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Characterizations of a heterozygous *Stx1b*-knockout mouse model with fever associated epilepsy

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Abbreviations

aCSF – artificial cerebrospinal fluid

ADNFLE – autosomal dominant nocturnal frontal lobe epilepsy

AED – antiepileptic drug

AgCl – silver chloride

AMPA – α-amino-3-hydroxy-5-methylisocazol-4-propionic-acid

AP – action potential

AP-5 - (2*R*)-amino-5-phosphonopentanoate

ATP - adenosyltriphosphate

CA1 – hippocampal region Cornu Ammonis 1

CA3 – hippocampal region Cornu Ammonis 3

CAE – childhood absence epilepsy

CNQX - 6-cyano-7-nitroquinoxaline-2,3-dione

DEE – developmental and epileptic encephalopathy

DNA - deoxyribonucleic acid

EEG - electroencephalography

EGTA – ethylene glycol-bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid

FCD – focal cortical dysplasia

FDR – false detection rate

FLP – flippase

FRT – flippase recognition target

FS – febrile seizure

FS+ - febrile seizures plus

GABA – Gamma-aminobutyric acid

GEFS+ - genetic epilepsy with febrile seizures plus

GGE – genetic generalized epilepsy

GTCS – generalized tonic clonic seizures

GTCSA – generalized tonic clonic seizures on awakening

GTP - guanyltriphosphate

HEPES – 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

HPRT – hypoxanthine-guanine phosphoribosyltranseferade

IBE – International Bureau on Epilepsy

IGE – idiopathic generalized epilepsy

ILAE – International League against Epilepsy

IPSC – inhibitory postsynaptic current

JAE – juvenile absence epilepsy

JME – juvenile myoclonic epilepsy

KO – knockout

mEPSC – miniature excitatory postsynaptic current

mIPSC – miniature inhibitory postsynaptic current

mM – millimolar

MMFSI – malignant migrating focal seizures of infancy

mOsm – milliosmole

n – number of

Na_2ATP – adenosine 5'-triphosphate disodium salt hydrate

NaCl – sodium chloride

NaGTP – guanosine 5'-triphosphate sodium salt hydrate

NaH_2PO_4 – sodium dihydrogen phosphate

$NaHCO_3$ – sodium bicarbonate

NMDA – N-methyl-D-aspartat

NSF – *N*-ethylmaleimide sensitive fusion protein

O_2 – oxygen

PCR – polymerase chain reaction

PTX – picrotoxin

rpm – rounds per minute

SD – standard deviation

SEM – standard error of the mean

SM-protein – Sec1/Munc18-proteins

SNAP – soluble NSF Attachment protein

SNARE – SNAP-Receptor proteins

STX1B – syntaxin 1B protein

Stx1B – syntaxin1B gene

STX1B$^{+/+}$ - wildtype *STX1B*

STX1B$^{+/-}$ - heterozygous *STX1B*-knockout

SUDEP – sudden unexpected death in epilepsy patients

TBE buffer – Tris/Borate/EDTA buffer

TLE – temporal lobe epilepsy

TMR – transmembrane region

TTX – tetrodotoxin

1 Introduction

1.1 Epilepsy

The International League against Epilepsy (ILAE) and the International Bureau on Epilepsy (IBE) defined epilepsy in their operational clinical definition in 2014 as “a disease of the brain defined by any of the following conditions:

1. at least two unprovoked (or reflex) seizures occurring > 24 h apart
2. one unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years
3. diagnosis of an epilepsy syndrome”.

Epilepsy affects about 70 million people worldwide (Beghi et al., 2019; Fisher et al., 2014; Singh et al., 2016). An epileptic seizure itself is defined as “a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain” (Fisher et al., 2014, 2005). Seizures are categorized into generalized and focal depending on the regions where epileptic discharges occur within the brain, which can be determined by their semiology or by electroencephalography (EEG). A seizure is considered generalized when it involves both hemispheres, leading to loss of consciousness and either motor symptoms affecting the whole body like tonic-clonic, tonic, atonic and epileptic spasms or nonmotor absence seizures. Focal seizures in contrast involve only one hemisphere and may be restricted to a small group of neurons. Based on the patient’s awareness, they are further classified into focal aware and focal seizures with impaired awareness. The semiology of the seizure onset gives another parameter to specify focal seizures. On the one hand there are motor symptoms in the form of automatisms, epileptic spasms, hyperkinetic, atonic, clonic, myoclonic or tonic convulsions, which in this case are restricted to the area of the body that is represented by the brain region where the epileptic discharge takes place. On the other hand, nonmotor focal seizures include symptoms of autonomic, cognitive, emotional or sensory quality or behavior arrest. Focal seizures may encroach upon the other hemisphere resulting in a focal to bilateral tonic clonic seizure. Additionally, there are seizures with unknown onset and

unclassified seizures, which are placeholders in case of lacking information (Fisher et al., 2017).

Considering the criteria above, epilepsy may be diagnosed as generalized, focal or combined generalized and focal. Including more information like specific EEG patterns, CT or MRI brain imaging, co-morbidities or age of seizure onset and convalescence may lead to the diagnosis of an epilepsy syndrome like Lennox-Gastaut-syndrome, Dravet-syndrome, childhood absence epilepsy (CAE) or juvenile myoclonic epilepsy (JME). In addition to this clinical aspect of diagnosis, investigation of structural, genetic, infectious, metabolic or immune etiology should be initiated to assess specific treatment options, such as surgery, antibiotics or immunogenic, or simply only anti-seizure pharmacotherapy (Scheffer et al., 2017).

The seizures expose patients to dangers like injuries from dropping down or asphyxia due to missing protection reflexes during unconsciousness. Furthermore, the condition may induce loss of control in potentially dangerous situations, like swimming, climbing or handling fire, cars or machines. Additionally, epilepsy patients are at risk of sudden unexpected death in epilepsy patients (SUDEP), for which the causes remain unknown. All these factors result in an elevated standardized mortality risk of 1.6 – 15 for epilepsy patients (Elmali et al., 2019; Holst et al., 2013; Levira et al., 2017; Thurman et al., 2017). Although antiepileptic drugs (AED) achieve remission of seizures in 67% of patients, about a third does not become seizure-free even with two or more AED and are thus considered drug-resistant (Kwan and Brodie, 2000). Risk factors for drug-resistance include symptomatic or cryptogenic epilepsy, family history of epilepsy, history of febrile seizures (FS) and > 20 seizures before treatment initialization (Brodie, 2005).

The high epidemiological burden and its impact on patients and their families drove intensive investigations of the pathophysiology of epilepsy to find effective therapies. This provided unique insight into the mechanisms of the central nervous system (Holmes and Ben-Ari, 2001; Oliva et al., 2012). With the development of next generation sequencing techniques, more and more genetic

factors have been found to cause the formerly so called “idiopathic” epilepsies, establishing single genotype-phenotype-relations and coining the term of “genetic epilepsies” (El Achkar et al., 2015; Fukata and Fukata, 2017; Lerche et al., 2013; Scheffer et al., 2017). These include not only voltage-gated ion channels like $Na_v1.1$ (*SCN1A*), $K_v1.2$ and $K_v2.1$ (*KCNA2* and *KCNB1*) or $Ca_v2.1$ and $Ca_v3.2$ (*CACNA1A* and *CACNA1H*), but also ligand-gated ion channels, as for example glutamate-dependent GluN1, GluN2A, GluN2B and GluN2D (*GRIN1*, *GRIN2A*, *GRIN2B*, *GRIN2D*) or GABA-receptors GABRA1, GABRB3 and GABRG2 (*GABRA1*, *GABRB3*, *GABRG2*) (Oyrer et al., 2018). As variants in these genes were functionally characterized using heterologous expression systems or mouse models, the epileptogenic processes could be explored, unravelling the need for specific treatments and implicate certain prognoses (Liu et al., 2019; Watanabe et al., 2000; Yu et al., 2006; Reif et al., 2017).

1.1.1 Febrile seizures and Genetic Epilepsy with Febrile Seizures plus (GEFS+)

Given the definition of epilepsy above, epileptic seizures are only considered for the diagnosis if they are unprovoked, thus excluding febrile seizures. These epileptic seizures occur in otherwise neurologically normal children at the age of six months to five years during body temperature rise due to an infection which does not affect the nervous system (Academy of Pediatrics, 2008). They are fairly common and befall 2-4% of the population at least once during childhood (Hauser, 2007). Simple FS are generalized seizures lasting < 10 minutes and occuring only once within 24 hours. Although they can be terrifying for parents, FS are considered benign since they seldomly oppose long-term damage to intellectual ability. Complex febrile seizures are defined as > 1 FS within 24 hours, FS with a duration of > 10 minutes or with a focal onset. They can be associated with mesial temporal sclerosis and temporal lobe epilepsy (Cendes et al., 1993) as well as a lower seizure threshold in rodent models (Dube et al., 2000). If febrile seizures are combined with or succeeded by other forms of seizures or occur past the age of 6 years, they are defined as FS+ (Eckhaus et al., 2013).

Genetic Epilepsy with Febrile Seizure plus is an epilepsy syndrome that includes FS, FS+ and afebrile seizures and affects several members of one family (Scheffer et al., 1997). Afebrile seizures in GEFS+ include generalized tonic-clonic seizures, atonic seizures, focal impaired awareness seizures, tonic seizures and absence seizures. Furthermore, whereas most individuals' neuronal development is normal, some patients suffer from mild intellectual disability (Wolking et al., 2019; Zhang et al., 2017). Most commonly, these syndromes are caused by variants in sodium channel genes, mainly *SCN1A or SCN1B*, but also *GABA(A)*-receptor subunit genes, such as *GABRG2* and *GABRB3* (Baulac et al., 2001; Escayg et al., 2000; Kasperaviciute et al., 2013; Møller et al., 2017; Wallace et al., 2002, 2001, 1998). In *SCN1A*, for example, missense variants in the highly conserved homologous domains, especially the voltage-sensing S4-domain, were found in patients with GEFS+, whereas truncating variants were more often associated with the severe Dravet syndrome (Zuberi et al., 2011). Furthermore, temporal lobe epilepsy (TLE) has been linked to variants in *SCN1B* as well as GEFS+ (Scheffer et al., 2007). Additionally, several variants in *STX1B* which encodes the presynaptic protein syntaxin-1B were linked to GEFS+ (Schubert et al., 2014). The broad variance of seizure types within one family seems to be mediated by modifying factors that remain to be unraveled (Myers et al., 2018; Puranam et al., 2015; Rigbye et al., 2016).

1.1.2 Genetic Generalized Epilepsies (GGE)

One of the most common epilepsy syndromes is genetic generalized epilepsy or idiopathic generalized epilepsy (IGE), which includes three main seizure types: generalized tonic-clonic seizures (GTCS), absence seizures and myoclonic seizures. These are accompanied by a generalized spike-and-wave-EEG with > 2.5 Hz. Intellectual disability or brain imaging alterations are normally not part of the syndrome. Given the seizure type and the age of onset, a further subclassification is used to specify the disease. When absence seizures start at the age of 4-8 years, a childhood absence epilepsy (CAE) is diagnosed, whereas an onset after 11 years of age is typical for juvenile absence epilepsy (JAE). In

contrast, when myoclonic seizures occur during adolescence this gives rise to the diagnosis of juvenile myoclonus epilepsy (JME), even if occasionally GTCS and absence seizures may arise. Finally, a GGE characterized by GTCS in the very morning on awakening is called EGTCS on awakening (Mullen et al., 2018). Additional to these well circumscribed syndromes, there are grey areas regarding adult or early onset generalized epilepsies. Genetic factors were assumed and identified to be the main cause of many of these syndromes (Berkovic et al., 1998; Lerche et al., 2013).

SCL2A1 coding for the type 1 glucose transoporter (GLUT1) is one example for a gene variant which can cause GGE. As GLUT1 is responsible for the energy supply in form of glucose across the blood-brain-barrier, it was identified to be one of the rare cases of epilepsy being treatable without pharmaceuticals (Arsov et al., 2012). Ketogenic diet aims at replacing glucose with ketone bodies as the brains main energy source, because ketones use different transporters to cross the blood-brain-barrier and are therefore independent of GLUT1 (Pong et al., 2012).

Other genes associated with GGE encode different GABA(A)-receptor subunits, in which the first variants were identified in families with CAE and febrile seizures (Baulac et al. 2001, Wallace et al., 2001). Later, variants in its alpha- and delta-subunit were also found to cause JME in several families (Cossette et al., 2002; Dibbens et al., 2004). The GABA(A)-receptor is a good example for the specific effects different variants within a gene may have on the neatly regulated neuronal function resulting in distinct seizure types and comorbidities.

1.1.3 Developmental and epileptic encephalopathies (DEE)

Whereas all of the above epilepsy syndromes are associated with mild or no intellectual deficits, a slowing or regressing neuronal development is the key feature of developmental and epileptic encephalopathies. The ILAE introduced this new term for the former called “epileptic encephalopathies” to highlight the two main symptoms: developmental delay and epileptic seizures. Many of these disorders have an identified genetic cause. Thus, on the one hand, the

dysfunctional proteins may interfere with development themselves. On the other hand, the frequent epileptic activity disturbs neuro- and synaptogenesis and may cause neuroinflammation leading to neuronal dysfunction as well (Kalser and Cross, 2018; Scheffer and Liao, 2020). Therefore, treatment of the epileptic activity may improve the cognitive impairment but in some patients the developmental regression continues (Scheffer et al., 2017).

The term DEE summarizes several epilepsies with distinct features regarding age of onset, ictal and interictal EEG pattern, seizure type or even mutated protein (McTague et al., 2016; Scheffer et al., 2017). The Dravet syndrome for example, is a common DEE that starts with febrile and afebrile seizures mostly within the first year of life, shows generalized and multifocal epileptiform EEG-abnormalities after the first one to two years and is hardly treatable with antiepileptic drugs. Variants of *SCN1A* have been shown to be responsible for > 85% of all cases, the others being caused by variants in *GABRA1*, *GABRG2* or Munc18-1 (also referred to as *STXBP1*) (Carvill et al., 2014; Claes et al., 2001; Huang et al., 2012; McTague et al., 2016). Variants in *STXBP1* (Munc18-1) themselves give rise to a broad spectrum of DEE including early myoclonic encephalopathy, early-onset epilepsy and encephalopathy, West syndrome, Ohtahara syndrome and the before mentioned Dravet syndrome (Deprez et al., 2010; Hamdan et al., 2009; Saitsu et al., 2008; reviewed in Stamberger et al., 2016).

1.1.4 Focal epilepsy

In contrast to epilepsies with primarily generalized seizures, focal epilepsies initially involve only a part or at most one hemisphere of the brain in epileptic discharge. Typical symptoms include epigastric or sensory auras, déjà vu sensations, motor automatisms, starring, vegetative symptoms, hallucinations or tonic or clonic movements of one part of the body (Berg et al., 2010; Fisher et al., 2005; Scheffer et al., 2017). The intellectual development is usually normal, but a broad variety of cognitive deficits may occur with seizure amount (reviewed in Elger et al., 2004). A variety of structural lesions may induce focal epilepsies, such as focal cortical dysplasia (FCD), hippocampal sclerosis, cerebral infarction,

viral or autoimmune inflammation of the central nervous system, brain tumors and post-traumatic or post-surgery scars to name a few out of a long list (reviewed in Shorvon, 2011). The identification of variants in *CHRNA4*, *CHRNB2* and *CHRNA2*, all encoding subunits of the nicotinic acetylcholine receptor (nAChR), in families with autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) was the first to hint on the genetically induced focal epilepsies (Diaz-Otero et al., 2008; Phillips et al., 1995; Steinlein et al., 1997, Villa et al., 2019). Other examples are *LGI1*, variants of which causing autosomal-dominant lateral temporal lobe epilepsy, *SCN1A* being associated with mesial temporal lobe epilepsy with hippocampal sclerosis and *KCNT1* inducing multiple epileptic syndromes including ADNFLE and malignant migrating focal seizures of infancy (MMFSI) (Barcia et al., 2012; Heron et al., 2012; Kasperaviciute et al., 2013; Kawamata et al., 2010; Møller et al., 2015).

1.2 Synaptic transmission

Information processing within the nervous system relies on electrical signaling and chemical transmission between neurons (reviewed in Millhorn et al., 1989).

1.2.1 Vesicle fusion

At the nerve's synaptic terminus, the signal must cross the pre- and postsynaptic membranes and is therefore translated from electrical to chemical coding (Katz, 1971). Once the action potential (AP) reaches the neurons' synapse, voltage-gated calcium channels open allowing calcium to enter the presynapse. Calcium is the initializing component to start the fusion of small vesicles filled with neurotransmitter with the presynaptic membrane leading to the release of the transmitter into the synaptic cleft. The neurotransmitter crosses the cleft and binds to ligand-gated ion channels, which are receptor proteins in the postsynaptic membrane that allow ion influx. As the neurotransmitter acts on specific receptors either Na^+ or Cl^- enter the cell and depending on their charge de- or hyperpolarize it. Neurotransmitters are regarded as inhibitory if their action results in a hyperpolarization due to Cl^--influx, vice versa Na^+-triggered depolarization is induced by excitatory ones.

1.2.2 SNARE proteins

The neurotransmitter release side or 'active zone' is a neatly organized part of the presynaptic membrane ensuring vesicle fusion and neurotransmitter release. Central in this process is the SNARE-protein complex (soluble NSF attachment receptor proteins)(Söllner et al., 1993b). This complex is formed by the three proteins from the SNARE-family: synaptobrevin in the vesicle membrane, syntaxin and SNAP-25 in the plasma membrane. They share a highly-conserved domain called SNARE-motif and a transmembrane anchor (Bennett et al., 1992; Sutton et al., 1998). The SNARE-motifs of all three SNARE-proteins may bind and twist together in a fashion that allows the vesicle's membrane to fuse with the plasma membrane. Interestingly, the highest stability and only hydrophilic interaction site of this bundle, the zero ionic layer, is formed by three glutamine residues and one arginine residue interacting asymmetrically. Based on this interaction, SNARE-proteins are also divided into Q-SNAREs, explicitly SNAP-25 and syntaxin-1 that contribute glutamine, and R-SNARES for synaptobrevin contributing arginine (Fasshauer et al., 1998).

As a first step, vesicles that are located close to the active zone of the presynaptic membrane may undergo the process of "docking and priming", to make them prone to fusion and neurotransmitter release upon calcium signal (Figure 1). This part is mainly regulated by the interplay of Sec1/Munc18-like-proteins (also referred to as STXBP1, SM-proteins). The enzyme Munc13 changes the conformation of syntaxin-1 from closed to open, because only in the open conformation its SNARE-motif may interact with other SNARE-motifs (Augustin et al., 1999; Deák et al., 2009, p. 1; Dulubova et al., 1999; Hata et al., 1993). Subsequently, the SNARE-motifs of syntaxin, SNAP-25 and synaptobrevin start to bind on their N-terminal end half way (Sørensen et al., 2006). At this point, complexin, a regulating protein, binds to the partly assembled SNARE-complex (Cai et al., 2008; Cao et al., 2013; Dulubova et al., 2007). Complexin in this regard seems to help the SNARE-complex assemble, suppress spontaneous fusion and enhance calcium dependent transmitter release, which results in a highly

synchronous and very quick fusion process upon AP-signal (Hu et al., 2002; Maximov et al., 2009)(Figure 1B).

Subsequently Rab23/RIM-proteins guide calcium channels close to vesicles and SNARE-proteins, thereby keeping ways short and enhancing the speed of fusion reaction (Kaeser et al., 2011; Liu et al., 2011; Wang et al., 1997). The target protein for the influxing calcium ions is the calcium sensing synaptotagmin in the vesicle membrane (Brose et al., 1992; Fernández-Chacón et al., 2001). When calcium ions bind to synaptotagmin, complexin gets released from the SNARE-complex. Thus the SNARE-motifs are free to complete their interaction forming a very stable four helix-bundle (Brose et al., 1992; Poirier et al., 1998).

This motion brings the membranes of vesicle and cell in close proximity. There are several theories about how the fusion pore opens eventually and allows the neurotransmitter to be released, but lipid mixing mediated by SM-proteins seems to gain support by evidence (Chanturiya et al., 1997; Kuhlmann et al., 2017; Woodbury and Hall, 1988). Once the two membranes fuse, exocytosis of the neurotransmitter through the fusion pore into the synaptic cleft is initiated and the neurotransmitter can bind to postsynaptic receptors. The SNARE-complex is recycled by *N*-ethylmaleimide-sensitive factor (NSF) using adenosyltriphosphate (ATP) in cooperation with α-SNAP (Glick and Rothman, 1987; Söllner et al., 1993a). Vesicle fusion may occur Ca^{2+}-dependent or - independent (Geppert et al., 1994).

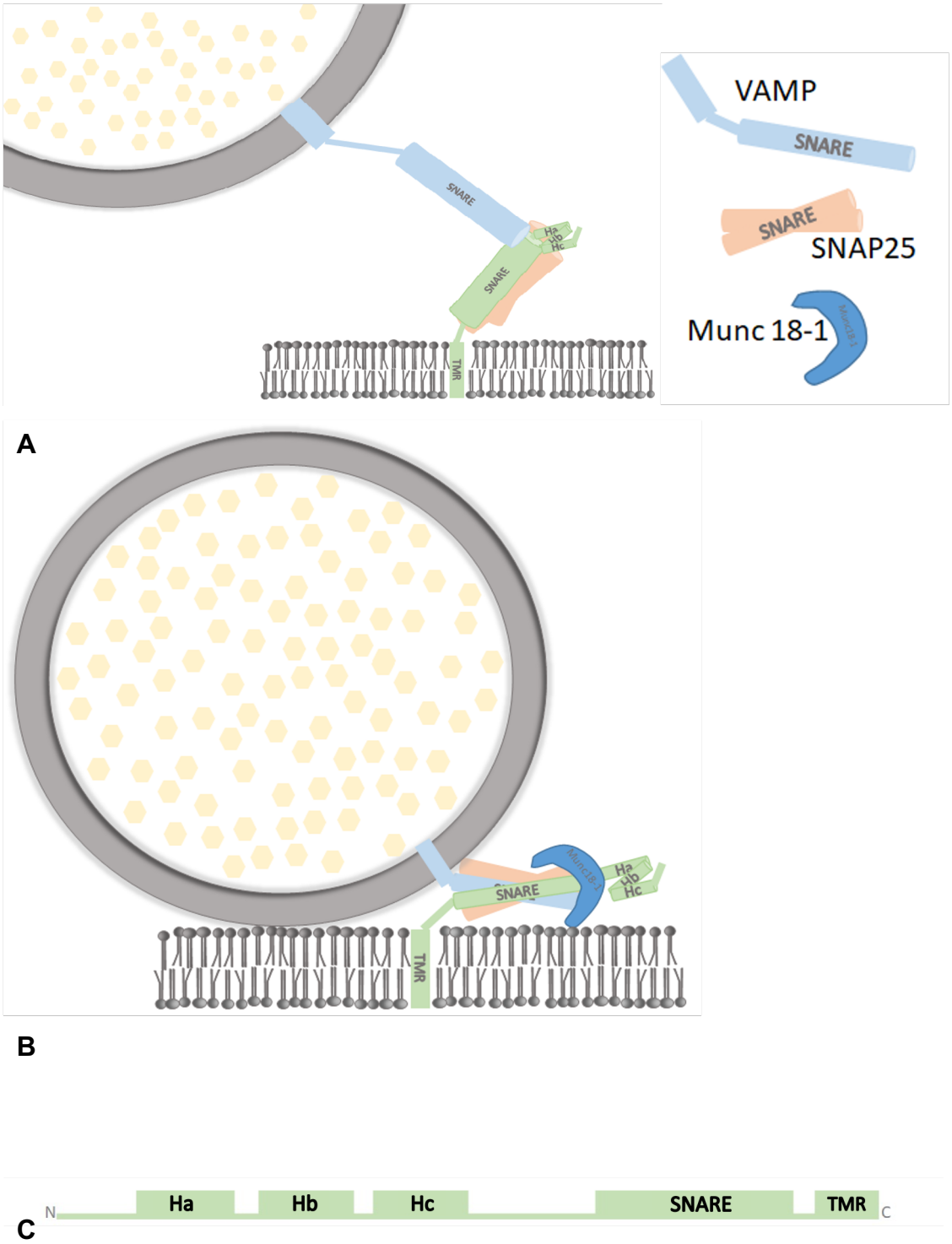

Figure 1: Vesicle fusion is driven by the SNARE-proteins: syntaxin-1 (green), SNAP25 (orange) and VAMP2 (blue). SNAP25 and VAMP2 are integrated into the vesicle membrane and syntaxin-1 in the presynaptic membrane with their transmembrane domain. The cytoplasmatic SNARE-motifs drive the fusion of both membranes. In vesicle docking (A), the helices bind to the N-terminus and start zigzagging into each other. In vesicle priming (B), almost all of the SNARE-motifs' length is involved in the interaction. Munc18-1 (dark blue) binds to the H_{abc}-domain of syntaxin-1 and thus stabilizes the conformation. (C) protein structure of syntaxin-1.

1.2.3 Syntaxin-1B

Syntaxin-1 is an integral membrane SNARE-protein with two homologues, syntaxin-1A and syntaxin-1B, which are 84% identical on their 288 amino acids' sequence (Bennett et al., 1992). Both consist of a C-terminal transmembrane region (TMR), the SNARE-motif, the H_{abc}-domain with three autonomously folded helices and the N-terminus (Fernandez et al., 1998, Figure 2). Whereas syntaxins are expressed ubiquitously in eukaryotic cells, syntaxin-1A and -1B are specific to the nervous system (Bennett et al., 1993) in which they play an essential role in Ca^{2+}-triggered synaptic transmission.

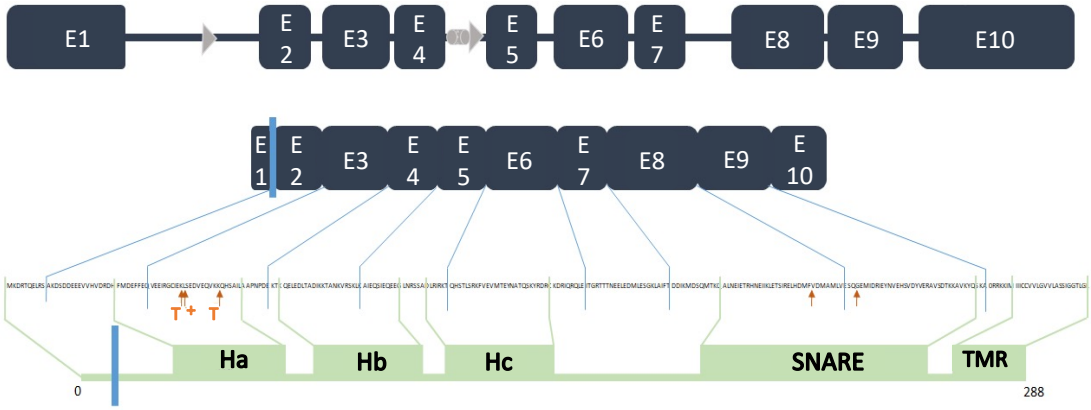

Figure 2: Genetic and protein structure of syntaxin-1B. The gene consists of 10 exons assembling to a protein of 288 amino acids. In the quaternary structure, the protein contains a transmembrane region (TMR), the SNARE-motif and the regulatory H_{abc}-domain. The grey arrow in the exon structure shows the gene sites that were loxed in order to generate the stx1B-ko-mouse model (see 2.1.1). The blue bars show where the protein thus ends and that no functional domain is left. Orange arrows show examples of variants that were found in individuals with GEFS+.

In the process of vesicle fusion, as described above, syntaxin-1 is initially attached to the presynaptic plasma membrane by the C-terminal domain and the N-terminal reaching into the cell with its H_{abc}-domain folding back onto the SNARE-motif. In this so called "closed state" Munc18-1 binds to syntaxin's N-terminus and stabilizes the conformation (Dulubova et al., 1999; Hata et al., 1993). Upon the initiation of docking, Munc-13 opens syntaxin by changing the H_{abc}-domains' position from closed (H_{abc} folded back onto the protein) to open state and thus allows the SNARE-motifs of the three SNARE-proteins to zip together (Richmond et al., 2001). Once the complex is fully assembled, Munc18-1 binds to the open syntaxin-1 within the four helix SNARE-bundle (Dulubova et

al., 2007). Depending on its binding site, Munc18-1 interactions have diverse effects: binding to the N-terminus of open syntaxin stabilizes Munc18-1 and facilitates fusion, whereas binding to the H_{abc}-domain of closed syntaxin regulates vesicle fusion (Zhou et al., 2013). Moreover, a constitutively open STX1B increases the number of assembled SNARE-complexes, thus accelerates vesicle fusion and causes epileptic seizures in a mouse model. In conclusion, the open-close dichotomy and the interaction of syntaxin-1, Munc13 and Munc18-1 regulates the rate of vesicle fusion (Gerber et al., 2008).

STX1B is also essential for early childhood survival in animal models, whereas deficiency in STX1A is not (Mishima et al., 2014, Vardar et al., 2020). In experiments with botulinum toxin, a potent neurotoxin that cleaves SNARE-proteins, decreased intracellular membrane traffic was identified as reason for the decreased neuron development in mice lacking SNAP-25 and syntaxin-1 (Peng et al., 2013). Interestingly, the exclusive knockout of *Stx1b* did cause a reduced quantity of excitatory and inhibitory neurons in cerebellar cultures leading to a reduced frequency of miniature inhibitory postsynaptic currents (mIPSC) in likely inhibitory neurons. However, there was no change of miniature inhibitory or excitatory postsynaptic currents (mEPSC) in autaptic, excitatory hippocampal and inhibitory striatal neuron cultures, in which cell growth was not impaired. The authors conclude, that the impairment of neuronal transmission occurs due to impaired neuronal survival but not due to an impaired vesicle fusion process (Wu et al., 2015).

1.2.4 Synaptopathies

In epilepsy, multiple ion channels with several variants have been identified as cause for the imbalance between excitation and inhibition in the human brain (Lerche et al., 2013; McTague et al., 2016). Additionally, variants in synaptic proteins are increasingly emerging as a clear cause for genetic epilepsies (Koko et al., 2021; Cassilas-Espinosa et al., 2012). To highlight their involvement in a variety of neurological diseases like Huntington's disease, autism spectrum disorder, Alzheimer's and Parkinson's disease the term "synaptopathies" has

been coined (Ferreira et al., 2015; Huguet et al., 2013; Li et al., 2003; Rockenstein et al., 2014).

A share of families with GEFS+ were identified by next generation sequencing to inherit heterozygous *STX1B*-variants (Schubert et al., 2014). With further investigation, additional patients were described with phenotypes ranging from febrile and afebrile seizures to GGE, DEE as well as focal epilepsies (Tian et al., 2019; Vlaskamp et al., 2016; Wolking et al., 2019).

Stx1b was knocked down in zebrafish larvae to better understand the pathomechanism. Local field potential analysis showed epileptogenic patterns with a more frequent occurrence at higher temperatures. The knock-down effect was reversible by transfection and overexpression of wild-type human *STX1B* but not the mutated gene, proving a causative role of the *STX1B* variant (Schubert et al., 2014). The phenotype seems to depend on the type of variant: whereas missense variantss in the SNARE-motif cause more likely severe syndromes like DEE, truncating variants with a complete loss of function result more often in milder syndromes, such as GEFS+ (Wolking et al., 2019).

A first functional study in rodents investigated two point mutations within syntaxin-1B SNARE-motif (G226R and V216E) and an InDel-variant in the H_{abc}-domaine in neuronal cultures. The InDel-variant's phenotype is described as similar to an early truncating variant which is associated with the rather mild types of epilepsy in GEFS+, whereas the V216E and G226R variants both cause DEE with pharmacoresistence (Schubert et al., 2014; Wolking et al., 2019). The InDel-variant decreased helicity of the H_{abc}-domain containing the Munc18-1-binding-site and decreased thermostability of the STX1B-protein. This led to an impaired interaction of $Stx1B^{InDel}$ with Munc18-1 and a reduced expression of both as they act as chaperones for each other. This and its instability were identified to be the reason for the highly impaired function. Similarly, G226R showed a decreased binding to Munc18-1 and a 60 % reduction in protein expression. Both, G226R and V216E variants alter Munc-13 interaction with syntaxin-1B but in a divergent manner: whereas $STX1B^{G226R}$ shows an enforced binding to Munc-13, $STX1B^{V216E}$ impairs the binding. Furthermore, V216E leads to an increased

fusogenicity with increased mEPSC frequency. Based on these effects on the protein interaction level, Stx1B^{V216E} was characterized as gain-of-function and Stx1B^{InDel} and Stx1B^{G226R} as loss-of-function variants. However, when *STX1B*$^{+/-}$*;STX1A*$^{+/+}$ neurons were transduced to simulate the genetic background of the patients, no changes in synaptic output or vesicle priming could be identified. The epileptogenic potential of the described changes in protein interaction thus remain to be explained (Vardar et al., 2020).

Recently, the group of Mishima et al. took the febrile seizures occurring in childhood into consideration for epileptogenesis. In their study, *Stx1b*$^{+/-}$-mice were first exposed to febrile and later to drug-induced seizures. Afterwards neurons from hippocampus were cultured and examined for their synaptic transmission and neuronal network activity at normal (35°C) and elevated (40°C) temperature. Interestingly, whereas wildtype neurons adapted to hyperthermia by reduced GABA-reuptake by the presynaptic GABA-transporter GAT1 and therefore enhanced inhibitory, postsynaptic $GABA_A$-currents, no adaption was observed in STX1B$^{+/-}$ mice. The exact mechanism of the interaction between syntaxin-1B and GAT1 remain to be explored. However, the study implies an impaired GABA reuptake and consecutively reduced inhibitory input during fever episodes as crucial contributors to epileptogenesis in STX1B-related GEFS+-patients (Mishima et al., 2020).

1.3 Research question

As a next step in understanding the underlying mechanisms of epileptogenesis in rodents due to *STX1B* knockout (KO), we studied the electrophysiological properties of hippocampal pyramidal cells in acute brain slices in a heterozygous *Stx1b*-KO mouse model. Whole-cell patch-clamp recordings allowed to record the miniature postsynaptic currents and to compare these currents between wild-type- and heterozygous KO-mice to estimate the vesicle fusogenity. The primary parameter of observation was the frequency of mIPSCs as this mainly depends on the number of vesicles fusing with the presynaptic membrane. Additionally, amplitude and curve properties of the postsynaptic currents were studied.

Furthermore, network effects were investigated in the same acute brain slices using extracellular recording of multiunit activity.

2 Material and Methods

2.1 Breeding

Five heterozygous, female *Stx1B*-KO-mice were kindly provided by Prof. Rosenmund, Charité Berlin, and transferred to the FORS/HIH animal facility in Ottfried-Müller-Straße 27, 72076 Tübingen, which were crossed with male C57BL/6N mice to generate the colony.

2.1.1 Generation of Stx1B-knockout mice

This procedure was performed and published previously (Wu et al., 2015).

Briefly, on a plasmid containing the *Stx1B*-gene LoxP sites were introduced between exon 1 and 2 (upstream) and exon 4 and 5 (downstream). A neomycin cassette with FRT sites was inserted between exon 4 and the 3' loxP site as a selectable marker. This construct was cloned into a P[acman] vector, linearized and electroporated into AB2.2 embryonic stem cell. Southern blotting was performed to select positive clones which were then injected into C57BL/6-blastocysts. Chimeric descendants were backcrossed to C57BL/6 mice. As homozygous *Stx1B*-KO mice died soon after birth, heterozygous mice were kept for the ongoing breeding colony (Wu et al., 2015).

2.1.2 Living and mating conditions

All experiments have been approved by the local Animal Care and Use Committee (Regierungspräsidium Tübingen, Germany). The animals were held in Typ II long cages with continuous fresh air supply and free access to food and

water. They were overseen by trained animal care takers of the FORS animal facility.

Males were mated with one or two females, with one gender being wildtype and the other being heterozygous *Stx1B*-KO mice. Litters were carefully documented and separated from their parents on postnatal day 17-20 grouped by gender. At this point they were genotyped by ear punch tissue (see 2.4).

2.2 Preparation of acute hippocampal slices

Experiments were carried out between postnatal day 15 and 20. Pups were picked without knowledge of the genotype. At no point was there any sign of sickness or suffering.

Mice were anesthetized with isoflurane within their cage. Deepness of anesthesia was checked by observation and tail tweaking. When no pain reflex was recognized anymore, the mice were taken out of their cages. Decapitation was carried out immediately by a smooth cut directly behind the skull with scissors.

The mouse head was transferred to a Petri dish with ice-cold cutting artificial cerebrospinal fluid (aCSF; see 2.3.2.1). The fluid was constantly bubbled with carbogen (95% O_2, 5% CO_2) calibrating the pH to 7.4. The skin was pulled from neck to eyes to uncover the skull itself, which was cut carefully with a scalpel on the median sagittal line from forehead to neck. The calvaria was removed laterally with forceps, revealing the brain. Using a dulled spatula, the mouse brain was removed out of the skull and placed in another Petri dish with fresh ice-cold, carbogenated cutting aCSF. All these procedures were performed as quickly as possible.

At this point the brain was inspected briefly for any injury caused by the preparation procedure. The cerebellum and brainstem were removed using a clean razor blade. A drop of glue (Uhu Sekundenkleber) was given onto a dry metal plate, which was freshly taken from the freezer to maintain the cold temperature. The drop of glue was evenly distributed over an area of about 1 cm^2. To obtain transversal slices the mouse brain was flipped onto its dorsal side,

placed on the glue and gently pressed by a brush to promote attachment to the metal surface.

The table was fixed magnetically to the slicing chamber of the Microm HM 650 V vibratome (Thermo Fisher Scientific), which was filled with iced cutting aCSF and aerated with carbogen continuously. With a razor blade on the slicer's cutting head and speed set to level 8, slicing was started on the ventral side of the brain. The slices were discarded until the hippocampus became visible, at which point the thickness was set to 350µm. The hemispheres of each slice were separated along the midline and transferred carefully to a storage chamber with aerated recording aCSF using a glass pipette.

This storage chamber was a glass beaker filled with recording aCSF (see 2.3.2.1) and placed in a water bath set to 36°C. It contained a dish fixed about 2 cm above the beakers ground in which the plastic bottom was replaced by a thin layer of stretched nylon mesh. With additional infusion of carbogen, this construction guaranteed good oxygen supply on both sides of the slices as well as minimal mechanic manipulation.

Slices were left to equilibrate and recover for 60 minutes. During recording the slices within the storage chamber were kept at room temperature.

2.3 Electrophysiological recordings

The function of excitable cells is mainly carried out by voltage-gated ion channels in the cell's membrane, that allow in- and outflow of ions with a certain charge. To measure the net current flow through the membrane and thus the voltage between the intracellular and extracellular compartment, a cell is connected to a current circuit in the patch-clamp technique.

2.3.1 Whole-cell patch-clamp technique

2.3.1.1 Whole-cell patch clamp configuration

A single cell gets integrated into an electric circuit by placing one electrode in the extracellular bath solution and the other electrode within the intracellular space.

The latter connection is obtained by filling a glass pipette with a solution that imitates the intracellular ion composition (see 2.3.2.1) and inserting an electrode into this solution. When the tip of the pipette touches the cell's surface with an angle of about 30°, it is slightly pressed onto the membrane obtain a larger area of contact between membrane and pipette tip. By applying a short impulse of suction via a syringe connected to the pipette, a very tight connection with a resistance of > 1 GΩ between pipette and membrane is established. Once this so called gigaseal is stable, the cell membrane gets ruptured by another suction impulse and the whole-cell configuration is established. Intracellular solution and pipette solution can mix and find equilibrium.

2.3.1.2 Voltage clamp

Both, bath electrode and pipette electrode, are connected to an amplifier, that allows to measure the voltage over the membrane. This amplifier is the central component in controlling the cell's membrane potential. For a set membrane potential, it corrects the measured voltage by current injections over the intracellular electrode creating a constant feedback circle. Thus, the membrane potential is clamped to a certain voltage and the injected current allows a direct measurement of the membrane ion flow, as it is the same in quantity but the opposite charge of the current flow over the membrane.

2.3.1.3 Miniature postsynaptic currents

As mentioned earlier, vesicle fusion is a process of multiple steps with checks and balances, that start either due to a presynaptically arriving action potential or in very small amounts spontaneously. When vesicles fuse and release the neurotransmitter, the neurotransmitter binds to the postsynaptic receptors and cause an ion influx into the postsynaptic cell. Because the ions carry a charge, they create a current flow over the membrane. The small currents due to spontaneous vesicle fusion are called miniature postsynaptic currents (mPSC) To examine the functionality of the vesicle fusion machinery, the likelihood of the spontaneous fusion events is measured by recording their postsynaptic effect. Using the whole-cell patch-clamp technique with a voltage clamp mode set to a physiological holding potential of about -70 mV allows to record mPSC.

Excitatory and inhibitory input may be distinguished by blocking the specific receptors for one or the other. This clarifies the spatial summation of simultaneously inflowing anions and cations, resulting in purely miniature inhibitory postsynaptic currents (mIPSC) or purely miniature excitatory postsynaptic currents (mEPSC).

2.3.2 Intracellular whole-cell patch-clamp recordings

2.3.2.1 Solutions

Two types of artificial cerebrospinal fluid (aCSF) were mixed for these experiments: 1) a cutting aCSF used for brain preparation and cutting on the vibratome and 2) a recording aCSF used for the bubble chamber as well as the recording chamber. Both were stored as 10-fold concentrated stock solution without the glucose, $CaCl_2$ and $MgCl_2$ to avoid precipitation.

The cutting aCSF contained in mM: 125 NaCl, 25 $NaHCO_3$, 2.5 KCl, 10 glucose, 1.25 NaH_2PO_4, 1 $CaCl_2$, and 7 MgCl. The recording aCSF contained in mM: 125 NaCl, 25 $NaHCO_3$, 10 glucose, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, and 1.25 NaH_2PO_4. For both variants of aCSF the pH was adjusted to 7.4 using NaOH or HCl and osmolarity to ~310 mOsm using water or mannitol.

The pipettes were filled with an internal solution containing in mM: 136 KCl, 17.8 HEPES, 1 EGTA, 4.6 $MgCl_2$, 4 Na_2-ATP, 0.3 Na-GTP, 12 creatine phosphate and 50 U/ml phosphocreatine kinase. Using KOH the pH was adjusted to 7.4 and osmolarity to 300 mOsm using water. To keep it stable the solution was aliquoted and frozen to -20°C and melted slowly on the day of experiment but kept on ice to avoid degradation of the creatine phosphate, ATP and GTP. As recordings occurred to be unstable and gigaseal was not stably accomplished, we excluded the phosphocreatine kinase from our recipe for internal solution which turned out to facilitate recordings considerably.

2.3.2.2 Electrophysiology

Pipettes were pulled with a Sutter P97 Puller (Sutter Instruments) from borosilicate glass capillaries GB100F-10P (Science Products GmbH) with a final

resistance of 2-6 MΩ when filled with internal solution (see 2.3.2.1). An Ag-AgCl-electrode was fixed to the pipette holder and inserted into the pipette's lumen. Data was collected by a MultiClamp 700B amplifier with, DigiData 1420 digitizer and Clampex 10.7 software (all Molecular Devices, San Jose/USA). The slice recording chamber, pipette and table manipulators as well as temperature controller were produced by Scientifica (United Kingdom), whereas the microscope was BX61WI from Olympus. The fluids were recycled by a Gilson MINIPULS 3 pump and carbogenated in a small reservoir within that circle.

2.3.2.3 Whole-cell patch-clamp recording

Electrophyisological whole-cell patch-clamp recordings were performed at 30 ±1°C on pyramidal neurons in the CA1 hippocampal region. The slices were continuously superfused with carbogenated recording aCSF in the recording chamber and the pyramidal cells in hippocampal CA1 can be identified according to their distinct morphology visualized under the microscope.

Cells were patched and opened once a gigaseal was established. After opening the cell to get the whole-cell configuration, the cell was left to rest for at least 3 min to allow the fluid exchange between the intracellular and the inner of the pipette. Series resistance was kept below 10 MΩ and compensated with 85%.

The first cell on every experimental day was patched without any drugs and checked for normal sodium currents. This was achieved by clamping the cells at -70 mV and applying depolarizing voltage steps from -80 to 90 mV with an increment of 10 mV. Leaving the whole-cell mode unchanged, tetrodotoxin (TTX) was used to block sodium channels and thus action potential induced vesicle fusion and additional drugs were added to filter for either inhibitory or excitatory input. The additional drugs were either the GABA-blocker picrotoxin (PTX; 5 µM) to record mEPSC or the NMDA-antagonist (2*R*)-amino-5-phosphonopentanoate (AP-5; 50 µM) and the AMPA/kainate-receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 10 µM) to record mIPSC. The drugs were diluted in the recording aCSF and washed into the recording chamber. The wash-in of the blockers was monitored by observation of sodium currents, which were to be nulled by the TTX, in the same voltage step protocol as above.

A gap free protocol of 5 minutes in voltage clamp mode with a holding potential of -70 mV, a sampling rate of 100 kHz and lowpass filter at 1 kHz was used to record mIPSCs and mEPSC.

2.3.2.4 Quality control parameters

The pipette shape and resistance were checked under the microscope after filling with internal solution within the bath solution.

For quality control the following recording parameters were monitored and had to be met throughout the experiment to be included in the analysis (modified from Molleman, 2003):

- seal resistance between cell and pipette tip >1GΩ
- leak current in whole-cell configuration <100pA
- series resistance <20MΩ
- stable cell capacitance and resistance

The membrane potential was checked to be more negative than -55 mV and the input resistance was calculated by episodic stimulation starting with -10pA and going up to -110pA in 10pA-steps in current clamp mode.

2.3.3 Extracellular recordings of multiunit activity

Extracellular recordings of multiunit activity (MUA) in the hippocampal CA1 and CA3 region were performed on the same day and with acute brain slices from the same animals as those used for intracellular recordings.

2.3.3.1 Set-up

Glass-pipettes were pulled as described above, polished slightly on sandpaper to have a wider opening and installed on Ag-AgCl-electrodes. The signal was amplified 10 000-fold and filtered between 300 and 2000 Hz by an extracellular amplifier (Modell 1700 A-M-Systems, Washington, USA). Rectification and integration were carried out online to facilitate assessment of the traces' quality by Model INR-011 (NPI, Germany) with a time constant of 50-100 ms. A Gilson

MINIPULS 3 pump circulated the recording aCSF with a temperature of 30°±1°C between the recording chamber and carbonating reservoir.

2.3.3.2 Recording of multiunit activity

Slices were transferred from the bubble chamber into a BSC-BU-brain slice chamber from Medical Systems Corp. The electrodes were lowered into the bath solution and allowed to fill with the extracellular bath fluid by capillary forces. Two electrodes were positioned on the same slice above hippocampal CA1 and CA3 regions, respectively. When the contact between glass and tissue caused some irritation on the cells as displayed by visual and acoustic signal on Clampex 10.7 sofware, they were left to rest for at least 10 min. By this time, we checked for network activity on the integrated traces. If there was no network activity the electrodes were repositioned several times. If no clear MUA-signal could be found, the slices were discarded. When a clear network activity was visible, a gap free protocol recorded this for 20 minutes.

2.4 Genotyping

Genotyping was performed with frozen tail tips cut from experimental mice after decapitation or ear punches obtained from littermates who were kept for breeding purposes.

2.4.1 DNA preparation

The tissue was lysed by adding 10 µl of protein kinase K (BIO-37084, Bioline) and 50 µl of a lysis buffer containing 5 ml 1M Tris-HCl pH 7.5, 10 ml 0.5M EDTA pH 8.0, 2ml 5M NaCl, 0.5 ml 1M DTT, 50 µl 1M Sperimidine, 20 ml 10% SDS and 62.45ml H_2O to each sample and incubating it over night at 56°C shaking at 500 rpm. To separate lysed DNA from hair and intact tissue the mixed solution was centrifuged for 5 minutes at 12 000 rpm. The supernatant was transferred to a new tube. To precipitate the DNA 350 µl 100% isopropanol were added into the supernatant. After another 5 minutes centrifugation at 12 000 rpm, the supernatant was discarded and the pellet was washed by 350 µl 70% ethanol,

which was discarded after centrifugation for 5 minutes at 12 0000 rpm. The pellet was left to dry in the air and finally dissolved in 40 µl H_2O to obtain the DNA.

2.4.2 PCR protocol

Since the bands for *Stx1B* wild-type and knock-out alleles are very close to each other, the polymerase chain reaction (PCR) should be performed separately for *Stx1B* wild-type and knock-out gene. Two batches were mixed per probe, the first one was to amplify the *Stx1B*-wildtype-gene, the second one was for the knockout-construct. For each 1 µl isolated DNA was mixed with 10 pmol forward and 10 pmol reverse primer, 12.5 µl My Taq Red Mix (Cat.No. BIO-25044, Bioline, United Kingdom) and 10.5µl water. The reverse primer was the same for both batches whereas the forward primers differentiated (foreward for wildtype: F1: 5-GTT TCC GCC TGA ATT GCA CCT G-3; KO: F2: 5-CAT AGC CTG TCT GAC TTC CAG-3, reverse for both: R1: 5-CTG GCA CCA GCA GAC AAG GAG-3 (Wu et al., 2015)). The PCR was run on a Biometra T3 thermocycler as specified in (**Fehler! Verweisquelle konnte nicht gefunden werden.**Table 1).

PCR step	Wildtype program	Knockout program
Inital Denaturation 94°C	3:00 min	2:00 min
Cycles	40	35
Initialization 94°C	0:30 min	0:15 min
Annealing 58°C	0:30 min	0:15 min
Extension 72°C	0:45 min	0:30 min
Final extension 72°C	3:00 min	2:00 min

Table 1 PCR protocol for genotyping of experiment animals and littermates.

2.4.3 Gel electrophoresis

About 1.5% agarose were dissolved in TBE buffer, stained with RedSafe (JH Science/iNtRON Biotechnology, USA) and poured into a gel electrophoresis chamber. After solidifying the gel was covered in TBE buffer and loaded with 12µl of every PCR product. An electrical field of 120 mV was applied for 1-1.5 hours on the chamber. The DNA bands were visualized by UV-light imaging. For every

mouse there were two different PCR-products with a length of 500bp for the wildtype and 550bp for the knockout-program.

2.5 Data analysis and statistics

2.5.1 Analysis of mEPSC and mIPSC on Clampfit

Recorded traces of both, mEPSC and mIPSC recordings, were loaded into Clampfit (Molecular Devices) and lowpass-filtered with a 1kHz Bessel filter if necessary. Templates were created for mEPSC and mIPSC respectively containing the data of at least 400 single events. These were then used in Template Search mode to identify further events. Events not fitting the visual criteria of a miniPSC were rejected manually and excluded from analysis. Data was copied to Microsoft Excel for further processing.

2.5.2 Analysis of extracellular recordings on MATLAB

The .abf-files were converted into .atf files containing one of the recorded traces and loaded into MATLAB (Massachusetts, USA). MATLAB scripts provided by Dr. Henner Koch were applied to cut out artefacts, integrate and detect UP-states in the traces. The output was transferred to Excel for further statistical processing.

2.5.3 Descriptive and inferential statistics

All data are depicted as means ± standard error of the means. Every data point represents one cell. Outliers were characterized as higher or lower values than 2 standard deviations from the mean and excluded from analysis. Combining literature and unpublished data from Dr. Yuanyuan Liu, we expected the knockout effect to be a decreased frequency in inhibitory postsynaptic currents. As we expected the knockout effect to be a decreased frequency in mIPSCs, this parameter was prioritized as the primary measuring and analyzed statistically. Secondly, frequency of mEPSC and amplitude of both input types as well as curve properties such as half-width, decay slope and decay time were taken into

the analysis, too. As normal distribution was not given, the Wilcoxon-ranksum-test was applied, and the secondary parameters p-values were corrected for multiple testing using the false detection rate (FDR).

2.5.4 Post-hoc power analysis

The animal numbers were very different in the two groups for both, mEPSC and mIPSC, and the results were different from what we saw in neuron cultures (unpublished data, Yuanyuan Liu). Therefore, a post-hoc power analysis was performed with the G*Power-Software (Faul et al., 2007) to gain insight into the results accuracy.

3 Results

To obtain further insight into the pathogenesis, an *Stx1b*$^{+/-}$ mouse model was employed for single-cell recordings by the whole-cell patch-clamp technique and extracellular recordings in acute brain slices.

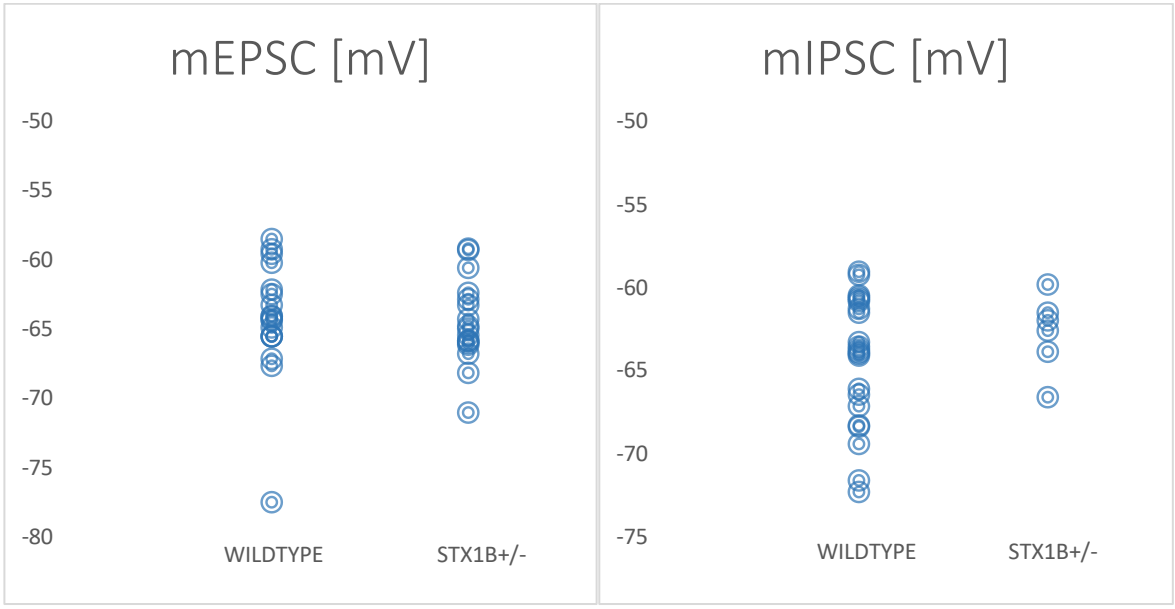

Figure 3: Resting membrane potential as determined by whole cell patch recording, also serving as a quality parameter for the recordings in general.

3.1 Miniature inhibitory postsynaptic currents

In epilepsy, the physiological balance of excitatory and inhibitory neurotransmission within the brain is disturbed in favor of excitation, which leads to the hypersynchronic activation of firing during an epileptic seizure. Since we decreased the number of functional *Stx1b*-allels and we assumed this to have an epileptogenic effect, we expected an accordingly decreased frequency of inhibitory input on neurons.

The mice were killed at postnatal days 15-20 as a resemblance of early human childhood, when febrile seizures occur most often (Dutta and Sengupta, 2016). To preserve as much of the network activity as possible, acute brain slices were obtained. Hippocampal pyramidal neurons were patched due to their clear anatomy and the well-known progression of febrile seizures into epilepsy with hippocampal sclerosis (Accardi et al., 2018; Baulac et al., 2004). To extract mIPSCs from all spontaneous and excitatory postsynaptic activity, sodium currents were blocked by TTX and mEPSCs by AP-5 and CNQX. A gap-free protocol was recorded for 20 minutes and the traces were analyzed off-line using Clampfit software (Figure 4).

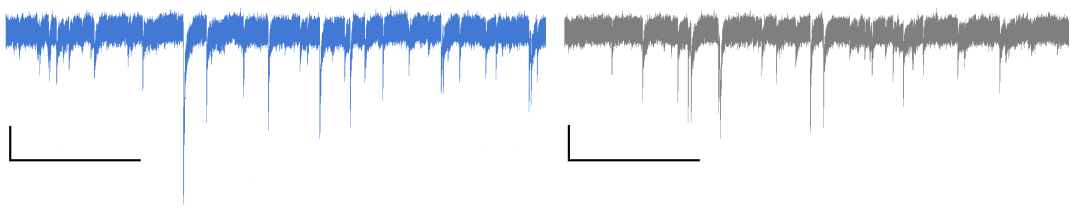

Figure 4: Sample traces of intracellular mEPSC recordings. Blue – wildtype. Grey – *stx1B*-knockout. Horizontal line shows 500ms, vertical line shows 50 mV.

For recordings of mIPSC 11 wildtype and 3 heterozygous mice were examined, resulting in data from of 53 wildtype and 13 heterozygous cells. The average mIPSC frequency did not show a significant difference between WT and *Stx1b*$^{+/-}$ mice (WT: μ = 5.36 Hz, SD = 3.42 Hz, n = 52 cells; Stx1B$^{+/-}$: μ = 3.98 Hz, SD = 2.23 Hz, n = 13 cells; p = 0.157, Wilcoxon-rank-sum-test). Since there is a small but not significant difference towards the hypothesized effect, the fact that this data arises from only 3 heterozygous animals should be taken into consideration.

On further analysis, there was no significant difference between the two groups regarding the amplitude of mIPSC. We used the Clampfit software to measure additional properties of mIPSC, like half-width, decay slope and decay time, but could not find any significant difference there, too (Figure 1, Figure 5, Table 2A).

A	frequency		amplitude		half-width		decay slope		decay time	
	Hz	n	pA	n	ms	n	pA/ms	n	ms	n
WT	5.36 ± 0.47	52	-42.63 ± 2.56	52	3.35 ± 0.06	50	2.70 ± 0.13	53	6.64 ± 0.08	51
+/-	3.98 ± 0.62	13	-34.96 ± 5.85	12	3.08 ± 0.1	13	2.29 ± 0.28	12	6.78 ± 0.08	12

B	frequency		amplitude		half-width		decay slope		decay time	
	Hz	n	pA	n	ms	n	pA/ms	n	ms	n
WT	1.61 ± 0.2	36	-18.34 ± 0.96	37	2.37 ± 0.07	36	2.05 ± 0.09	37	5.49 ± 0.12	37
+/-	2.09 ± 0.39	15	-20.34 ± 1.29	15	3.05 ± 0.18*	16	1.89 ± 0.12	15	6.31 ± 0.15*	15

Table 2: Miniature inhinibitory postsynaptic currents of hippocampal CA1-neurons in wildtype and heterozygous STX1B-ko-mice. The table depicts miniature postsynaptic currents. A: mIPSC were recorded under 10 μmol CNQX and 50 μmol AP-5. B: mEPSC were recorded under 5 μmol picrotoxin. Data are presented as mean ± SEM, * $p < 0.001$, Wilcoxon rank-sum test. +/- heterozygous STX1B-ko. n – number of cells.

3.2 Miniature excitatory postsynaptic currents

In order to obtain a complete picture of the excitation-inhibition-balance, the recordings were extended to mEPSC. Therefore, sodium currents were blocked by TTX and inhibitory GABA-receptors by picrotoxin. In total 8 wildtype and 5 heterozygous mice were used, yielding data of 38 wildtype and 16 heterozygous cells. We could not find any significant difference on amplitude, frequency and decay slope (Table 2 B, Figure 5). The two groups did show significant differences regarding curve properties of mEPSC, as the half-width in *Stx1b*$^{+/-}$-mice was longer with an accordingly longer decay-time.

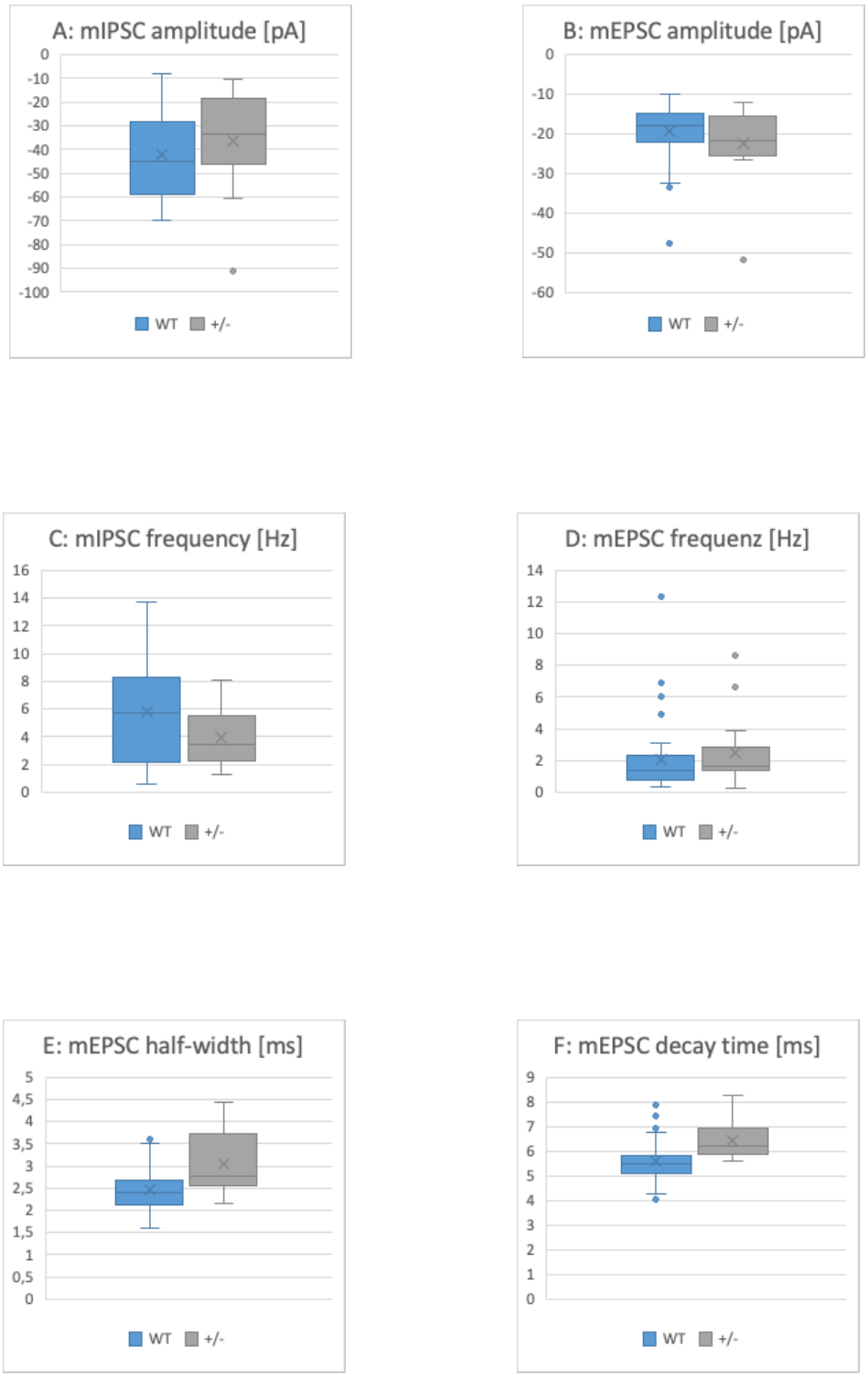

Figure 5: Results of whole-cell patch clamp recordings. A: amplitude of mIPSC (nWT=52, nStx1b+/- = 12). B: amplitude of mEPSC (nWT = 37, nStx1b+/- = 15). C: frequency of mIPSC (nWT = 52, nStx1b+/- = 13). D: frequency of mEPSC (nWT = 36, nStx1b+/- = 15). E: half-width of mEPSC is significantly longer in Stx1b+/- neurons (nWT = 36, nStx1b+/- = 16, $p < 0.001$). F: decay time of mEPSC is significantly longer in Stx1B+/- neurons (nWT = 37, nStx1b+/- = 15, $p < 0.001$). Blue – wildtype. Grey – Stx1b+/-.

3.3 Extracellular recordings

The human brain consists of single cells working together as a network. A critical feature of this interplay is the degree of synchronicity or order, in which the neurons fire. With very little order, the distinction of more and less important signals is not possible, leading for example to schizophrenia and autism (Wilson et al., 2007; Yizhar et al., 2011), whereas too much order might end up in hypersynchronicity, which is a typical sign of epileptic seizures. The interplay of neuronal connectivity and network activity has thus become a focus of epilepsy research (Meisel, 2016; Meisel et al., 2015; Yang et al., 2012).

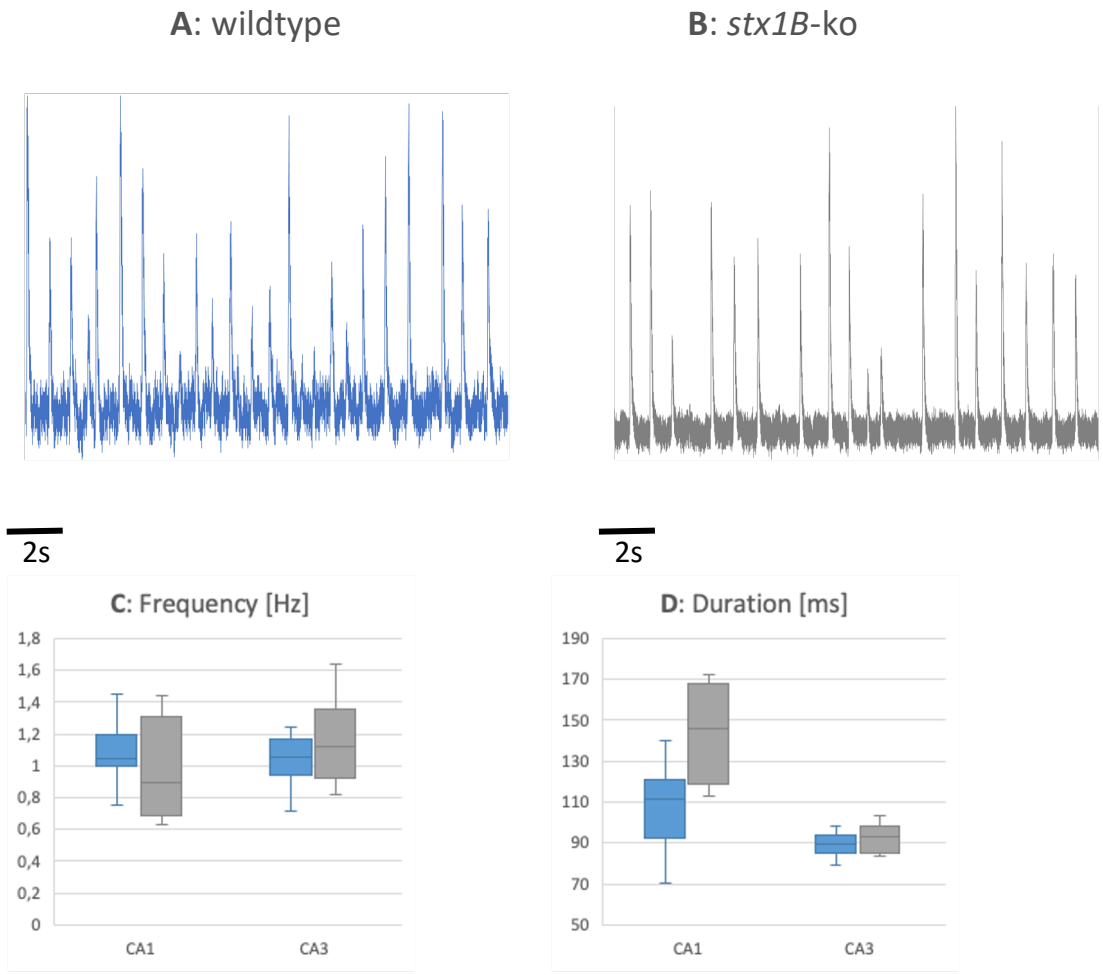

Figure 6: Extracellular recordings of hippocampal CA1 and CA3 region in acute brain slices of wildtype and *Stx1b*$^{+/-}$-mice. A and B: sample traces of extracellular multi-unit activity. C and D: frequency and duration of UP-states of multi-unit activity. Blue – wildtype. Grey – *Stx1b*$^{+/-}$.

Due to the role of STX1B in neurotransmission, we performed extracellular recordings in acute hippocampal brain slices of WT and *Stx1b*$^{+/-}$-mice in CA1 and CA3 regions of the hippocampus to gain insight into network activity. Within the gap-free traces, up-states were identified offline by a computerized parameter search in MATLAB-software using the script in the appendix. No significant differences were found for frequency or duration in CA1 and CA3 (Figure 6). Since these results come from preliminary data with restricted numbers, further research will be needed.

3.4 Post-hoc power analysis

Since there was no marker to differentiate WT and mutant mice from each other, the genotype of the mice was unknown at the time of the experiment. When we genotyped the experimental animals and their breeding littermates, we found roughly half of them to be *Stx1b*$^{+/+}$ and the other half to be *Stx1b*$^{+/-}$ mice, as we would expect according to our breeding pattern. Thus, the differences in the high number of wildtype animals in our experiments seem to be due to coincidence. However, the low number of *Stx1b*$^{+/-}$-mice does weaken the power of our results.

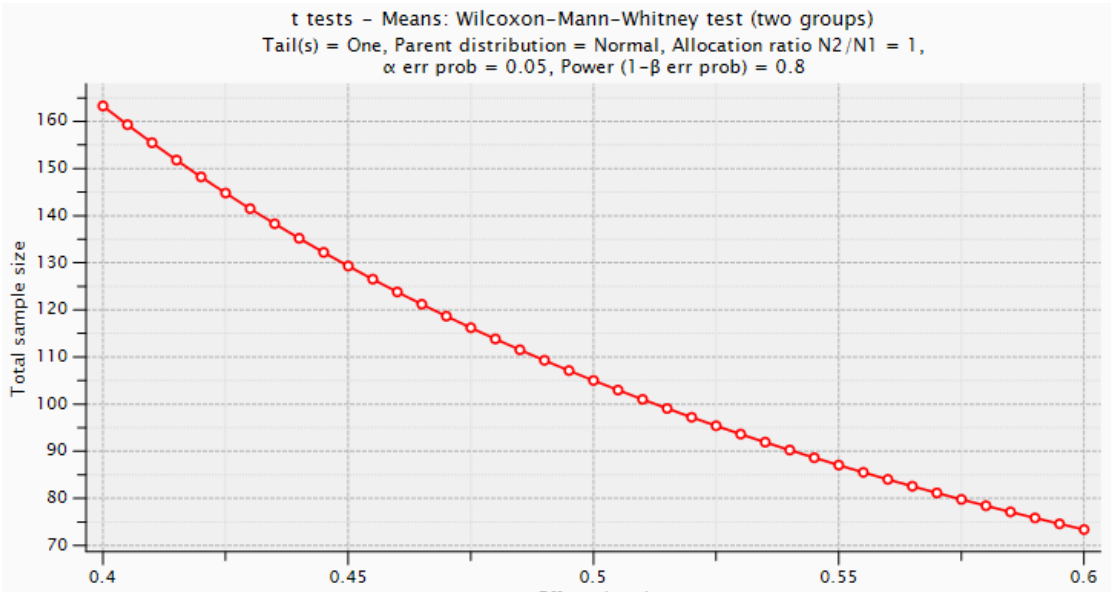

*Figure 7: G*Power software displaying the required sample size as a function of the effect size d with a Power of 0.8 and α of 0.05. The estimated sample size was calculated at d=0.474. For this effect size, the required sample size is 118 in total.*

Therefore, we used a post-hoc power analysis performed by G*Power software to estimate the results strength statistically (Faul et al., 2007).

We assumed that the values would be normally distributed, and the expected effect was a lower mIPSC frequency in the *Stx1b*$^{+/-}$-mice. The effect size was estimated by using the data that we acquired up to now, α was set to 0.05 and we aimed for a power of at least 0.8. The required sample size thus calculated was 59 per group (Figure 7). Thus, whereas the wildtype group almost reached this aim, the ko-group includes insufficient data.

4 Discussion

Synaptic processes gained attention when their principal mechanisms and later their relation to multiple neurologic disorders were discovered. Whereas in some of these the synapses may be affected during disease course, in some types of genetic epilepsies their dysfunction is the cause of seizure generation. *STX1B*, coding for a SNARE-protein that is involved in vesicle fusion at the presynaptic membrane, was identified as a candidate gene in several GEFS+-families from a large cohort of epilepsy patients (Schubert et al., 2014). In the constantly growing number of epilepsy patients with variants in *STX1B,* benign febrile seizures and febrile seizures+ as well as the epilepsy syndromes GEFS+, GGE, DEE and focal epilepsy have been identified. Whereas many patients respond well to pharmacotherapy, those with missense variants seem to have a worse progression and do hardly improve with AED treatment (Wolking et al., 2019). Former studies revealed the relevance of *STX1B* for neuronal survival and the regulatory role of the open-close-dichotomy of the H_{abc}-domain (Gerber et al., 2008; Mishima et al., 2014; Wu et al., 2015).

According to our hypothesis, a decreased inhibitory network activity in the brain causes greater excitability and thus a lower seizure threshold of STX1B-deficient epilepsy patients. To gain insight into such network effects in naturally grown neurons and synapses of rodents, we investigated the hippocampal CA1-region in acute brain slices of a *Stx1b*$^{+/-}$ mouse model, mimicking the genetic status of mostly mildly affected human individuals carrying heterozygous nonsense

variants. First, we employed the whole-cell patch-clamp technique to record postsynaptic effects on single pyramidal hippocampal CA1 neurons. Second, extracellular recordings were used to record multi-unit activity in the hippocampal CA1 and CA3-regions of these same slices.

4.1 Haploinsufficiency in syntaxin-1B does not have an effect on mIPSC frequency

Although we expected neurons derived from *Stx1b*$^{+/-}$ mice to receive a decreased frequency of inhibitory input in form of mIPSC compared to neurons from WT mice, we could not confirm this hypothesis. Overall, we did not find any significant difference pointing towards an epileptogenic mechanism regarding the single cell level except for a prolonged decay time of mEPSCs in *Stx1b*$^{+/-}$-neurons. One could conclude that the exocytosis of glutamate does not work as synchronized in these mice as it does in the wildtype animals, leading to a broader stretch of neurotransmitter-activated channel opening on the postsynaptic membrane. However, this change is small and affects only the repolarization phase of the mEPSC. As action potential generation depends on the all-or-nothing-dichotomy, it hardly depends on repolarization features. Therefore, we do not regard them as relevant for the epileptic seizures.

When Wu et al. examined mEPSC and mIPSC in autaptic neuronal cultures, there was no significant difference between wildtype, heterozygous and homozygous *Stx1b*-ko mice in striatal GABAergic neurons or hippocampal glutamatergic neurons either. Yet they did identify a comparatively high expression of *Stx1b* and a decreased number of synapses in cerebellar cultures of STX1B-deficient neurons. Accordingly, they found the expected decreased mIPSC frequency in heterozygous and homozygous *Stx1b*-ko cerebellar inhibitory neurons (Wu et al., 2015).

Furthermore, even though Vardar et al. found differences in protein interaction and vesicle fusogenicity in their transfected hippocampal autaptic neuronal cultures, they only detected function differences on a single cell level in an artificial system deficient of Syntaxin 1A, and they did not examine potential

changes in networks. When the STX1A was expressed, the postsynaptic input in the *STX1A*$^{+/+}$*;STX1B*$^{+/InDel}$ and *STX1A*$^{+/+}$*;STX1B*$^{+/G226R}$ neuron cultures remained equal (Vardar et al., 2020). The connection between protein action, synaptic transmission and seizure generation remains to be established.

4.2 Hippocampal CA1 region shows longer duration of up-states in preliminary data

On the network level, we could find a longer duration of excitable up-states in the CA1-region but would like to stress the fact, that this observation relies on very limited data. However, since the brain is a complex organ and synaptic plasticity is not yet fully understood, a greater excitability of the whole network may exactly pose a first hint on that other scale that we are looking for in epileptic synaptopathies.

4.3 Conclusion

Our study does not provide evidence to support the hypothesis of an impaired inhibitory input on the single cell level as a simple cause for GEFS+ in patients with *STX1B*-variants. This supports several former studies that could also not find an altered input in neuronal cultures when STX1A was present (Mishima et al., 2014; Vardar et al., 2020; Wu et al., 2015). However, although we relied on a mouse model of the patients' genetic situation for our experiments, we did not induce fever, that typically triggers seizures in patients. As we know from complex FS and FS+, these may change and damage the brain with long-term effects (Cendes et al., 1993; Dube et al., 2000; Eckhaus et al., 2013). Furthermore, the InDel-variant reduced thermostability of STX1B's regulatory H_{abc}-domain (Vardar et al., 2020). One could thus imagine that the initially occurring FS or perhaps even the fever itself initiate the epileptogenic process in the GEFS+ patients as supported by the neuronal culture experiments concerning GABA-release and reuptake in *Stx1b*$^{+/-}$-mice during hyperthermia (Mishima et al., 2020).

Additionally, whereas only syntaxin-1B is crucial for postnatal survival, its homologue STX1A has been shown to be functionally redundant for neuro transmission (Kofuji et al., 2014; Wu et al., 2015). *STX1B*-deficiency gains relevance on a *STX1A*$^{-/-}$-background (Vardar et al., 2020). One could thus imagine, that the decreased amount of STX1B in our heterozygous ko-mice is sufficient for neuronal development and its effects on vesicle fusion is rescued by STX1A. This redundancy may be different in humans and therefore mice may not be a sufficient model organism.

In conclusion, the present study suggests that even on a genetic mouse model of *STX1B*-related epilepsies, the epileptogenic process could not be revealed on a single cell level. Further electrophysiological investigations may include fever models, an approach on network level and the examination of knock-in point variants.

5 Summary

Genetic variants have been identified for many different epilepsy syndromes and understanding their pathomechanisms may lead to targeted therapies in epilepsy. In patients with GEFS+, GGE, DEE and focal epilepsies, variants in the presynaptic protein STX1B were discovered. STX1B as SNARE-protein is part of the vesicle fusion machinery responsible for neurotransmitter release into the synaptic cleft. STX1B, but not its homologue STX1A, is crucial for neuronal development and postnatal survival. In functional studies in zebra fish larvae with *stx1b*-knockdown and neuronal cultures with *STX1B* variants reduced expression, altered protein interactions and increased fever sensibility were detected. However, an effect on synaptic transmission was not observed up to now. We relied on a *Stx1b*$^{+/-}$mouse model and acute hippocampal brain slices to examine naturally grown neurons. The whole-cell patch clamp technique was employed to study inhibitory and excitatory synaptic input in hippocampal CA1 pyramidal neurons. Additionally, multi-unit network activity was recorded in hippocampal CA1 and CA3 regions. We could not find physiologically relevant differences for the recorded mIPSC and mEPSC. However, we did find a longer duration of up-states in *Stx1b*$^{+/-}$-mice demonstrating an increased excitability. As

these results come from only small numbers, they need to be confirmed by further investigations. In conclusion, we do complement former studies conducted in neuronal cultures and find that a simple impact on synaptic input is not the sole cause for the patients' epilepsy. Further research may concentrate on different induced fever models, steric effects of mutated *STX1B* and network effects.

6 Zusammenfassung

Genetische Varianten wurden für viele verschiedene Epilepsiesyndrome identifiziert und ihre Pathomechanismen zu verstehen kann zu zielgerichteten Therapien führen. Bei Patienten mit GEFS+, GGE, DEE und fokalen Epilepsien wurden Varianten in dem präsynaptischen Protein STX1B entdeckt. STX1B ist als SNARE-Protein Teil des Vesikelfusionsapparates, der für die Neurotransmitterfreisetzung in den synaptischen Spalt verantwortlich ist. STX1B, aber nicht sein Homolog STX1A, ist essentiell für neuronale Entwicklung und postnatales Überleben. In funktionellen Studien an Zebrafischlarven mit stx1b-knockdown und Neuronenkulturen mit STX1B-Varianten wurde eine reduzierte Expression, veränderte Proteininteraktionen und eine erhöhte Fiebersensibilität festgestellt. Nichtsdestotrotz konnte bisher kein Effekt auf die synaptische Transmission beobachtet werden. Wir nutzten ein *Stx1B*$^{+/-}$-Mausmodell und akute Hirnschnitte des Hippocampus, um natürlich gewachsene Neurone zu untersuchen. Mit der Whole-cell Patch Clamp-Technik untersuchten wir inhibitorischen und exzitatorischen, synaptischen Input an Pyramidenzellen der Hippocampusregion CA1 zu messen. Zusätzlich leiteten wir Multi-unit-Netzwerk-Aktivität von Hippocampusregion CA1 und CA3 ab. Wir fanden keine relevanten Unterschiede für mIPSC und mEPSC. Wir fanden jedoch eine verlängerte Dauer der Up-Zustände in *Stx1b*$^{+/-}$-Mäusen als Zeichen einer erhöhten Erregbarkeit. Da diese Ergebnisse auf einer geringen Anzahl basieren, sollten weitere Untersuchungen zur Bestätigung stattfinden. Schließlich bestätigen wir vorangegangene Studien an Neuronenkulturen und stellen fest, dass ein einfacher Effekt auf den synaptischen Input nicht die einzelne Ursache der Epilepsie deser Patienten darstellt. Weitere Forschung sollte sich auf induzierte

Fiebermodelle, sterische Effekte der *STX1B*-*V*arianten und Netzwerkeffekte konzentrieren.

References:

Academy of Pediatrics, 2008. Febrile Seizures: Clinical Practice Guideline for the Long-term Management of the Child With Simple Febrile Seizures. Pediatrics 121, 1281–1286. https://doi.org/10.1542/peds.2008-0939

Accardi, M.V., Huang, H., Authier, S., 2018. Seizure liability assessments using the hippocampal tissue slice: Comparison of non-clinical species. J Pharmacol Toxicol Methods 93, 59–68. https://doi.org/10.1016/j.vascn.2017.11.003

Allone, C., Lo Buono, V., Corallo, F., Pisani, L.R., Pollicino, P., Bramanti, P., Marino, S., 2017. Neuroimaging and cognitive functions in temporal lobe epilepsy: A review of the literature. J. Neurol. Sci. 381, 7–15. https://doi.org/10.1016/j.jns.2017.08.007

Arancillo, M., Min, S.-W., Gerber, S., Münster-Wandowski, A., Wu, Y.-J., Herman, M., Trimbuch, T., Rah, J.-C., Ahnert-Hilger, G., Riedel, D., Südhof, T.C., Rosenmund, C., 2013. Titration of Syntaxin1 in mammalian synapses reveals multiple roles in vesicle docking, priming, and release probability. J. Neurosci. 33, 16698–16714. https://doi.org/10.1523/JNEUROSCI.0187-13.2013

Arsov, T., Mullen, S.A., Rogers, S., Phillips, A.M., Lawrence, K.M., Damiano, J.A., Goldberg-Stern, H., Afawi, Z., Kivity, S., Trager, C., Petrou, S., Berkovic, S.F., Scheffer, I.E., 2012. Glucose transporter 1 deficiency in the idiopathic generalized epilepsies. Ann. Neurol. 72, 807–815. https://doi.org/10.1002/ana.23702

Augustin, I., Rosenmund, C., Südhof, T.C., Brose, N., 1999. Munc13-1 is essential for fusion competence of glutamatergic synaptic vesicles. Nature 400, 457–461. https://doi.org/10.1038/22768

Barcia, G., Fleming, M. R., Deligniere, A., Gazula, V. R., Brown, M. R., Langouet, M., Chen, H., Kronengold, J., Abhyankar, A., Cilio, R., Nitschke, P., Kaminska, A., Boddaert, N., Casanova, J. L., Desguerre, I., Munnich, A., Dulac, O., Kaczmarek, L. K., Colleaux, L., & Nabbout, R. (2012). De novo gain-of-function KCNT1 channel mutations cause malignant migrating partial seizures of infancy. Nature genetics, 44(11), 1255–1259. https://doi.org/10.1038/ng.2441

Baulac, S., Gourfinkel-An, I., Nabbout, R., Huberfeld, G., Serratosa, J., Leguern, E., Baulac, M., 2004. Fever, genes, and epilepsy. Lancet Neurol 3, 421–430. https://doi.org/10.1016/S1474-4422(04)00808-7

Baulac, S., Huberfeld, G., Gourfinkel-An, I., Mitropoulou, G., Beranger, A., Prud'homme, J.F., Baulac, M., Brice, A., Bruzzone, R., LeGuern, E., 2001. First genetic evidence of GABA(A) receptor dysfunction in epilepsy: a mutation in the gamma2-subunit gene. Nat. Genet. 28, 46–48. https://doi.org/10.1038/88254

Beghi, Ettore, Giorgia Giussani, Foad Abd-Allah, Jemal Abdela,Ahmed Abdelalim, Haftom Niguse Abraha, Mina G Adib, Sutapa Agrawal, Fares Alahdab, Ashish Awasthi, Yohanes Ayele, Miguel A Barboza, Abate Bekele Belachew, Belete Biadgo, Ali Bijani, Helen Bitew, Félix Carvalho, Yazan Chaiah, Ahmad Daryani, Huyen Phuc Do, Manisha Dubey, Aman Yesuf Yesuf Endries, Sharareh Eskandarieh, Andre Faro, Farshad

Farzadfar, Seyed-Mohammad Fereshtehnejad, Eduarda Fernandes, Daniel Obadare Fijabi, Irina Filip, Florian Fischer, Abadi Kahsu Gebre, Afewerki Gebremeskel Tsadik, Teklu Gebrehiwo Gebremichael, Kebede Embaye Gezae, Maryam Ghasemi-Kasman, Kidu Gidey Weldegwergs, Meaza Girma Degefa, Elena V Gnedovskaya, Tekleberhan B Hagos, Arvin Haj-Mirzaian, Arya Haj-Mirzaian, Hamid Yimam Hassen, Simon I Hay, Mihajlo Jakovljevic, Amir Kasaeian, Tesfaye Dessale Kassa, Yousef Saleh Khader, Ibrahim Khalil, Ejaz Ahmad Khan, Jagdish Khubchandani, Adnan Kisa, Kristopher J Krohn, Chanda Kulkarni, Yirga Legesse Nirayo, Mark T Mackay, Marek Majdan, Azeem Majeed, Treh Manhertz, Man Mohan Mehndiratta, Tesfa Mekonen, Hagazi Gebre Meles, Getnet Mengistu, Shafiu Mohammed, Mohsen Naghavi, Ali H Mokdad, Ghulam Mustafa, Seyed Sina Naghibi Irvani, Long Hoang Nguyen, Emma Nichols, Molly R Nixon, Felix Akpojene Ogbo, Andrew T Olagunju, Tinuke O Olagunju, Mayowa Ojo Owolabi, Michael R Phillips, Gabriel David Pinilla-Monsalve, Mostafa Qorbani, Amir Radfar, Anwar Rafay, Vafa Rahimi-Movaghar, Nickolas Reinig, Perminder S Sachdev, Hosein Safari, Saeed Safari, Saeid Safiri, Mohammad Ali Sahraian, Abdallah M Samy, Shahabeddin Sarvi, Monika Sawhney, Masood A Shaikh, Mehdi Sharif, Gagandeep Singh, Mari Smith, Cassandra E I Szoeke, Rafael Tabarés-Seisdedos, Mohamad-Hani Temsah, Omar Temsah, Miguel Tortajada-Girbés, Bach Xuan Tran, Amanuel Amanuel Tesfay Tsegay, Irfan Ullah, Narayanaswamy Venketasubramanian, Ronny Westerman, Andrea Sylvia Winkler, Ebrahim M Yimer, Naohiro Yonemoto,, Valery L Feigin, Theo Vos, Christopher J L Murray, 2019. Global, regional, and national burden of epilepsy, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. The Lancet Neurology. https://doi.org/10.1016/S1474-4422(18)30454-X

Bennett, M.K., Calakos, N., Scheller, R.H., 1992. Syntaxin: a synaptic protein implicated in docking of synaptic vesicles at presynaptic active zones. Science 257, 255–259.

Bennett, M.K., Garcia-Arrerás, J.E., Elferink, L.A., Peterson, K., Fleming, A.M., Hazuka, C.D., Scheller, R.H., 1993. The syntaxin family of vesicular transport receptors. Cell 74, 863–873. https://doi.org/10.1016/0092-8674(93)90466-4

Berg, A.T., Berkovic, S.F., Brodie, M.J., Buchhalter, J., Cross, J.H., van Emde Boas, W., Engel, J., French, J., Glauser, T.A., Mathern, G.W., Moshé, S.L., Nordli, D., Plouin, P., Scheffer, I.E., 2010. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009. Epilepsia 51, 676–685. https://doi.org/10.1111/j.1528-1167.2010.02522.x

Berkovic, S.F., Howell, R.A., Hay, D.A., Hopper, J.L., 1998. Epilepsies in twins: genetics of the major epilepsy syndromes. Ann. Neurol. 43, 435–445. https://doi.org/10.1002/ana.410430405

Brodie, M.J., 2005. Diagnosing and predicting refractory epilepsy. Acta Neurol. Scand., Suppl. 181, 36–39. https://doi.org/10.1111/j.1600-0404.2005.00507.x

Brose, N., Petrenko, A.G., Sudhof, T.C., Jahn, R., 1992. Synaptotagmin: a calcium sensor on the synaptic vesicle surface. Science 256, 1021–1025. https://doi.org/10.1126/science.1589771

Cai, H., Reim, K., Varoqueaux, F., Tapechum, S., Hill, K., Sørensen, J.B., Brose, N., Chow, R.H., 2008. Complexin II plays a positive role in Ca2+-triggered exocytosis by facilitating vesicle priming. Proc Natl Acad Sci U S A 105, 19538–19543. https://doi.org/10.1073/pnas.0810232105

Cao, P., Yang, X., Südhof, T.C., 2013. Complexin activates exocytosis of distinct secretory vesicles controlled by different synaptotagmins. J. Neurosci. 33, 1714–1727. https://doi.org/10.1523/JNEUROSCI.4087-12.2013

Carvill, G.L., Weckhuysen, S., McMahon, J.M., Hartmann, C., Møller, R.S., Hjalgrim, H., Cook, J., Geraghty, E., O'Roak, B.J., Petrou, S., Clarke, A., Gill, D., Sadleir, L.G., Muhle, H., von Spiczak, S., Nikanorova, M., Hodgson, B.L., Gazina, E.V., Suls, A., Shendure, J., Dibbens, L.M., De Jonghe, P., Helbig, I., Berkovic, S.F., Scheffer, I.E., Mefford, H.C., 2014. GABRA1 and STXBP1: novel genetic causes of Dravet syndrome. Neurology 82, 1245–1253. https://doi.org/10.1212/WNL.0000000000000291

Casillas-Espinosa, P. M., Powell, K. L., & O'Brien, T. J. (2012). Regulators of synaptic transmission: roles in the pathogenesis and treatment of epilepsy. Epilepsia, 53 Suppl 9, 41–58. https://doi.org/10.1111/epi.12034

Celiker Uslu, S., Yuksel, B., Tekin, B., Sariahmetoglu, H., Atakli, D., 2019. Cognitive impairment and drug responsiveness in mesial temporal lobe epilepsy. Epilepsy Behav 90, 162–167. https://doi.org/10.1016/j.yebeh.2018.10.034

Cendes, F., Andermann, F., Dubeau, F., Gloor, P., Evans, A., Jones-Gotman, M., Olivier, A., Andermann, E., Robitaille, Y., Lopes-Cendes, I., 1993. Early childhood prolonged febrile convulsions, atrophy and sclerosis of mesial structures, and temporal lobe epilepsy: an MRI volumetric study. Neurology 43, 1083–1087.

Chanturiya, A., Chernomordik, L.V., Zimmerberg, J., 1997. Flickering fusion pores comparable with initial exocytotic pores occur in protein-free phospholipid bilayers. Proc Natl Acad Sci U S A 94, 14423–14428.

Claes, L., Del-Favero, J., Ceulemans, B., Lagae, L., Van Broeckhoven, C., De Jonghe, P., 2001. De novo mutations in the sodium-channel gene SCN1A cause severe myoclonic epilepsy of infancy. Am. J. Hum. Genet. 68, 1327–1332. https://doi.org/10.1086/320609

Cossette, P., Liu, L., Brisebois, K., Dong, H., Lortie, A., Vanasse, M., Saint-Hilaire, J.-M., Carmant, L., Verner, A., Lu, W.-Y., Wang, Y.T., Rouleau, G.A., 2002. Mutation of GABRA1 in an autosomal dominant form of juvenile myoclonic epilepsy. Nat. Genet. 31, 184–189. https://doi.org/10.1038/ng885

Deák, F., Xu, Y., Chang, W.-P., Dulubova, I., Khvotchev, M., Liu, X., Südhof, T.C., Rizo, J., 2009. Munc18-1 binding to the neuronal SNARE complex controls synaptic vesicle priming. J Cell Biol 184, 751–764. https://doi.org/10.1083/jcb.200812026

Deprez, L., Weckhuysen, S., Holmgren, P., Suls, A., Van Dyck, T., Goossens, D., Del-Favero, J., Jansen, A., Verhaert, K., Lagae, L., Jordanova, A., Van

Coster, R., Yendle, S., Berkovic, S.F., Scheffer, I., Ceulemans, B., De Jonghe, P., 2010. Clinical spectrum of early-onset epileptic encephalopathies associated with STXBP1 mutations. Neurology 75, 1159–1165. https://doi.org/10.1212/WNL.0b013e3181f4d7bf

Díaz-Otero F, Quesada M, Morales-Corraliza J, Martínez-Parra C, Gómez-Garre P, Serratosa JM. Autosomal dominant nocturnal frontal lobe epilepsy with a mutation in the CHRNB2 gene. Epilepsia. 2008;49(3):516-520. doi:10.1111/j.1528-1167.2007.01328.x

Dibbens, L.M., Feng, H.-J., Richards, M.C., Harkin, L.A., Hodgson, B.L., Scott, D., Jenkins, M., Petrou, S., Sutherland, G.R., Scheffer, I.E., Berkovic, S.F., Macdonald, R.L., Mulley, J.C., 2004. GABRD encoding a protein for extra- or peri-synaptic GABAA receptors is a susceptibility locus for generalized epilepsies. Hum. Mol. Genet. 13, 1315–1319. https://doi.org/10.1093/hmg/ddh146

Dube, C., Chen, K., Eghbal-Ahmadi, M., Brunson, K., Soltesz, I., Baram, T.Z., 2000. Prolonged febrile seizures in the immature rat model enhance hippocampal excitability long term. Ann. Neurol. 47, 336–344.

Dulubova, I., Khvotchev, M., Liu, S., Huryeva, I., Südhof, T.C., Rizo, J., 2007. Munc18-1 binds directly to the neuronal SNARE complex. Proc Natl Acad Sci U S A 104, 2697–2702. https://doi.org/10.1073/pnas.0611318104

Dulubova, I., Sugita, S., Hill, S., Hosaka, M., Fernandez, I., Südhof, T.C., Rizo, J., 1999. A conformational switch in syntaxin during exocytosis: role of munc18. EMBO J 18, 4372–4382. https://doi.org/10.1093/emboj/18.16.4372

Dutta, S., Sengupta, P., 2016. Men and mice: Relating their ages. Life Sciences 152, 244–248. https://doi.org/10.1016/j.lfs.2015.10.025

Eckhaus, J., Lawrence, K.M., Helbig, I., Bui, M., Vadlamudi, L., Hopper, J.L., Scheffer, I.E., Berkovic, S.F., 2013. Genetics of febrile seizure subtypes and syndromes: a twin study. Epilepsy Res. 105, 103–109. https://doi.org/10.1016/j.eplepsyres.2013.02.011

El Achkar, C.M., Olson, H.E., Poduri, A., Pearl, P.L., 2015. The Genetics of the Epilepsies. Current Neurology and Neuroscience Reports 15, 39. https://doi.org/10.1007/s11910-015-0559-8

Elger CE, Helmstaedter C, Kurthen M. Chronic epilepsy and cognition. Lancet Neurol. 2004 Nov;3(11):663-72. doi: 10.1016/S1474-4422(04)00906-8. PMID: 15488459.

Elmali, A.D., Bebek, N., Baykan, B., 2019. Let's talk SUDEP. Noro Psikiyatr Ars 56, 292–301. https://doi.org/10.29399/npa.23663

Elverman, K.H., Resch, Z.J., Quasney, E.E., Sabsevitz, D.S., Binder, J.R., Swanson, S.J., 2019. Temporal lobe epilepsy is associated with distinct cognitive phenotypes. Epilepsy Behav 96, 61–68. https://doi.org/10.1016/j.yebeh.2019.04.015

Escayg, A., MacDonald, B.T., Meisler, M.H., Baulac, S., Huberfeld, G., An-Gourfinkel, I., Brice, A., LeGuern, E., Moulard, B., Chaigne, D., Buresi, C., Malafosse, A., 2000. Mutations of *SCN1A*, encoding a neuronal sodium channel, in two families with GEFS+2. Nature Genetics 24, 343–345. https://doi.org/10.1038/74159

Fasshauer, D., Sutton, R.B., Brunger, A.T., Jahn, R., 1998. Conserved structural features of the synaptic fusion complex: SNARE proteins reclassified as Q- and R-SNAREs. Proc Natl Acad Sci USA 95, 15781. https://doi.org/10.1073/pnas.95.26.15781

Faul, F., Erdfelder, E., Lang, A.-G., Buchner, A., 2007. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior Research Methods 39, 175–191. https://doi.org/10.3758/BF03193146

Fernandez, I., Ubach, J., Dulubova, I., Zhang, X., Südhof, T.C., Rizo, J., 1998. Three-Dimensional Structure of an Evolutionarily Conserved N-Terminal Domain of Syntaxin 1A. Cell 94, 841–849. https://doi.org/10.1016/S0092-8674(00)81742-0

Fernández-Chacón, R., Königstorfer, A., Gerber, S.H., García, J., Matos, M.F., Stevens, C.F., Brose, N., Rizo, J., Rosenmund, C., Südhof, T.C., 2001. Synaptotagmin I functions as a calcium regulator of release probability. Nature 410, 41–49. https://doi.org/10.1038/35065004

Ferreira, S.T., Lourenco, M.V., Oliveira, M.M., De Felice, F.G., 2015. Soluble amyloid-β oligomers as synaptotoxins leading to cognitive impairment in Alzheimer's disease. Front Cell Neurosci 9, 191. https://doi.org/10.3389/fncel.2015.00191

Fisher, R.S., Acevedo, C., Arzimanoglou, A., Bogacz, A., Cross, J.H., Elger, C.E., Engel, J., Forsgren, L., French, J.A., Glynn, M., Hesdorffer, D.C., Lee, B.I., Mathern, G.W., Moshé, S.L., Perucca, E., Scheffer, I.E., Tomson, T., Watanabe, M., Wiebe, S., 2014. ILAE official report: a practical clinical definition of epilepsy. Epilepsia 55, 475–482. https://doi.org/10.1111/epi.12550

Fisher, R.S., Boas, W. van E., Blume, W., Elger, C., Genton, P., Lee, P., Engel, J., 2005. Epileptic Seizures and Epilepsy: Definitions Proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). Epilepsia 46, 470–472. https://doi.org/10.1111/j.0013-9580.2005.66104.x

Fisher, R.S., Cross, J.H., French, J.A., Higurashi, N., Hirsch, E., Jansen, F.E., Lagae, L., Moshé, S.L., Peltola, J., Perez, E.R., Scheffer, I.E., Zuberi, S.M., 2017. Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE Commission for Classification ... - PubMed - NCBI. Epilepsia 58, 522–430. https://doi.org/10.1111/epi13670

Fukata, Y., Fukata, M., 2017. Epilepsy and synaptic proteins. Current Opinion in Neurobiology 45, 1–8. https://doi.org/10.1016/j.conb.2017.02.001

Geppert, M., Goda, Y., Hammer, R.E., Li, C., Rosahl, T.W., Stevens, C.F., Südhof, T.C., 1994. Synaptotagmin I: a major Ca2+ sensor for transmitter release at a central synapse. Cell 79, 717–727.

Gerber, S.H., Rah, J.-C., Min, S.-W., Liu, X., de Wit, H., Dulubova, I., Meyer, A.C., Rizo, J., Arancillo, M., Hammer, R.E., Verhage, M., Rosenmund, C., Südhof, T.C., 2008. Conformational Switch of Syntaxin-1 Controls Synaptic Vesicle Fusion. Science 321, 1507–1510. https://doi.org/10.1126/science.1163174

Glick, B.S., Rothman, J.E., 1987. Possible role for fatty acyl-coenzyme A in intracellular protein transport. Nature 326, 309–312. https://doi.org/10.1038/326309a0

Hamdan, F.F., Piton, A., Gauthier, J., Lortie, A., Dubeau, F., Dobrzeniecka, S., Spiegelman, D., Noreau, A., Pellerin, S., Côté, M., Henrion, E., Fombonne, E., Mottron, L., Marineau, C., Drapeau, P., Lafrenière, R.G., Lacaille, J.C., Rouleau, G.A., Michaud, J.L., 2009. De novo STXBP1 mutations in mental retardation and nonsyndromic epilepsy. Ann. Neurol. 65, 748–753. https://doi.org/10.1002/ana.21625

Hata, Y., Slaughter, C.A., Südhof, T.C., 1993. Synaptic vesicle fusion complex contains unc-18 homologue bound to syntaxin. Nature 366, 347–351. https://doi.org/10.1038/366347a0

Hauser, W.A., 2007. The Prevalence and Incidence of Convulsive Disorders in Children. Epilepsia 35, S1–S6. https://doi.org/10.1111/j.1528-1157.1994.tb05932.x

Heron, S.E., Smith, K.R., Bahlo, M., Nobili, L., Kahana, E., Licchetta, L., Oliver, K.L., Mazarib, A., Afawi, Z., Korczyn, A., Plazzi, G., Petrou, S., Berkovic, S.F., Scheffer, I.E., Dibbens, L.M., 2012. Missense mutations in the sodium-gated potassium channel gene KCNT1 cause severe autosomal dominant nocturnal frontal lobe epilepsy. Nat. Genet. 44, 1188–1190. https://doi.org/10.1038/ng.2440

Holmes, G.L., Ben-Ari, Y., 2001. The Neurobiology and Consequences of Epilepsy in the Developing Brain. Pediatric Research 49, 320–325. https://doi.org/10.1203/00006450-200103000-00004

Holst, A.G., Winkel, B.G., Risgaard, B., Nielsen, J.B., Rasmussen, P.V., Haunsø, S., Sabers, A., Uldall, P., Tfelt-Hansen, J., 2013. Epilepsy and risk of death and sudden unexpected death in the young: A nationwide study. Epilepsia 54, 1613–1620. https://doi.org/10.1111/epi.12328

Hu, K., Carroll, J., Rickman, C., Davletov, B., 2002. Action of complexin on SNARE complex. J. Biol. Chem. 277, 41652–41656. https://doi.org/10.1074/jbc.M205044200

Huang, X., Tian, M., Hernandez, C.C., Hu, N., Macdonald, R.L., 2012. The GABRG2 nonsense mutation, Q40X, associated with Dravet syndrome activated NMD and generated a truncated subunit that was partially rescued by aminoglycoside-induced stop codon read-through. Neurobiol. Dis. 48, 115–123. https://doi.org/10.1016/j.nbd.2012.06.013

Huguet, G., Ey, E., Bourgeron, T., 2013. The genetic landscapes of autism spectrum disorders. Annu Rev Genomics Hum Genet 14, 191–213. https://doi.org/10.1146/annurev-genom-091212-153431

Kaeser, P.S., Deng, L., Wang, Y., Dulubova, I., Liu, X., Rizo, J., Südhof, T.C., 2011. RIM proteins tether Ca2+ channels to presynaptic active zones via a direct PDZ-domain interaction. Cell 144, 282–295. https://doi.org/10.1016/j.cell.2010.12.029

Kalser, J., Cross, J.H., 2018. The epileptic encephalopathy jungle - from Dr West to the concepts of aetiology-related and developmental encephalopathies. Curr. Opin. Neurol. 31, 216–222. https://doi.org/10.1097/WCO.0000000000000535

Kasperaviciute, D., Catarino, C.B., Matarin, M., Leu, C., Novy, J., Tostevin, A., Leal, B., Hessel, E.V.S., Hallmann, K., Hildebrand, M.S., Dahl, H.-H.M., Ryten, M., Trabzuni, D., Ramasamy, A., Alhusaini, S., Doherty, C.P., Dorn, T., Hansen, J., Krämer, G., Steinhoff, B.J., Zumsteg, D., Duncan, S., Kälviäinen, R.K., Eriksson, K.J., Kantanen, A.-M., Pandolfo, M., Gruber-Sedlmayr, U., Schlachter, K., Reinthaler, E.M., Stogmann, E., Zimprich, F., Théâtre, E., Smith, C., O'Brien, T.J., Meng Tan, K., Petrovski, S., Robbiano, A., Paravidino, R., Zara, F., Striano, P., Sperling, M.R., Buono, R.J., Hakonarson, H., Chaves, J., Costa, P.P., Silva, B.M., da Silva, A.M., de Graan, P.N.E., Koeleman, B.P.C., Becker, A., Schoch, S., von Lehe, M., Reif, P.S., Rosenow, F., Becker, F., Weber, Y., Lerche, H., Rössler, K., Buchfelder, M., Hamer, H.M., Kobow, K., Coras, R., Blumcke, I., Scheffer, I.E., Berkovic, S.F., Weale, M.E., UK Brain Expression Consortium, Delanty, N., Depondt, C., Cavalleri, G.L., Kunz, W.S., Sisodiya, S.M., 2013. Epilepsy, hippocampal sclerosis and febrile seizures linked by common genetic variation around SCN1A. Brain 136, 3140–3150. https://doi.org/10.1093/brain/awt233

Katz, B., 1971. Quantal mechanism of neural transmitter release. Science 173, 123–126.

Kawamata, J., Ikeda, A., Fujita, Y., Usui, K., Shimohama, S., Takahashi, R., 2010. Mutations in LGI1 gene in Japanese families with autosomal dominant lateral temporal lobe epilepsy: the first report from Asian families. Epilepsia 51, 690–693. https://doi.org/10.1111/j.1528-1167.2009.02309.x

Kofuji, T., Fujiwara, T., Sanada, M., Mishima, T., Akagawa, K., 2014. HPC-1/syntaxin 1A and syntaxin 1B play distinct roles in neuronal survival. Journal of Neurochemistry 130, 514–525. https://doi.org/10.1111/jnc.12722

Koko, M., Krause, R., Sander, T., Bobbili, D. R., Nothnagel, M., May, P., Lerche, H., & Epi25 Collaborative (2021). Distinct gene-set burden patterns underlie common generalized and focal epilepsies. EBioMedicine, 72, 103588. https://doi.org/10.1016/j.ebiom.2021.103588

Kuhlmann, J.W., Junius, M., Diederichsen, U., Steinem, C., 2017. SNARE-Mediated Single-Vesicle Fusion Events with Supported and Freestanding Lipid Membranes. Biophys. J. 112, 2348–2356. https://doi.org/10.1016/j.bpj.2017.04.032

Kwan, P., Brodie, M.J., 2000. Early identification of refractory epilepsy. N. Engl. J. Med. 342, 314–319. https://doi.org/10.1056/NEJM200002033420503

Lerche, H., Shah, M., Beck, H., Noebels, J., Johnston, D., Vincent, A., 2013. Ion channels in genetic and acquired forms of epilepsy: Ion channels in epilepsy. The Journal of Physiology 591, 753–764. https://doi.org/10.1113/jphysiol.2012.240606

Levira, F., Thurman, D.J., Sander, J.W., Hauser, W.A., Hesdorffer, D.C., Masanja, H., Odermatt, P., Logroscino, G., Newton, C.R., Epidemiology Commission of the International League Against Epilepsy, 2017. Premature mortality of epilepsy in low- and middle-income countries: A systematic review from the Mortality Task Force of the International League Against Epilepsy. Epilepsia 58, 6–16. https://doi.org/10.1111/epi.13603

Li, J.-Y., Plomann, M., Brundin, P., 2003. Huntington's disease: a synaptopathy? Trends Mol Med 9, 414–420. https://doi.org/10.1016/j.molmed.2003.08.006

Liu, K.S.Y., Siebert, M., Mertel, S., Knoche, E., Wegener, S., Wichmann, C., Matkovic, T., Muhammad, K., Depner, H., Mettke, C., Bückers, J., Hell, S.W., Müller, M., Davis, G.W., Schmitz, D., Sigrist, S.J., 2011. RIM-Binding Protein, a Central Part of the Active Zone, Is Essential for Neurotransmitter Release. Science 334, 1565. https://doi.org/10.1126/science.1212991

Maximov, A., Tang, J., Yang, X., Pang, Z.P., Südhof, T.C., 2009. Complexin Controls the Force Transfer from SNARE Complexes to Membranes in Fusion. Science 323, 516–521. https://doi.org/10.1126/science.1166505

McTague, A., Howell, K.B., Cross, J.H., Kurian, M.A., Scheffer, I.E., 2016. The genetic landscape of the epileptic encephalopathies of infancy and childhood. Lancet Neurol 15, 304–316. https://doi.org/10.1016/S1474-4422(15)00250-1

Meisel, C., 2016. Linking cortical network synchrony and excitability. Commun Integr Biol 9, e1128598. https://doi.org/10.1080/19420889.2015.1128598

Meisel, C., Schulze-Bonhage, A., Freestone, D., Cook, M.J., Achermann, P., Plenz, D., 2015. Intrinsic excitability measures track antiepileptic drug action and uncover increasing/decreasing excitability over the wake/sleep cycle. Proc. Natl. Acad. Sci. U.S.A. 112, 14694–14699. https://doi.org/10.1073/pnas.1513716112

Millhorn, D.E., Bayliss, D.A., Erickson, J.T., Gallman, E.A., Szymeczek, C.L., Czyzyk-Krzeska, M., Dean, J.B., 1989. Cellular and molecular mechanisms of chemical synaptic transmission. Am. J. Physiol. 257, L289-310. https://doi.org/10.1152/ajplung.1989.257.6.L289

Mishima, T., Fujiwara, T., Sanada, M., Kofuji, T., Kanai-Azuma, M., Akagawa, K., 2014. Syntaxin 1B, but not syntaxin 1A, is necessary for the regulation of synaptic vesicle exocytosis and of the readily releasable pool at central synapses. PLoS ONE 9, e90004. https://doi.org/10.1371/journal.pone.0090004

Mishima, T., Fujiwara, T., Kofuji, T., Saito, A., Terao, Y., & Akagawa, K. (2020). Syntaxin 1B regulates synaptic GABA release and extracellular GABA concentration, and is associated with temperature-dependent seizures. Journal of Neurochemistry.doi:10.1111/jnc.15159

Molleman, A., 2003. Patch clamping: an introductory guide to patch clamp electrophysiology. J. Wiley, New York.

Møller, R.S., Heron, S.E., Larsen, L.H.G., Lim, C.X., Ricos, M.G., Bayly, M.A., van Kempen, M.J.A., Klinkenberg, S., Andrews, I., Kelley, K., Ronen, G.M., Callen, D., McMahon, J.M., Yendle, S.C., Carvill, G.L., Mefford, H.C., Nabbout, R., Poduri, A., Striano, P., Baglietto, M.G., Zara, F., Smith, N.J., Pridmore, C., Gardella, E., Nikanorova, M., Dahl, H.A., Gellert, P., Scheffer, I.E., Gunning, B., Kragh-Olsen, B., Dibbens, L.M., 2015. Mutations in KCNT1 cause a spectrum of focal epilepsies. Epilepsia 56, e114–e120. https://doi.org/10.1111/epi.13071

Møller, R.S., Wuttke, T.V., Helbig, I., Marini, C., Johannesen, K.M., Brilstra, E.H., Vaher, U., Borggraefe, I., Talvik, I., Talvik, T., Kluger, G., Francois, L.L., Lesca, G., de Bellescize, J., Blichfeldt, S., Chatron, N., Holert, N., Jacobs,

J., Swinkels, M., Betzler, C., Syrbe, S., Nikanorova, M., Myers, C.T., Larsen, L.H.G., Vejzovic, S., Pendziwiat, M., von Spiczak, S., Hopkins, S., Dubbs, H., Mang, Y., Mukhin, K., Holthausen, H., van Gassen, K.L., Dahl, H.A., Tommerup, N., Mefford, H.C., Rubboli, G., Guerrini, R., Lemke, J.R., Lerche, H., Muhle, H., Maljevic, S., 2017. Mutations in GABRB3: From febrile seizures to epileptic encephalopathies. Neurology 88, 483–492. https://doi.org/10.1212/WNL.0000000000003565

Mullen, S.A., Berkovic, S.F., ILAE Genetics Commission, 2018. Genetic generalized epilepsies. Epilepsia 59, 1148–1153. https://doi.org/10.1111/epi.14042

Myers, K.A., Scheffer, I.E., Berkovic, S.F., ILAE Genetics Commission, 2018. Genetic literacy series: genetic epilepsy with febrile seizures plus. Epileptic Disord 20, 232–238. https://doi.org/10.1684/epd.2018.0985

Oliva, M., Berkovic, S.F., Petrou, S., 2012. Sodium channels and the neurobiology of epilepsy. Epilepsia 53, 1849–1859. https://doi.org/10.1111/j.1528-1167.2012.03631.x

Oyrer, J., Maljevic, S., Scheffer, I.E., Berkovic, S.F., Petrou, S., Reid, C.A., 2018. Ion Channels in Genetic Epilepsy: From Genes and Mechanisms to Disease-Targeted Therapies. Pharmacological Reviews 70, 142–173. https://doi.org/10.1124/pr.117.014456

Peng, L., Liu, H., Ruan, H., Tepp, W.H., Stoothoff, W.H., Brown, R.H., Johnson, E.A., Yao, W.-D., Zhang, S.-C., Dong, M., 2013. Cytotoxicity of Botulinum Neurotoxins Reveals a Direct Role of Syntaxin 1 and SNAP-25 in Neuron Survival. Nat Commun 4, 1472. https://doi.org/10.1038/ncomms2462

Phillips, H.A., Scheffer, I.E., Berkovic, S.F., Hollway, G.E., Sutherland, G.R., Mulley, J.C., 1995. Localization of a gene for autosomal dominant nocturnal frontal lobe epilepsy to chromosome 20q 13.2. Nat. Genet. 10, 117–118. https://doi.org/10.1038/ng0595-117

Poirier, M.A., Xiao, W., Macosko, J.C., Chan, C., Shin, Y.-K., Bennett, M.K., 1998. The synaptic SNARE complex is a parallel four-stranded helical bundle. Nature Structural Biology 5, 765.

Pong, A.W., Geary, B.R., Engelstad, K.M., Natarajan, A., Yang, H., De Vivo, D.C., 2012. Glucose transporter type I deficiency syndrome: epilepsy phenotypes and outcomes. Epilepsia 53, 1503–1510. https://doi.org/10.1111/j.1528-1167.2012.03592.x

Puranam, R.S., He, X.P., Yao, L., Le, T., Jang, W., Rehder, C.W., Lewis, D.V., McNamara, J.O., 2015. Disruption of Fgf13 causes synaptic excitatory-inhibitory imbalance and genetic epilepsy and febrile seizures plus. J. Neurosci. 35, 8866–8881. https://doi.org/10.1523/JNEUROSCI.3470-14.2015

Reif, P.S., Tsai, M.-H., Helbig, I., Rosenow, F., Klein, K.M., 2017. Precision medicine in genetic epilepsies: break of dawn? Expert Review of Neurotherapeutics 17, 381–392. https://doi.org/10.1080/14737175.2017.1253476

Richmond, J.E., Weimer, R.M., Jorgensen, E.M., 2001. An open form of syntaxin bypasses the requirement for UNC-13 in vesicle priming. Nature 412, 338–341. https://doi.org/10.1038/35085583

Rigbye, K.A., van Hasselt, P.M., Burgess, R., Damiano, J.A., Mullen, S.A., Petrovski, S., Puranam, R.S., van Gassen, K.L.I., Gecz, J., Scheffer, I.E., McNamara, J.O., Berkovic, S.F., Hildebrand, M.S., 2016. Is FGF13 a major contributor to genetic epilepsy with febrile seizures plus? Epilepsy Res. 128, 48–51. https://doi.org/10.1016/j.eplepsyres.2016.10.008

Rockenstein, E., Nuber, S., Overk, C.R., Ubhi, K., Mante, M., Patrick, C., Adame, A., Trejo-Morales, M., Gerez, J., Picotti, P., Jensen, P.H., Campioni, S., Riek, R., Winkler, J., Gage, F.H., Winner, B., Masliah, E., 2014. Accumulation of oligomer-prone α-synuclein exacerbates synaptic and neuronal degeneration in vivo. Brain 137, 1496–1513. https://doi.org/10.1093/brain/awu057

Saitsu, H., Kato, M., Mizuguchi, T., Hamada, K., Osaka, H., Tohyama, J., Uruno, K., Kumada, S., Nishiyama, K., Nishimura, A., Okada, I., Yoshimura, Y., Hirai, S., Kumada, T., Hayasaka, K., Fukuda, A., Ogata, K., Matsumoto, N., 2008. De novo mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy. Nat. Genet. 40, 782–788. https://doi.org/10.1038/ng.150

Scheffer, I.E., Berkovic, S., Capovilla, G., Connolly, M.B., French, J., Guilhoto, L., Hirsch, E., Jain, S., Mathern, G.W., Moshé, S.L., Nordli, D.R., Perucca, E., Tomson, T., Wiebe, S., Zhang, Y.-H., Zuberi, S.M., 2017. ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. Epilepsia 58, 512–521. https://doi.org/10.1111/epi.13709

Scheffer, I.E., Berkovic, S.F., 1997. Generalized epilepsy with febrile seizures plus. A genetic disorder with heterogeneous clinical phenotypes. Brain 120 (Pt 3), 479–490.

Scheffer, I.E., Harkin, L.A., Grinton, B.E., Dibbens, L.M., Turner, S.J., Zielinski, M.A., Xu, R., Jackson, G., Adams, J., Connellan, M., Petrou, S., Wellard, R.M., Briellmann, R.S., Wallace, R.H., Mulley, J.C., Berkovic, S.F., 2007. Temporal lobe epilepsy and GEFS+ phenotypes associated with SCN1B mutations. Brain 130, 100–109. https://doi.org/10.1093/brain/awl272

Scheffer, I.E., Liao, J., 2020. Deciphering the concepts behind “Epileptic encephalopathy” and “Developmental and epileptic encephalopathy.” Eur. J. Paediatr. Neurol. 24, 11–14. https://doi.org/10.1016/j.ejpn.2019.12.023

Schubert, J., Siekierska, A., Langlois, M., May, P., Huneau, C., Becker, F., Muhle, H., Suls, A., Lemke, J.R., de Kovel, C.G.F., Thiele, H., Konrad, K., Kawalia, A., Toliat, M.R., Sander, T., Rüschendorf, F., Caliebe, A., Nagel, I., Kohl, B., Kecskés, A., Jacmin, M., Hardies, K., Weckhuysen, S., Riesch, E., Dorn, T., Brilstra, E.H., Baulac, S., Møller, R.S., Hjalgrim, H., Koeleman, B.P.C., EuroEPINOMICS RES Consortium, Jurkat-Rott, K., Lehman-Horn, F., Roach, J.C., Glusman, G., Hood, L., Galas, D.J., Martin, B., de Witte, P.A.M., Biskup, S., De Jonghe, P., Helbig, I., Balling, R., Nürnberg, P., Crawford, A.D., Esguerra, C.V., Weber, Y.G., Lerche, H., 2014. Mutations in STX1B, encoding a presynaptic protein, cause fever-associated epilepsy syndromes. Nat. Genet. 46, 1327–1332. https://doi.org/10.1038/ng.3130

Shorvon, S.D., 2011. The etiologic classification of epilepsy. Epilepsia 52, 1052–1057. https://doi.org/10.1111/j.1528-1167.2011.03041.x

Singh, A., Trevick, S., 2016. The Epidemiology of Global Epilepsy. Neurol Clin 34, 837–847. https://doi.org/10.1016/j.ncl.2016.06.015

Söllner, T., Bennett, M.K., Whiteheart, S.W., Scheller, R.H., Rothman, J.E., 1993a. A protein assembly-disassembly pathway in vitro that may correspond to sequential steps of synaptic vesicle docking, activation, and fusion. Cell 75, 409–418. https://doi.org/10.1016/0092-8674(93)90376-2

Söllner, T., Whiteheart, S.W., Brunner, M., Erdjument-Bromage, H., Geromanos, S., Tempst, P., Rothman, J.E., 1993b. SNAP receptors implicated in vesicle targeting and fusion. Nature 362, 318–324. https://doi.org/10.1038/362318a0

Sørensen, J.B., Wiederhold, K., Müller, E.M., Milosevic, I., Nagy, G., de Groot, B.L., Grubmüller, H., Fasshauer, D., 2006. Sequential N- to C-terminal SNARE complex assembly drives priming and fusion of secretory vesicles. EMBO J 25, 955–966. https://doi.org/10.1038/sj.emboj.7601003

Stamberger, H., Nikanorova, M., Willemsen, M.H., Accorsi, P., Angriman, M., Baier, H., Benkel-Herrenbrueck, I., Benoit, V., Budetta, M., Caliebe, A., Cantalupo, G., Capovilla, G., Casara, G., Courage, C., Deprez, M., Destrée, A., Dilena, R., Erasmus, C.E., Fannemel, M., Fjær, R., Giordano, L., Helbig, K.L., Heyne, H.O., Klepper, J., Kluger, G.J., Lederer, D., Lodi, M., Maier, O., Merkenschlager, A., Michelberger, N., Minetti, C., Muhle, H., Phalin, J., Ramsey, K., Romeo, A., Schallner, J., Schanze, I., Shinawi, M., Sleegers, K., Sterbova, K., Syrbe, S., Traverso, M., Tzschach, A., Uldall, P., Van Coster, R., Verhelst, H., Viri, M., Winter, S., Wolff, M., Zenker, M., Zoccante, L., De Jonghe, P., Helbig, I., Striano, P., Lemke, J., Møller, R.S., Weckhuysen, S., 2016. STXBP1 encephalopathy: A neurodevelopmental disorder including epilepsy. Neurology 86, 954–962. https://doi.org/10.1212/WNL.0000000000002457

Steinlein, O.K., Magnusson, A., Stoodt, J., Bertrand, S., Weiland, S., Berkovic, S.F., Nakken, K.O., Propping, P., Bertrand, D., 1997. An insertion mutation of the CHRNA4 gene in a family with autosomal dominant nocturnal frontal lobe epilepsy. Hum. Mol. Genet. 6, 943–947. https://doi.org/10.1093/hmg/6.6.943

Sutton, R.B., Fasshauer, D., Jahn, R., Brunger, A.T., 1998. Crystal structure of a SNARE complex involved in synaptic exocytosis at 2.4 Å resolution. Nature 395, 347–353. https://doi.org/10.1038/26412

Thurman, D.J., Logroscino, G., Beghi, E., Hauser, W.A., Hesdorffer, D.C., Newton, C.R., Scorza, F.A., Sander, J.W., Tomson, T., Epidemiology Commission of the International League Against Epilepsy, 2017. The burden of premature mortality of epilepsy in high-income countries: A systematic review from the Mortality Task Force of the International League Against Epilepsy. Epilepsia 58, 17–26. https://doi.org/10.1111/epi.13604

Tian, Y., Hou, C., Wang, X.Y., Yang, Z.X., Ma, Y.L., Cao, B.B., Li, X.J., 2019. [A novel inherited STX1B mutation associated with generalized epilepsy with febrile seizures plus: a family analysis and literature review]. Zhonghua Er Ke Za Zhi 57, 206–210. https://doi.org/10.3760/cma.j.issn.0578-1310.2019.03.010

Vardar, G., Gerth, F., Schmitt, X.J., Rautenstrauch, P., Trimbuch, T., Schubert, J., Lerche, H., Rosenmund, C., Freund, C., 2020. Epilepsy-causing STX1B mutations translate altered protein functions into distinct phenotypes in mouse neurons. Brain 143, 2119–2138. https://doi.org/10.1093/brain/awaa151

Villa, C., Colombo, G., Meneghini, S., Gotti, C., Moretti, M., Ferini-Strambi, L., Chisci, E., Giovannoni, R., Becchetti, A., & Combi, R. (2019). CHRNA2 and Nocturnal Frontal Lobe Epilepsy: Identification and Characterization of a Novel Loss of Function Mutation. Frontiers in molecular neuroscience, 12, 17. https://doi.org/10.3389/fnmol.2019.00017

Vlaskamp, D.R.M., Rump, P., Callenbach, P.M.C., Vos, Y.J., Sikkema-Raddatz, B., van Ravenswaaij-Arts, C.M.A., Brouwer, O.F., 2016. Haploinsufficiency of the STX1B gene is associated with myoclonic astatic epilepsy. Eur. J. Paediatr. Neurol. 20, 489–492. https://doi.org/10.1016/j.ejpn.2015.12.014

Wallace, R.H., Marini, C., Petrou, S., Harkin, L.A., Bowser, D.N., Panchal, R.G., Williams, D.A., Sutherland, G.R., Mulley, J.C., Scheffer, I.E., Berkovic, S.F., 2001. Mutant GABA(A) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. Nat. Genet. 28, 49–52. https://doi.org/10.1038/88259

Wallace, R.H., Scheffer, I.E., Parasivam, G., Barnett, S., Wallace, G.B., Sutherland, G.R., Berkovic, S.F., Mulley, J.C., 2002. Generalized epilepsy with febrile seizures plus: Mutation of the sodium channel subunit SCN1B. Neurology 58, 1426–1429. https://doi.org/10.1212/WNL.58.9.1426

Wallace, R.H., Wang, D.W., Singh, R., Scheffer, I.E., George, A.L., Phillips, H.A., Saar, K., Reis, A., Johnson, E.W., Sutherland, G.R., Berkovic, S.F., Mulley, J.C., 1998. Febrile seizures and generalized epilepsy associated with a mutation in the Na+-channel beta1 subunit gene SCN1B. Nat. Genet. 19, 366–370. https://doi.org/10.1038/1252

Wang, Y., Okamoto, M., Schmitz, F., Hofmann, K., Südhof, T.C., 1997. Rim is a putative Rab3 effector in regulating synaptic-vesicle fusion. Nature 388, 593–598. https://doi.org/10.1038/41580

Weber, Y.G., Jacob, M., Weber, G., Lerche, H., 2008. A BFIS-like syndrome with late onset and febrile seizures: suggestive linkage to chromosome 16p11.2-16q12.1. Epilepsia 49, 1959–1964. https://doi.org/10.1111/j.1528-1167.2008.01646.x

Wilson, T.W., Rojas, D.C., Reite, M.L., Teale, P.D., Rogers, S.J., 2007. Children and adolescents with autism exhibit reduced MEG steady-state gamma responses. Biol. Psychiatry 62, 192–197. https://doi.org/10.1016/j.biopsych.2006.07.002

Wolking, S., May, P., Mei, D., Møller, R.S., Balestrini, S., Helbig, K.L., Altuzarra, C.D., Chatron, N., Kaiwar, C., Stöhr, K., Widdess-Walsh, P., Mendelsohn, B.A., Numis, A., Cilio, M.R., Van Paesschen, W., Svendsen, L.L., Oates, S., Hughes, E., Goyal, S., Brown, K., Sifuentes Saenz, M., Dorn, T., Muhle, H., Pagnamenta, A.T., Vavoulis, D.V., Knight, S.J.L., Taylor, J.C., Canevini, M.P., Darra, F., Gavrilova, R.H., Powis, Z., Tang, S., Marquetand, J., Armstrong, M., McHale, D., Klee, E.W., Kluger, G.J.,

Lowenstein, D.H., Weckhuysen, S., Pal, D.K., Helbig, I., Guerrini, R., Thomas, R.H., Rees, M.I., Lesca, G., Sisodiya, S.M., Weber, Y.G., Lal, D., Marini, C., Lerche, H., Schubert, J., 2019. Clinical spectrum of STX1B-related epileptic disorders. Neurology 92, e1238–e1249. https://doi.org/10.1212/WNL.0000000000007089

Woodbury, D.J., Hall, J.E., 1988. Vesicle-membrane fusion. Observation of simultaneous membrane incorporation and content release. Biophys. J. 54, 345–349. https://doi.org/10.1016/S0006-3495(88)82965-5

Wu, Y.-J., Tejero, R., Arancillo, M., Vardar, G., Korotkova, T., Kintscher, M., Schmitz, D., Ponomarenko, A., Tabares, L., Rosenmund, C., 2015. Syntaxin 1B is important for mouse postnatal survival and proper synaptic function at the mouse neuromuscular junctions. Journal of Neurophysiology 114, 2404. https://doi.org/10.1152/jn.00577.2015

Yang, H., Shew, W.L., Roy, R., Plenz, D., 2012. Maximal variability of phase synchrony in cortical networks with neuronal avalanches. J. Neurosci. 32, 1061–1072. https://doi.org/10.1523/JNEUROSCI.2771-11.2012

Yizhar, O., Fenno, L.E., Prigge, M., Schneider, F., Davidson, T.J., O'Shea, D.J., Sohal, V.S., Goshen, I., Finkelstein, J., Paz, J.T., Stehfest, K., Fudim, R., Ramakrishnan, C., Huguenard, J.R., Hegemann, P., Deisseroth, K., 2011. Neocortical excitation/inhibition balance in information processing and social dysfunction. Nature 477, 171–178. https://doi.org/10.1038/nature10360

Zhang, Y.-H., Burgess, R., Malone, J.P., Glubb, G.C., Helbig, K.L., Vadlamudi, L., Kivity, S., Afawi, Z., Bleasel, A., Grattan-Smith, P., Grinton, B.E., Bellows, S.T., Vears, D.F., Damiano, J.A., Goldberg-Stern, H., Korczyn, A.D., Dibbens, L.M., Ruzzo, E.K., Hildebrand, M.S., Berkovic, S.F., Scheffer, I.E., 2017. Genetic epilepsy with febrile seizures plus: Refining the spectrum. Neurology 89, 1210–1219. https://doi.org/10.1212/WNL.0000000000004384

Zhou, P., Pang, Z.P., Yang, X., Zhang, Y., Rosenmund, C., Bacaj, T., Südhof, T.C., 2013. Syntaxin-1 N-peptide and Habc-domain perform distinct essential functions in synaptic vesicle fusion. The EMBO Journal 32, 159. https://doi.org/10.1038/emboj.2012.307

Zuberi, S.M., Brunklaus, A., Birch, R., Reavey, E., Duncan, J., Forbes, G.H., 2011. Genotype-phenotype associations in SCN1A-related epilepsies. Neurology 76, 594–600. https://doi.org/10.1212/WNL.0b013e31820c309b

7 Erklärung zum Eigenanteil

Die Arbeit wurde im Hertie-Institut für klinische Hirnforschung unter Betreuung von Prof. Holger Lerche durchgeführt.

Die Konzeption der Studie erfolgte durch Dr. Yuanyuan Liu und Prof. Holger Lerche.

Die Versuche wurden nach Einarbeitung durch Dr. Yuanyuan Liu von mir eigenständig durchgeführt.

Die statistische Auswertung erfolgte nach Beratung durch Dr. Yuanyuan Liu und Dr. Marc Himmelbach durch mich.

Ich versichere, das Manuskript selbständig verfasst zu haben und keine weiteren als die von mir angegebenen Quellen verwendet zu haben.

Frankfurt, den

8 Appendix

8.1 List of abbreviations of genes and proteins

CACNA1A – voltage-dependent calcium channel alpha-1A subunit gene

CACNA1H – voltage-dependent calcium channel alpha-1H subunit gene

$Ca_V2.1$ – voltage-dependent calcium channel, L-type

$Ca_V3.2$ – voltage-dependent calcium channel, T-type

CHRNA2 - neuronal acetylcholine receptor subunit alpha-2 gene

CHRNA4 – neuronal acetylcholine receptor subunit alpha-4 gene

CHRNB2 – neuronal acetylcholine receptor subunit beta-2 gene

GABRA1 – GABA-receptor subunit alpha-1 gene

GABRA1 – GABA-receptor subunit alpha-1

GABRB3 – GABA-receptor subunit beta-3 gene

GABRB3 – GABA-receptor subunit beta-3

GABRG2 – GABA-receptor subunit gamma-2 gene

GABRG2 – GABA-receptor subunit gamma-2

GluN1 – NMDA-glutamate-receptor subunit zeta-1

GluN2A – NMDA-glutamate-receptor subunit epsilon-1

GluN2B – NMDA-glutamate-receptor subunit epsilon-2

GluN2D – NMDA-glutamate-receptor subunit epsilon-4

GLUT1 – glucose transporter 1

GRIN1 – NMDA-glutamate recepter subunit zeta-1 gene

GRIN2A – NMDA-glutamate receptor subunit epsilon-1 gene

GRIN2B – NMDA-glutamate receptor subunit epsilon-2 gene

GRIN2D – NMDA-glutamate receptor subunit epsilon-4 gene

KCNA2 – voltage-gated potassium channel, subfamily A, member 2 gene

KCNB1 – voltage-gated potassium channel, Shab-related subfamily, type 1 gene

KCNT1 – calcium-activated potassium channel, subfamily T, member 1 gene

$K_v1.2$ – voltage-gated potassium channel, Shab-related subfamily, type 1

$K_V2.1$ – voltage-gated potassium channel, subfamily A, member 2

LGI1 – leucine rich, glioma inactivated 1

Munc13 – mammalian uncoordinated 13

Munc18-1 – part of Sec1/Munc18-like protein family

nAChR – nicotingergi acetylcholine receptor

$Na_V1.1$ – voltage-gated sodium channel type I

SCN1A – sodium channel, type 1, alpha subunit gene

SCN1B – sodium channel, type 1, beta subunit gene

SCN2A – sodium channel, type 2, alpha subunit gene

SLC2A1 – solute carrier family 2, facilitated glucose transporter member 1 gene

SNAP-25 – synaptosomal-associated protein, 25 kDa

STX1A – syntaxin-1A gene

STX1A – syntaxin-1A protein

STX1B – syntaxin-1B gene

STX1B – syntaxin-1B protein

$STX1B^{+/+}$ - wildtype syntaxin-1B gene

$STX1B^{+/-}$ - heterozygous knockout of syntaxin-1B gene

$STX1B^{InDel}$ – insertion-deletion-variant in syntaxin-1B gene

$STX1B^{G226R}$ – point-mutation in syntaxin-1B gene

STX1B^{V216} – point-mutation in syntaxin-1B gene

STX1BP – syntaxin-1B binding protein, also known as Munc18-1