and its binding to postsynaptic receptors (chemical synapses) or through ion flow through intercellular pores

(electrical synapse). Precise regulation and maintenance of synaptic transmission are necessary for the brain to function without any pathologies. Over the last decades, excitatory and inhibitory chemical synapses have been extensively studied and their role in epilepsy is now much more understood, but more research is still needed to fill our knowledge gaps. Researchers also suggest that synapses and their components might be the target of future ASMs or other selective epilepsy therapies (Zaitsev et al., 2021).
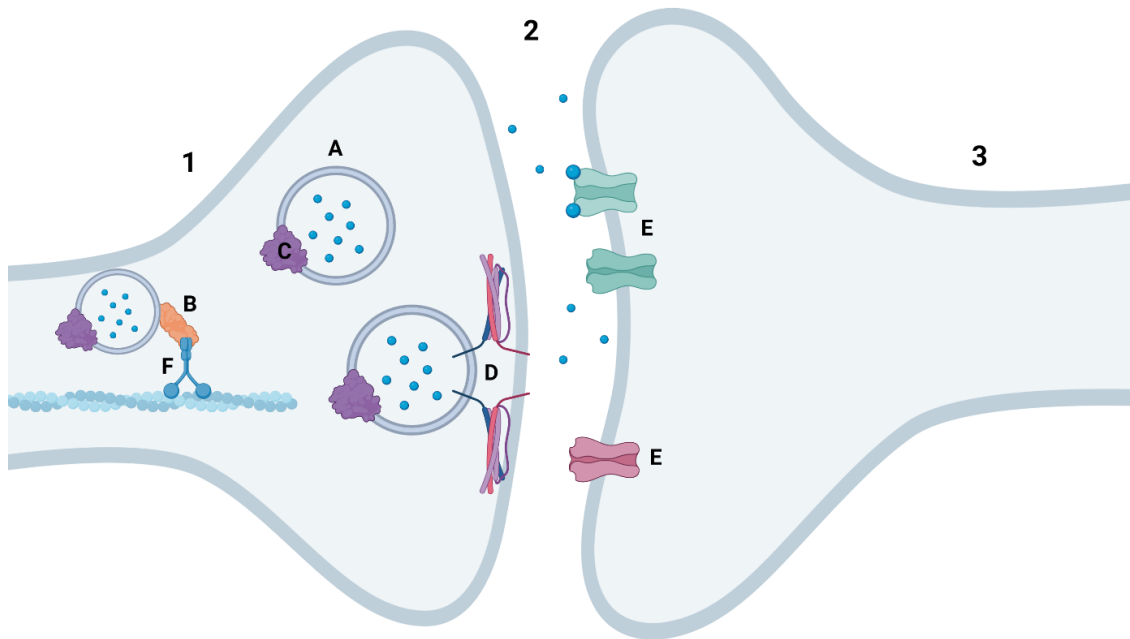
Figure 2: Illustration of a synapse with proteins that will be further discussed in this chapter. 1) presynaptic neuron, 2) synaptic cleft, 3) postsynaptic neuron. A) synaptic vesicle filled with molecules of neurotransmitter, B) Synapsin I, C) Synaptic vesicle glycoprotein 2A, D) SNARE complex, E) ionotropic receptors, F) molecular motor moving vesicle across actin fiber (author's illustration).

4.1. Synaptic vesicle-related proteins

A complex, yet precise cascade of molecular interactions is necessary for the release of the neurotransmitter from the presynaptic neuron into the synaptic cleft. The neurotransmitter is stored inside synaptic vesicles (SVs). These vesicles need to be transported to the proximity of the membrane and fused so the neurotransmitter can enter the synaptic cleft. The following proteins are indispensable in this interaction and interestingly, researchers have shown multiple times that dysfunctions in those proteins are associated with epilepsy.

It is often important to take these proteins into consideration when studying epilepsy, even when we know they are not the original culprit causing this disorder. Expression patterns

of some of these proteins may be altered by seizures (Yu et al., 2018) or disruptions in signaling pathways (Toering et al., 2009). Other presynaptic proteins might influence the strength of the synapse (Selak et al., 2009). These are some of the possible mechanisms that could generally explain why some epilepsies tend to worsen over time.

4.1.1. Synapsins

Firstly, it is necessary for the SVs to be transported to the synaptic membrane. This is done using common molecular motors that move the vesicle along the actin fiber, although one special class of proteins, called synapsins is needed in this interaction. Synapsins are a family of phosphoproteins found exclusively in axon terminals of neurons (De Camilli et al., 1983). Mammals possess genes for 3 different synapsins labeled from I to III (Kao et al., 1999). Synapsins I and II are activated by protein kinase A or Ca^{2+}/calmodulin-dependent protein kinase II (Ueda et al., 1973; Huttner and Greengard, 1979). Function of synapsin I is to crosslink the SV with actin fiber so the SV can then migrate along toward the axon terminal and fuse with the cell membrane (Bähler et al., 1990). This makes synapsin I one of the key elements in neurotransmitter release. Synapsin II plays a role in formation of new synapses (Han et al., 1991) and regulation of the number of SVs (Sugiyama et al., 2000). Synapsin III is important for axonal growth and recycling of SVs (Feng et al., 2002). Although all synapsin share cell localization, they are expressed at different levels in glutamatergic and GABAergic neurons (Bragina et al., 2007). Knockout (KO) of synapsins I or II in mice resulted in lower number of SVs on presynaptic neurons in both excitatory and inhibitory (E/I) synapses. At the same time, the imbalance between E/I currents shifted in favor of excitation. This led to epileptic phenotype and furthermore, KO of multiple synapsins at once increases the severity of seizures (Farisello et al., 2013). The proper function of synapsins is therefore needed for the brain to stay seizure-free.

4.1.2. Synaptic vesicle glycoprotein 2A

Next player in exocytosis is synaptic vesicle glycoprotein 2A (SV2A). SV2A is crucial in regulating the quantity of releasable SVs during exocytosis (Xu and Bajjalieh, 2001). The precise mechanism of this interaction remains unknown. However, researchers hypothesize that SV2A either modifies responsiveness of SVs to Ca^{2+} ions during exocytosis (Chang and Südhof, 2009) or that it might play a role in the process of filling SVs with neurotransmitter due to its homology with bacterial sugar transporters (Bajjalieh et al., 1992; Chang and Südhof, 2009). SV2A KO mice were found to display epileptic phenotype and shortened lifespan

(Crowder et al., 1999). In human patients, loss-of-function mutation in SV2A led to epileptic phenotype (Al-Maawali et al., 2024).

In human patients with FCD, TSC or hippocampal sclerosis, expression and localization of SV2A is severely altered. Neuropil in proximity of the lesion shows reduced expression, however dysmorphic neurons present in FCD type II show increased SV2A protein levels expression (Toering et al., 2009). Since lack of SV2A leads to impaired inhibitory neurotransmission (Crowder et al., 1999) and dysmorphic neurons are the ones thought to be responsible for abnormal activity in epilepsy (Chvojka et al., 2024), this discovery suggests that SV2A might have a crucial role in creating E/I imbalance and affecting epileptogenesis in MCD patients. Changes in expression of SV2A might also explain why FCD-related epilepsy is refractory to some drugs, since SV2A is the target molecule for ASM levetiracetam (LEV) (Toering et al., 2009). LEV is an anticonvulsant drug (Gower et al., 1992), that was approved in 1999 (Crepeau and Treiman, 2010), but only in 2004 it was discovered that LEV works by interacting with SV2A (Lynch et al., 2004).

4.1.3. SNARE proteins

After the SV reaches the membrane, a group of specialized proteins mediate the fusion of SV and the membrane. These proteins are called soluble N-ethylmaleimide-sensitive factor attachment proteins receptors (SNAREs, Fig. 3). They are a large group of membrane-bound proteins that mediate exocytosis. They are divided into 2 groups based on their localization in the cell, t-SNAREs (target), which are bound to the inner side of the cell membrane, and v-SNAREs (vesicle), which are located on the outside of vesicle (Söllner et al., 1993). These proteins interact to form a structure known as the SNARE complex. The main neuronal SNARE complex consists of three proteins that modulate the fusion of SV with the cell membrane by interacting with their highly conserved, α-helical domains (Sutton et al., 1998). After induction of seizures in rats, the expression of all the SNARE proteins was decreased and cognitive dysfunction emerged (Yu et al., 2018). However, individual proteins of the SNARE complex have varying effects on the function of the whole complex.
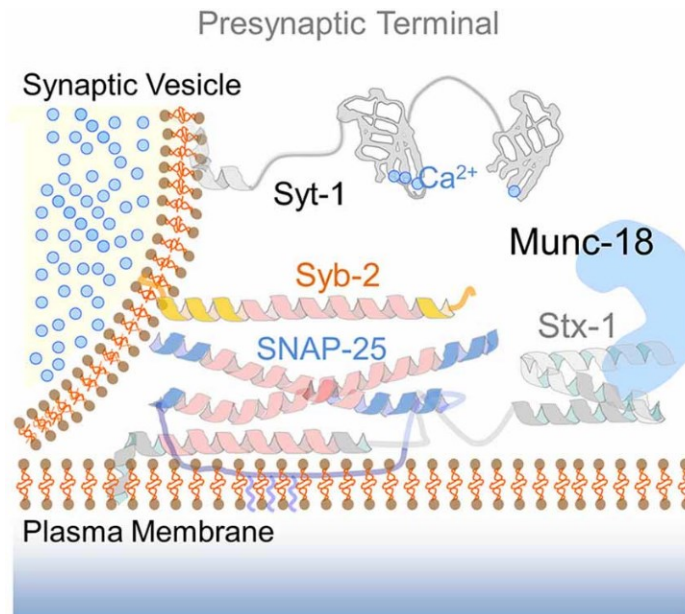
Figure 3: SNARE complex mediating the fusion of SV with the membrane. Syt-1: synaptotagmin 1, Munc-18: syntaxin-binding protein 1, Syb-2: synaptobrevin 2, Stx-1: syntaxin 1, SNAP25: synaptosomal-associated protein 25. Borrowed from Jurado, 2014.

Genetic screening of epilepsy patients revealed a wide array of mutations that impaired the proper function of syntaxin 1B (Stx1B), a main neuronal t-SNARE (Schubert et al., 2014; Vlaskamp et al., 2016; Vardar et al., 2020). Interestingly, different mutations in Stx1B led to different epileptic disorders (Wolking et al., 2019; Krenn et al., 2021). To properly facilitate the process of SV fusion, Stx1B must be assisted by syntaxin-binding protein 1 (StxBP1). Default conformation of Stx1B is 'closed' and cannot bind with other SNARE proteins to form the SNARE complex. By interacting with StxBP1, Stx1B adopts 'open' conformation, which allows it to participate in the SNARE complex (Dulubova et al., 1999). Some mutations in Stx1B were found to significantly affect this interaction in mice. Deletion of one residue led to absolute inability to interact between Stx1B and StxBP1 and caused mild epilepsy (Vardar et al., 2020). Two different point mutations resulted in altered neurotransmission and epilepsy by differently compromising the interaction with StxBP1. Surprisingly, the first mutation resulted in decreased neurotransmission, while the second resulted in increased neurotransmission (Vardar et al., 2020). It has also been shown that Stx1B is necessary for proper regulation of exocytosis (Mishima et al., 2014) and reuptake of GABA (Mishima et al., 2021). Complete lack of this protein was found to be lethal in mice (Wu et al., 2015). KO of syntabulin, a protein which regulates the trafficking of Stx1B, led to increased expression of Stx1B in the vicinity of the presynaptic membrane. This resulted in E/I imbalance and epilepsy (Ke et al., 2023).

Other proteins needed for the assembly of the SNARE complex are synaptosomal-associated protein 25 (SNAP25) and synaptobrevin 2 (Syb2). SNAP25 is a t-SNARE that has been linked to several neurological disorders, including epilepsy (Corradini et al., 2009; Antonucci et al., 2016). Deficiency in this protein leads to absence of fast SV release (Sørensen et al., 2003). Several mutations in the gene coding this protein have been proven to cause epilepsy. Mutated SNAP25 either exhibits lowered expression (Watanabe et al., 2015), or generally cannot sustain its proper function (Rohena et al., 2013). However the precise mechanisms of how SNAP25 affects exocytosis and formation of SNARE complex are unknown and researchers hypothesize that only decrease in expression of SNAP25 is insufficient to cause epileptiform activity (Watanabe et al., 2015). Besides acting as the part of the SNARE complex, this protein was also found on the postsynaptic side (Hussain et al., 2019), where it significantly contributes to maturation of dendrites (Tomasoni et al., 2013) and also influences the postsynaptic localization of ionotropic receptors, and therefore contributes to the strength of synapse (Selak et al., 2009; Lau et al., 2010). Regulation of translation by microRNA (miRNA) is a crucial step in expression of this protein, which, if not executed correctly, may lead to epilepsy. On the other hand, this mechanism can be used to selectively decrease expression levels of SNAP25 to compensate for excessive neurotransmitter release. A specific molecule of miRNA, miR-128 was found to decrease neurotransmission and therefore attenuate seizures, which makes it a potential candidate as future therapy (Wang et al., 2021).

Syb2 is the last member of the SNARE complex. In the mouse kindling model of epilepsy, reduced expression of Syb2 led to resistance towards seizures (Matveeva et al., 2012). Other than that, not much is known about the role of Syb2 in epilepsy.

4.2. Ionotropic receptors

Two classes of receptors are found on the postsynaptic membrane. Metabotropic receptors can activate various signaling pathways by binding neurotransmitters. Ionotropic receptors form channels that open after binding neurotransmitters and allow the flow of ions into the postsynaptic cell. This generates E/I postsynaptic potentials. Disturbances in the function of ionotropic receptors may lead to altered activation of postsynaptic neurons and therefore allow for generation and spreading of epileptiform activity in the brain. Moreover, seizures are capable of altering gene expression and subunit composition of ionotropic receptors (Loddenkemper et al., 2014), which can significantly affect the properties of the synapse.

4.2.1. Glutamatergic receptors

Glutamate is the major excitatory neurotransmitter in the mammalian brain. As many as 89 % of all synapses might utilize this neurotransmitter (Braitenberg and Schüz, 1998). Three different classes of ionotropic receptors interacting with glutamate have been identified: N-methyl-D-aspartate receptors (NMDAR), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR) and kainic acid receptors (KAR) (Hassel and Dingledine, 2012). AMPARs mediate rapid responses while NMDARs mediate longer-lasting responses (Hestrin et al., 1990). All three classes of glutamatergic receptors are tetrameric (Moriyoshi et al., 1991; Wu et al., 1996) molecules that form a glutamate-gated channel for Na^+, K^+ and Ca^{2+} cations. However, the Ca^{2+} permeability in AMPARs and KARs is strictly tied to their subunit composition (Hollmann et al., 1991). NMDARs and AMPARs are common targets of ASMs since they mediate most of the excitatory inputs in the brain. KARs, beside their main role as postsynaptic glutamatergic receptor, possess many regulatory functions, such as being able to regulate the surface expression of AMPARs (Petrovic et al., 2017) or regulate the neurotransmitter release when located on presynaptic membrane (Rodríguez-Moreno et al., 1997). But due to their similarities with AMPARs, they are a challenging target of research and much about them still remains to be discovered (Carta et al., 2014). All classes of ionotropic receptors have been linked to epilepsy multiple times.

Many mutations of NMDARs' subunits associated with epilepsy were identified in human patients, specifically on GluN1, GluN2A and GluN2D subunits. Mutations in GluN1 subunit are often associated with PMG (Fry et al., 2018). Receptors with mutated GluN1 subunit are more potent and require lower concentration of agonist in comparison to non-mutated subunits to be activated, which results in increased excitation (Fry et al., 2018). Mutations in GluN2A and Glun2D subunits also increase potency of the receptor and therefore result in increased excitation (Li et al., 2016; Chen et al., 2017b). Overall, increased potency of NMDARs is a common cause for epilepsy. Therefore, many antagonists of NMDARs are in these cases used to treat epilepsy (Sivakumar et al., 2022).

Under normal physiological conditions, AMPARs are not just bound to the postsynaptic zone of the membrane, but rather extensively transported across the cytoplasm and the whole neuronal membrane (Charsouei et al., 2020). In epilepsy patients, membrane expression levels of AMPARs are increased in the focal seizure onset zone, but reduced in most other brain areas, resulting in abnormal neuronal activity (Eiro et al., 2023). Hypofunction of the GluA2 and GluA4 subunits was observed to correlate with epilepsy. In human patients suffering from

MCDs and rats with hypoxia-induced seizures, expression of the GluA2 subunit was significantly decreased (Sanchez et al., 2001; Loddenkemper et al., 2014). However, another group of researchers recorded an increase in expression of GluA2 subunit in rats injected with convulsant pilocarpine (Russo et al., 2013). This paradoxical behavior of GluA2 subunits has not been clarified yet. The GluA2 subunit is crucial for AMPARs, since its presence renders the receptor non-permeable for Ca^{2+} ions (Hollmann et al., 1991). Therefore, lack of this subunit may lead to increased influx of Ca^{2+}, which could alter various metabolic processes since Ca^{2+} acts as a second messenger inside the cell (Rasmussen et al., 1976). GluA4 contributes to the correct function of feed-forward inhibition microcircuit (discussed in 5.1.1.).

Out of the 3 ionotropic glutamate receptor classes, KARs weren't getting much attention from researchers in the past, despite kainate (KA) injections being used to evoke epilepsy in rodent models since the late seventies (Ben-Ari et al., 1979). This was likely the result of KA working as an agonist to both AMPARs and KARs (Patneau and Mayer, 1991; Paternain et al., 1995) and hence it being complicated to distinguish between effects of those receptor classes on neurotransmission. However, using AMPAR blockers, researchers uncovered that activation of KARs by KA in rat hippocampus not only increased excitation, but also resulted in activation of KARs that were located on the presynaptic membrane, which decreased the rate of GABA release and impaired GABAergic inhibition (Rodríguez-Moreno et al., 1997). Similarly to other receptors, KARs role in epilepsy is dependent on their subunit composition. Deficiency in GluR5 subunit led to epileptic phenotype in patients (Sander et al., 1997), deficiency in GluR6 subunit led to resistance towards KA-induced seizures in mice (Mulle et al., 1998). Despite this, no effort to develop ASMs that would act solely as a KAR antagonist or would alter this receptor's properties in any way, has been reported (Lerma and Marques, 2013).

4.2.2. GABAergic receptors

With 20 to 30 % of cortical neurons releasing this neurotransmitter (Bloom and Iversen, 1971; Hendry et al., 1987), γ-aminobutyric acid (GABA) is the most common inhibitory neurotransmitter in the mammalian cortex. Increasing evidence suggests that hypofunction of GABA-mediated inhibition, which is indispensable for maintaining the right E/I balance, can provoke or sustain epileptiform activity (McCormick, 1989). In rodent models of genetic and acquired epilepsy, a decrease of either of GABAergic neurons, or GABAergic receptors has been reported (Olsen et al., 1985; Pitkänen et al., 1987).

Two types of GABA receptors are present on neuronal membrane, ionotropic GABA type A receptors (GABA$_A$Rs), and metabotropic, G-protein coupled GABA type B receptors. GABA$_A$Rs are pentameric (Nayeem et al., 1994) molecules consisting of several different subunits that form a chloride channel, which is opened upon binding of the GABA molecule (Fig. 4). Since neurons maintain a low level of intracellular Cl$^-$, Cl$^-$ starts flowing into the cell through open GABA$_A$Rs and generates inhibitory postsynaptic potential on the membrane. This hyperpolarization, together with shunting of excitatory inputs, efficiently decreases the excitability of postsynaptic neuron. Agonists of GABA$_A$Rs, such as benzodiazepines, have been used as ASMs for decades (Haefely et al., 1975) and new ASMs that act as GABA$_A$R agonists are still being developed (Janković et al., 2021). Binding of these compounds leads to increased influx of Cl$^-$ and fast inhibition, which can in some cases restore the E/I balance. Accordingly, some convulsants, such as bicuculline (Curtis et al., 1971) and pentylenetetrazole (Huang et al., 2001) were proven to act as GABA$_A$R antagonists.
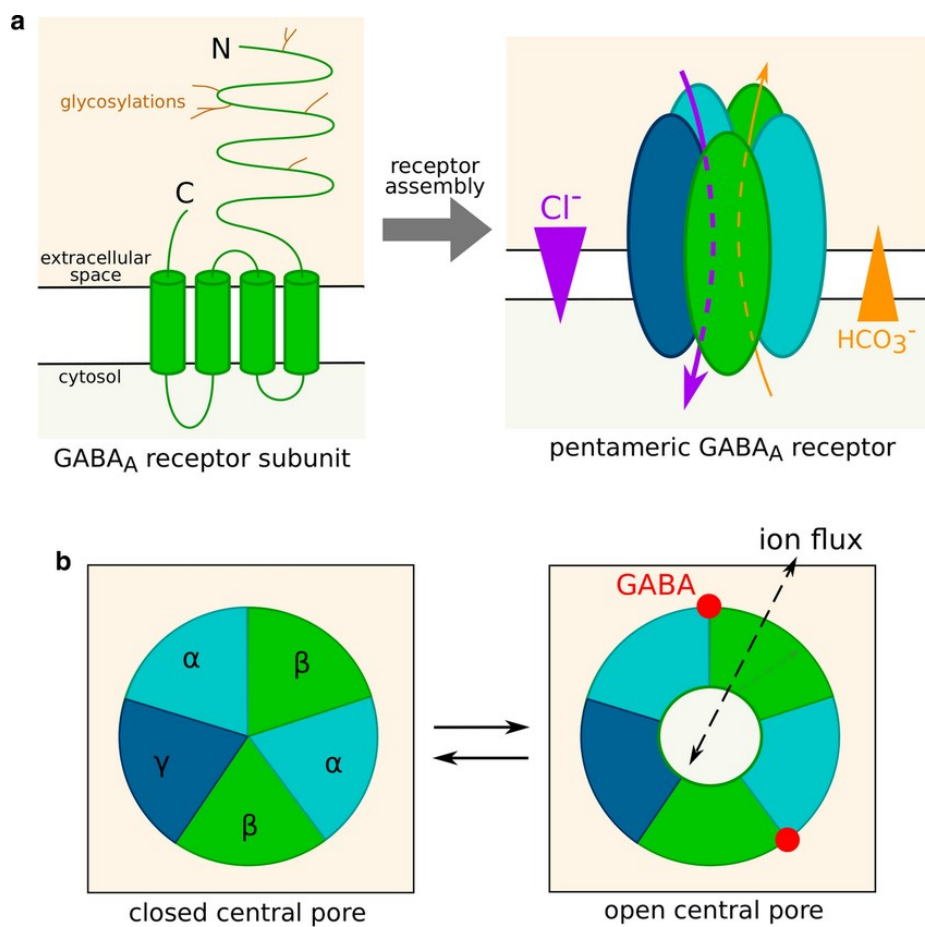
Figure 4: Structure of GABA$_A$R. a) structure and glycosylation spots of the GABA$_A$R subunit, assembled pentameric receptor with directions of ion flow, b) top-down scheme of canonical GABA$_A$R oscillating between open/close conformation by binding a molecule of GABA. Borrowed from Sallard et al., 2021.

GABA$_A$Rs consist of 5 subunits that can influence their properties. While multiple subunits have been identified (α1–α6, β1–β3, γ1–γ3, δ, ε, θ, π, ρ), only some combinations were found to be viable in the mammalian brain, and most GABA$_A$Rs consist of 2 α-, 2 β- and 1 γ-subunit (Sieghart and Sperk, 2002). The β3 subunit was found to play a significant role in onset and progression of multiple neurodevelopmental disorders, including epilepsy (Homanics et al., 1997; DeLorey et al., 1998). Mutations in this subunit have been identified as a cause for infantile and childhood epilepsy (Tanaka et al., 2008; Papandreou et al., 2016). These mutations are located close to the N-terminus of β3 subunit, where they often result in increased glycosylation. This increase in glycosylation leads to improper folding, transport and assembly of the GABA$_A$Rs, which results in decreased GABA$_A$R-mediated inhibition (Tanaka et al., 2008). Mutations in other subunits, such as α1 and γ2 were recently discovered to cause epilepsy as well, but more details about these remain to be uncovered (Zhang et al., 2023).

It has been shown that the number of GABA$_A$Rs on neuronal membranes can be reduced by their internalization during seizures. When epileptiform activity was induced in hippocampal slices of epileptic mice, GABA$_A$Rs began to intensively accumulate intracellularly (Goodkin et al., 2005). In a later study on mouse model, researchers unraveled that internalization of GABA$_A$Rs depends on the subunit composition. In later experiment using the similarly acquired hippocampal cultures, subunits β2, β3 and γ2 were found to be the ones that had their surface expression reduced (Goodkin et al., 2008). Due to a variety of β3 mutations and epileptic phenotypes, ranging from mild to serious, and less than 60% efficacy of ASMs in β3-related epilepsies (Yang et al., 2022), subunit β3 is a critical chokepoint in epileptic brain. Thus, designing novel personalized therapies, acting onto this receptor, should be one of the aims of future research.

5. Cortical microcircuits

Epilepsy is not just a result of one dysfunction in one synaptic protein inside one cell. These changes on a molecular scale frequently lead to larger changes in connections and interactions amidst larger populations of neurons or whole brain regions. The circuitry of the epileptogenic zone and its surroundings must be considered when we're trying to understand this disorder deeply. Previously mentioned mechanisms are critical to the onset of epilepsy and generation of seizures, but we need to put them in the larger context to gain a deeper understanding of this disorder.

5.1. Microcircuits in epilepsy

In recent years, many researchers have tried to unravel the mysteries of ictogenesis. Focusing on connections between neurons might help us understand how seizures propagate. While mapping every neuronal connection in the brain is impossible in the near future for its complexity, some microcircuit motifs that repeatedly appear in the mammalian brain have been isolated. Researchers examined their roles in brain networks and managed to link alterations in these microcircuits to epilepsy. In this chapter, I am going to detail some of the microcircuit motifs relevant to epilepsy (Fig. 5), some other larger-scale changes in the brain connectivity and how they are related to the generation of seizures.
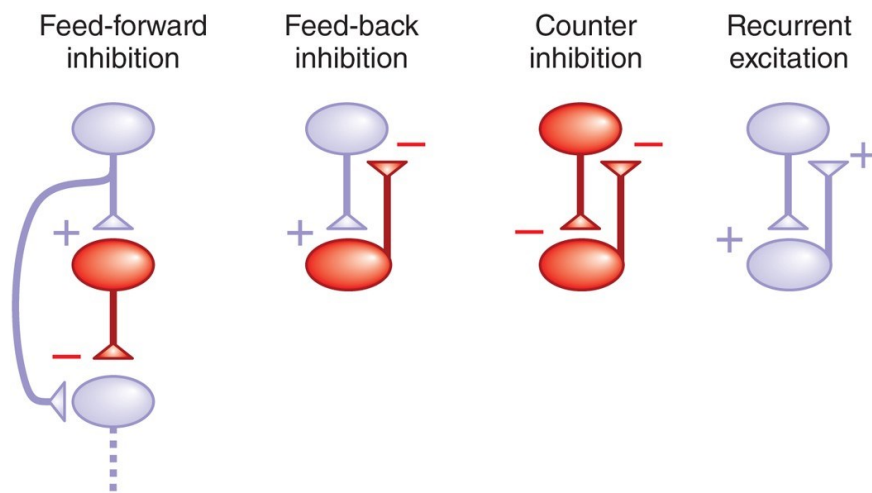
Figure 5: Common motifs of cortical microcircuits. Inhibitory neurons are red, excitatory neurons purple. Borrowed from Paz and Huguenard, 2015.

5.1.1. Feed-forward inhibition

Feed-forward inhibition (FFI) has been extensively studied in epileptic patients and animal models. This microcircuit motif is significant mainly in the context of cortico-thalamo-cortical (CTC) network. Sensory signals travel into thalamus and from there, they are relayed directly to appropriate cortical regions as excitatory signals (Constantinople and Bruno, 2013). As a part of modulation and processing of sensory information, cortical neurons send signals back to the thalamic neurons (Caviness Jr. and Frost, 1980). When this happens, inhibitory neurons in the reticular nucleus are activated and send inhibitory signals to the thalamic neurons. Although this network seems to work as feed-back on a larger-scale, on microcircuit-scale, it is feed-forward, since cortical neurons activate inhibitory neurons which inhibit the thalamic neurons.

Multiple studies found the loss of FFI in the CTC network to be a trigger for epilepsy (Beyer et al., 2008; Paz et al., 2011). Mice with impaired FFI presented enhanced synchrony of the network that resulted in seizures. Impaired FFI was a result of deficiency in the GluA4 subunit of AMPAR, which is a subunit that is expressed predominantly in thalamus (Golshani et al., 2001). Presence of this subunit leads to faster kinetics of AMPAR (Mosbacher et al., 1994) and therefore lack of it may lead to insufficient signaling. FFI is at the same time the determining factor for the speed of the propagation of epileptiform discharges (Trevelyan et al., 2007). In a later study, using *in silico* model of corticothalamic pathway, it was found that strengthening excitation of inhibitory neurons in thalamus leads to higher control and suppression of seizures (Chen et al., 2017a).

However, FFI is necessary in other parts of the brain besides the CTC network. Selective deactivation of calcium or sodium channels in mouse neocortical inhibitory neurons leads to loss of FFI and causes seizures (Rossignol et al., 2013; Tai et al., 2014). Similarly, deactivating potassium channels in mouse amygdala prevents inhibitory neurotransmission, impairs FFI and results in seizures (Thouta et al., 2021).

5.1.2. Other microcircuit motifs

Feed-back inhibition (FBI) is a simple pathway consisting of an excitatory neuron and an inhibitory neuron. When an excitatory neuron activates a connected inhibitory neuron, the inhibitory neuron releases GABA that hyperpolarizes or shunts the membrane of the original excitatory neuron in response. This mechanism is prominent in the hippocampus, which is a part of the brain often strongly affected by epilepsy. In a rat model of epilepsy induced by pilocarpine injections, the dynamic of the FBI was significantly altered. The release probability of neurotransmitter was reduced, but only on excitatory synapses onto inhibitory neurons (Pothmann et al., 2019). This resulted in insufficient inhibition and seizures. However, the mechanisms on molecular/cellular level that could lead to these changes are still unknown.

Counter inhibition is a microcircuit motif that has been studied for example in the reticular nucleus of the thalamus. Counter inhibition has the potential to disrupt the synchrony in thalamo-cortical neuronal oscillations and therefore prevent seizures (Huntsman et al., 1999). Moreover, the ASM clonazepam was found to increase the strength of counter inhibition, which led to even stronger desynchronization (Huguenard and Prince, 1994).

Lastly, recurrent excitation is a microcircuit motif that can be found commonly in the healthy brain (Kisvárday et al., 1986). Nevertheless, in temporal lobe epilepsy, excitatory

granule cells in the hippocampus can undergo the process of mossy fiber sprouting (MFS). MFS is a result of local abnormal signaling of the mTOR pathway (Buckmaster et al., 2009). During the MFS, axons of granule cells (mossy fibers) grow extensively, and new synapses are generated. Out of these new synapses, more than 95 % of them are excitatory. This newly formed excitatory network is recurrently connected and serves as the foundation for generating seizures (Buckmaster et al., 2002). Furthermore, the excitation from the mossy cells is strengthened and their synapses are potentiated by epileptic activity, which further increases the likelihood of seizures (Botterill et al., 2019; Nasrallah et al., 2022).

5.2. Other neuronal changes related to epilepsy

Besides abnormalities in specific microcircuit motifs, other alterations in neuronal connectivity are found in epilepsy. In the FCD, a significant decrease in density of inhibitory neurons takes place. While this happens in both FCD type I and II, it is more distinct in type II (Garbelli et al., 2006; Barinka et al., 2010). Furthermore, in FCD type II, the 2 abnormal cellular types, the dysmorphic neurons and balloon-like cells, are surrounded by a halo of inhibitory synapses connecting onto them (Spreafico et al., 2000; André et al., 2010). This may be a compensatory change where the interneurons try to silence the hyperactive, discharge-generating abnormal neurons. When researchers measured the GABAergic synaptic transmission onto the dysmorphic neurons, they found out that the increased number of synapses fully outweighs the overall decrease in inhibitory neuron density (Zhong et al., 2021). Therefore, they theorize that insufficient GABAergic transmission in FCD is a result of a dysfunction in synaptic proteins, most likely GABA$_A$Rs and GABA transporters (Calcagnotto et al., 2005; André et al., 2010), rather than being a result of a decrease in density of inhibitory synapses. Another reason why seizures appear so often in FCD might be an increased number of excitatory synapses (Brill and Huguenard, 2010) and their strengthening inside the whole FCD lesion (Cheng et al., 2022).

A multitude of neuron types is present anywhere in the brain. This heterogeneity is very prominent among inhibitory neurons, over 60 different types have been identified so far (Tasic et al., 2018). In epilepsy patients, the heterogeneity of neurons, both excitatory and inhibitory, is decreased in the whole brain. However, a smaller decrease in heterogeneity is needed among inhibitory neurons in comparison to excitatory neurons to result in epilepsy (Rich et al., 2022). This further suggests that inhibitory neurons and their connections are a chokepoint in the epileptic brain and need to be addressed in future epilepsy therapies.

6. Conclusion

The similarity of epileptic phenotypes, yet a multitude of different mechanisms on molecular, cellular, microcircuit and whole-brain level leading to them is the biggest issue of epilepsy research and treatment. While symptoms of most epilepsies can be treated, there is still a significant number of patients whose epilepsies are refractory. Due to the diversity in etiology of epilepsies, there is a poor chance of coming up with a 'magic' pill or procedure to cure all epilepsies. One of the possible solutions for researchers and physicians is to focus on personalized therapies, precisely tailored for each patient's needs.

Genetic studies play a fundamental role in epilepsy. I reviewed multiple mutations of synaptic proteins leading to epilepsy, however, tens of different genes for proteins regarding various synaptic transmission and cellular signaling have been linked to epilepsy so far (McTague et al., 2016). With genetic methods like CRISPR/Cas9 assisted gene editing becoming more accessible and already being used to treat some forms of epilepsy in animal models (Colasante et al., 2020), it is only a matter of time until this form of therapy becomes available for human patients. This might be a chance for patients with refractory epilepsy to finally become seizure-free and for patients that take ASMs to live seizure-free without the downside of adverse effects.

However, this probably will not render ASMs obsolete. Although genetic methods seem very promising, literature suggests that seizures and abnormal activity in the brain during epilepsy lead to several changes that may increase the seizure susceptibility and some of them may be long lasting or permanent. For example, synaptic proteins, such as SNAP25, AMPARs or KARs are capable of affecting the strength of the synapse. Therefore, replacing mutated gene with healthy variant may fix the cause, but seizures may endure due to consequential alterations on synaptic level. In this case, ASMs might find use to balance E/I and adjust the activity and potency of these synapses, until the expression levels of synaptic proteins return to normal state. However, this may be overcome by diagnosing epilepsy as soon as possible and preventing those changes from happening by treating patients at an early stage of the disease.

In terms of microcircuits and changes on cellular level in epilepsy, further research is needed to help us understand the relationship between those, and specific proteins or mechanisms that are responsible for them. Nevertheless, current literature implies that the context of E/I and the process of strengthening or weakening the synapses in the microcircuit are necessary to understand how seizures arise.

An alteration of expression or function of one protein may have a different, or completely opposite effect in an excitatory neuron and in an inhibitory neuron and the effect also depends on the location inside the microcircuit. Both increase and decrease in neurotransmission can in the end result in epilepsy. Situations like this urge us to revisit some ASMs that work by generally decreasing neurotransmission, since they might impair specific microcircuits where lower activity would lead to seizures. But interestingly, when researchers tested those ASM, they found that they spare the majority of inhibitory neurons of their effect. This happens because they only fire in brief episodes which makes them resistant to the blocking of Na^+ channels (Pothmann et al., 2014).

The future goals of epilepsy research should therefore be: 1) Continue to search for hallmarks that will help us diagnose epilepsy as soon as possible. 2) Learn to distinguish if specific alteration or dysfunction in protein is causal or consequential for epilepsy in a given context. 3) Strive to conceive new means of anti-epilepsy therapies that act on the exact origin of this disorder, rather than sticking to ones that act non-specifically or only suppress the consequences. However, the consequential changes in neurotransmission must be taken into consideration, so we can fully understand why some means of therapy may or may not work.

7. References

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