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THE ROLE OF ASTROCYTES IN TYPICAL ABSENCE EPILEPSY

MERVE ÖZGÜR

NEUROSCIENCE

PhD. Programme

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required by the final examining committee have been made.

Committee Members: Signature:

Safiye Çavdar, Prof. Dr. (Advisor)

Selçuk Sürücü, Prof. Dr.

Behice Durgun, Prof. Dr.

Alp Bayramoğlu, Prof. Dr.

Ali İlke Gürses, Doç. Dr.

Director of the Institute:

Prof. Yasemin Gürsoy Özdemir

STATEMENT

I hereby declare that this thesis work is my own work, I did not behave unethically throughout all the phases starting from the planning until the writing, I obtained all the information according to the academic and ethical rules, the references of the knowledge and comments gained from other works than this thesis work has been provided and these references are shown in the reference list, and again I declare that I have no derogatory behavior of the patent and authors royalties during working and writing phases of this thesis.

Signature

Merve Özgür

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* I dedicate this thesis to the children who suffer from epilepsy.

Merve Özgür

ABSTRACT

The Role of Astrocytes in Typical Absence Epilepsy

Merve Özgür, Supervisor: Prof. Dr. Safiye Çavdar, Koç University Graduate School of Health Sciences, Neuroscience PhD Programme

Astrocytes play an important role in the modulation of synaptic transmission in terms of GABA clearance/turnover and even though they are electrically unexcitable, with their vast astrocyte-to-astrocyte network, they might modulate the spread of cortically-drived spike-and-wave discharges.

The aim of the study is to show that astrocytes play an important role in the pathophysiology of epilepsy and have a central role in the modulation of spike-and-wave discharges. We hypothesize that exciting the activity of astrocytes in the thalamus is sufficient to intervene the ongoing seizures in real-time. For this, we stimulated the astrocytes specifically by using optogenetic tool of exciting virus and simultaneously record the electrophysiological activity of the cortical neurons in vivo. Further, we observed histological changes in fluorescent microscopy. In this study, we used genetic absence epileptic rats and WAG-Rij rats to investigate our hypothesis and reveal any differences between these strains.

Keywords: optogenetics, typical absence epilepsy**,** animal model.

ÖZET

Tipik Absans Epilepside Astrositlerin Rolü

Merve Özgür, Danışman: Prof. Dr. Safiye Çavdar, Koç Üniversitesi Sağlık Bilimleri Enstitüsü Sinirbilim Doktora Programı

Astrositler, sinaptik alandan GABA'nın temizlenmesi ve yıkılıp tekrar sentezlenmesinde rol alarak sinaptik transmisyonu module ederler. Astrosit membranları elektriksel olarak eksite olmamalarına rağmen astrosit-astrosit bağlantıları ile kortikal orjinli diken-dalga deşarjlarının (SWD) regulasyonunda görev alırlar.

Bu çalışmanın amacı, astrositlerin tipik absans epilepsi patofizyolojisindeki fonksiyonuna ışık tutmak ve diken-dalga deşarjlarının gelişmesinde merkezi rol aldığını göstermek. Talamus'taki astrositlerin eksite edilmesinin diken-dalga deşarjlarının modulasyonu için yeterli olduğunu öne sürmekteyiz. Bu amaçla, optogenetik olarak modifiye edilmiş virus sayesinde astrositler hedef haline getirilerek spesifik olarak stimule edilmiştir. Eş zamanlı olarak, kortikal nöronların electrofizyolojik aktivitesi ölçülüp, beyinde oluşan histolojik değişiklikler flurosan mikroskop ile incelenmiştir. Bu çalışmamızda genetik absans epileptik sıçanlar ve WAG-Rij cinsi sıçanlar kullanılarak hem hipotezimiz incelenmek istenmiş, hem de bu iki tür arasındaki olası farklılıklar ortaya çıkarılmak hedeflenmiştir.

Anahtar kelimeler: optogenetik, tipik absans epilepsi, hayvan modeli

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NOMENCLATURE

SWD	Spike-and-wave discharges
EEG	Electroencephalogram
TC	Thalamocortical
CT	Corticothalamic
TRN	Thalamic reticular nucleus
GAERS	Genetic Absence Epileptic Rats from Strasbourg
VB	Ventrobasal
WAG-Rij	Wistar Albino Glaxo rats from Rijswijk
ATP	Adenosine triphosphate
ILAE	International League Against Epilepsy
MRI	Magnetic resonance imaging
SUDEP	Sudden unexpected death in epilepsy
AED	Anti-epileptic drug
NMDA	N-methyl-D-aspartate
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
GABA	γ-aminobutyric acid
GLUT-1	Glucose transporter-1
LTS	Low-threshold spikes
RTS	Resting membrane potential
IPSP	Inhibitory postsynaptic potentials
EPSP	Excitatory postsynaptic potentials
PNS	Peripheral nervous system
CNS	Central nervous system
SIC	Slow inward current
EATT1/2	Excitatory aminoacid transporter 1/2
GDH	Glutamate dehydrogenase
GS	Glutamine synthease
TCA	Tricarboxylic acid
GAD	Glutamic acid decarboxylase
GS	Glutamine synthetase

PAG	Phosphate-activated glutaminase
PC	Pyruvate carboxylase
PDH	Pyruvate dehydrogenase
GFAP	Glial fibrillary acid protein
Kir	Rectifying potassium ion channel
TNFα	Tumor necrosis factor alpha
TGFβ	Transporter growth factor beta
IL-1b	Interleukin-1beta
KO	Knock-out
GHB	γ-hydroxybutyric acid
REM	Rapid-eye-movement
ChR2	Channelrhodopsin-2
NpHR.	Halorhodopsin
AAV	Adeno-associated virus

Chapter 1.

1. INTRODUCTION AND AIM OF THE THESIS

Epilepsy, a heterogeneous disease of recurrent, unprovoked seizures, is grouped into two major classes, generalized or localization-related (focal) (386). Differing molecular starting points with shared common cellular mechanisms take places in many inherited forms (47) and yet develop with distinctive, recognizable clinical syndrome. Absence epilepsy is one of the major inherited subtypes, with multiple syndromes extending from infancy to adolescence characterized by nonconvulsive seizures with behavioral arrest and concomitant periodic 3-4/s generalized spike-wave discharges (SWD), in the electroencephalogram (EEG) (10). The occurrence, propagation and termination of epileptic seizures are thought to rely on the neuronal networks that are connected through synaptic as well as non-synaptic interactions (1).

Absence seizures are genetically determined and originate from abnormal electrical activity in reciprocally connected thalamic and cortical territories (the cortico-thalamic-cortical loop). Key cellular elements of these network include pyramidal cells and interneurons of different cortical layers, the thalamocortical (TC) neurons of sensory thalamic nuclei and their main inhibitory input, the gamma-aminobutyric acid (GABA) releasing neurons of the nucleus reticularis thalami (TRN) (387). These complicated interactions include modified functions of neurons and glia as well as the mediation of excitatory and inhibitory mechanisms with feedback homeostasis (1). Three intrinsic mechanisms have been the focus for years that increase likelihood of thalamo-cortical oscillations. The first mechanism involves T-currents elicited by activated T-type calcium channel which trigger sustained burst-firing of thalamic neurons during absence seizures. The second mechanism is ý-aminobutyric acid-B receptor ($GABA_{B)}$ receptors which is capable of eliciting long lasting hyperpolarization in thalamic neurons required to prime T-channels for sustained burst-firing. A third mechanism involves the reticular thalamic $GABA_A$ receptors to mediate recurrent inhibition (2). Thalamo-cortical rhythmicity is affected by corticothalamic input (glutamate mediated) which results in a slow depolarization of thalamic relay cells (147). Activation of glutamate receptors depolarize the thalamic neurons and thereby decreases the absence seizures.

Neural dysfunctions as well as physiological neural processes and mechanisms are tightly modulated/controlled by diverse neuronal and non-neuronal cells such as glial cells (50). Astrocytes and microglia have been shown to play prominent roles in epileptic events such as epileptogenesis and seizure generation. Activated astrocytes and microglia are major sources of inflammatory molecules in the brain during epileptic activity in the animal model of limbic seizures and temporal lobe epilepsy (388, 389). Tian et al., (2005) has also demonstrated that astrocytic glutamate release trigger ictal events in different seizure models (307). The involvement of glial mechanisms has been widely studied in convulsive seizures, however there is a need for exploration the role of glial cells in non-convulsive epilepsy, especially in absence epilepsy. In this context, we aimed to measure the simultaneous effect of astrocytic mechanisms in pathophysiology of absence epilepsy in vivo using genetically epileptic animal models.

Epilepsy affects around 65 million people worldwide, and in large part due to critical lack of understanding of many fundamental aspects of epilepsy current treatment options such as pharmacological therapy and electrical stimulation have some shortcomings. Optogenetics provides researchers a powerful and flexible tool in terms of specificity and accuracy of manipulation of the targeted cells. Thus, allows significant advances in understanding the etiology of epilepsy. This novel groundbreaking technique provides an opportunity to investigate neuronal circuit elements that may be capable of seizure control. In our study, we used optogenetics in genetically epileptic animal models of absence epilepsy to manipulate the astrocytes and record ongoing cortical activity for the first time in literature. To accomplish this, we used viral transduction technology to deliver genetically engineered light-sensitive ion channels to the astrocytes in ventrobasal thalamic nuclei. Then, after stimulation of these cells with a light emitting diode, phenotypic changes of spike-and-wave discharges were recorded. Furthermore, electrophysiological findings were integrated by histological studies using fluorescent microscopy to identify astrocytes in the affected areas.

Both the Wistar Albino Glaxo rats from Rijswijk (WAG-Rij) and the Genetic Absence Epilepsy Rat from Strasbourg (GAERS) are well-validated, most predicted genetic rat models of typical absence epilepsy (28,225). In GAERS, both the

electrophysiological (spike-and-wave discharges) and behavioral (behavioral arrest) traits fit well with those observed in human patients with typical absence epilepsy. The SWDs in GAERS and WAG-Rij are very similar, high-amplitude asymmetric synchronized rhythmic activity with fundamental frequency of 7-11 Hz. WAG-Rij rat model enables predictions about pharmacotherapy of epileptic patients and therefore this strain is considered as a valid model of human absence epilepsy (191).

The models are also helpful to explore simultaneously the neural circuits involved in the generation of seizures at different level of integration due to their easily detectable spontaneous recurrent seizures on electroencephalic recordings. Disease states result in the loss of appropriate interactions of these rhythms, either in their prevalence, synchrony, frequency, or distribution (116,347). A seizure on the other hand, is a process of amplification and synchronization of neuronal firing, which involves the GABAergic inhibitory mechanisms and glutamatergic excitatory mechanism. Burst of high energy result from increased electrical energy during the epileptic seizure. This high energy results in an increased requirement for blood flow, oxygen, glucose and ATP as well as increase in glycolysis, and all these mechanisms take place in astrocytes. Healthy astrocytes control appropriate feedback to compensate for transiently excessive coupling, while dysfunctional astrocytes lose this capability (164). Astroglia dysfunction in epilepsy has therefore needs to be studied in detail.

This is an ambitious project for several reasons and those create building blocks of the thesis. First, this would allow us to test astrocytes function spontaneously in an awake animal which is close to the real disease condition. Therefore, results might give us the opportunity to reveal one of the possible mechanisms involved in the pathophysiology of typical absence epilepsy. Second, use of optogenetics for astrocytes has never been performed in the animal models of absence epilepsy. Therefore, the project will open new windows to epilepsy research.

Chapter 2.

2. LITERATURE REVIEW

2.1. Epilepsy

2.1.1. Definition

Epilepsy is a neurological condition that is characterized by perpetual predisposition of the brain to develop seizures accompanying with cognitive, psychological and social aftereffects (6). These symptoms can limit everyday tasks and cause difficulty in learning and memory, maintenance of lasting employment, as well as socioeconomic integration (8). There are 65 million people affected from epilepsy worldwide and disease cause a major burden in seizure-related disability, mortality, comorbidities, and cost (6). Therefore, epilepsy is associated with increased mortality rate and reduced quality of life.

Epileptic seizure is defined as a ‘’temporal occurrence of symptoms due to aberrant excessive or concurrent neuronal activity in the brain’’ by the International League Against Epilepsy (ILAE) (7). This definition is commonly appealed when a person has two unprovoked seizures without any systemic or acute insults such as head trauma or stroke within 24 hours or more apart. According to the ILAE, epilepsy arises when certain conditions are present including: ‘’ (i) minimum two gratuitous seizures are found within 24 hours apart; (ii) a seizure and possibility of more seizures familiar to the risk of general recurrence following two unprovoked seizure (at least 60%) develop over the next 10 years; (iii) an epilepsy syndrome is diagnosed (6).

Seizure phases comprises of preictal period (before seizure), ictal period (during the seizure), interictal period (between consecutive seizures), and postictal period (the transition from the ictal phase to the patient’s normal level of awareness). Ictal and postictal phases are evaluated by the patient’s speech and ability to follow commands, head/eye deviation, posture of extremities, whether the patient sustained injuries (e.g., falls, tongue, biting), incontinence, weakness, aphasia, and postictal confusion (8). Typical diagnostic evaluation of seizure include a history of family occurrence, head trauma, febrile seizures, meningitis/encephalitis, medication) as well as other test like blood tests measuring the complete blood count, electrolytes, glucose,

calcium magnesium, phosphate, hepatic, and renal function tests, lumbar puncture in the case of infectious etiology (e.g., meningitis), blood or urine screen for drugs, EEG and brain MRI (8).

2.1.2. Classification

The classification and organization of epilepsy has been the subject of change in view of the scientific advancement. ILAE (2010) extend the classification and organized seizures and epilepsies with the proposed changes in nomenclature and approach is shown figure 1.

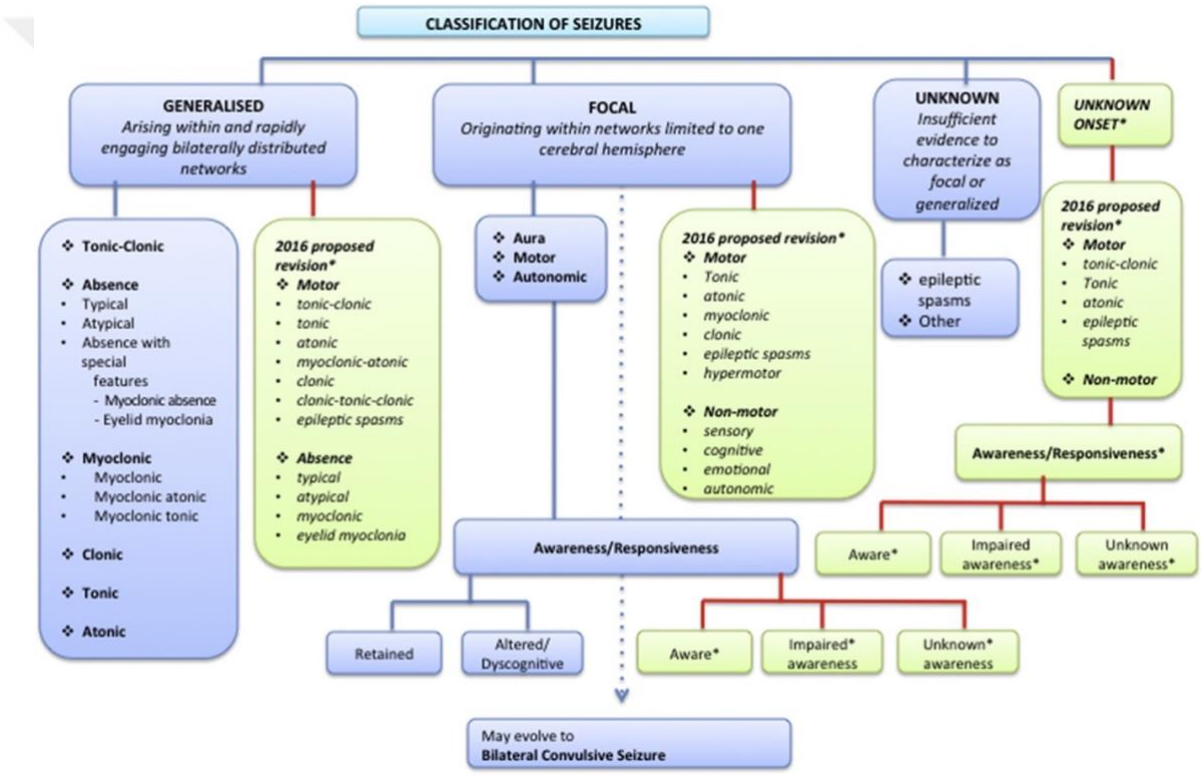


Figure 1. Classification of seizure types. Green boxes represent the unfinished revisions. Retrieved from Ngoh, A. et al. 2017 [7].

2.1.3 Types

According to the ILAE proposal, the types of seizures are summarized in table 1. Focal seizures involve seizure onset from a focal area in one hemisphere and this type of seizures has no longer dichotomized into simple versus complex on the basis of changes in the consciousness (6). Generalized seizures originate in bilaterally

disturbed cortical or cortical/subcortical networks without certain focality. Although both focal and generalized seizures can be represented in many syndromes together, it should be investigated whether epilepsy is a result of focal pathology or not due to the possible implications for surgical options.

Table 1. Overview of Seizure Type

Seizure Type	**Brief Description**	**EEG Characteristics**
Focal (partial)	Onset of the seizure starts and restrained to one hemisphere	Start focally then may secondarily generalize
Neocortical	Seizure generation from neocortex; manifestation depends on exact location of origin and pattern of spread	Focal onset from neocortex
Temporal lobe	Seizure generation from temporal lobe; often consist automatisms, dystonia of contralateral hand, and postictal confusion	Focal onset from temporal lobe
Generalized	Seizure onset simultaneously from both hemispheres	Generalized
Absence	Brief loss of consciousness, eye blinking and staring, and/or facial movements with no postictal confusion	3-Hz generalized spike-and-slow wave complexes
Myoclonic	Quick, repetitive, arrhythmic muscle twitching involving one or both sides of the body; consciousness remains intact	Generalized spike-and-wave discharges
Clonic	Seizures consist of rhythmic muscle jerks during impaired consciousness	Fast activity (>10 Hz) and slow waves with occasional spike-wave patterns
Tonic	An increase in muscle tone causes flexion of head, trunk, and/or extremities for several seconds	Bilateral synchronous medium to high-voltage fast activity (10-25 Hz)
Tonic-Clonic	Tonic extension of muscles followed by clonic rhythmic movements and postictal confusion	Tonic phase: Generalized rhythmic discharges decreasing in frequency and increasing in amplitude Clonic phase: Slow waves
Atonic	Brief loss of postural tone, which can result in falls and injuries	Slow rhythmic (1-2 Hz) spike-and-wave complexes or more rapid, irregular multifocal spike-and-wave activity

In the ILAE proposal the causes of epilepsy was changed from idiopathic to genetic for epilepsies in which genetic factors play major role in the development of disorder and the inheritance of the causative/susceptibility genes occur with Mendelian, mitochondrial or complex patterns or as a consequence of de-novo mutations that might or might not be inherited in the next generation; from symptomatic to structural or metabolic (e.g., stroke, trauma, brain tumor, cortical malformations, aminoacidopathy); and from cryptogenic to unknown causes (6).

2.1.4. Epidemiology and Prognosis

2.1.4.1. Incidence and prevalence

The prevalence of epilepsy shows regional changes as a consequence of differences in encountering the risk factors such as infections and inadequate antenatal and perinatal care (62). In high-income countries the incidence range is between 5-8 per 1000 population whereas it increases to 10 per 1000 for low-income countries (63).

2.1.4.2. Comorbidity and Mortality

Although not all epilepsy forms result in increased mortality, and no proven relationship for decreased life expectancy in self-remitting cases like childhood is available in absence epilepsy (6), life expectancy is decreased by up to 2 years in some genetic epilepsy patients and patients with unknown cause, while it reduced to 10 years in structural or metabolic type epilepsy patients (64).

The mortality ratio in epilepsy patients living in high-income countries is 2-5 times higher than general population, while this ratio increases by up to 37 times in low-income country residents (65). High mortality is profoundly significant during the first years after seizure onset due to underlying causes of epilepsy and comorbidities (6) such as psychiatric disorders (21). The most common cause of excess mortality is the sudden unexpected death in epilepsy (SUDEP) in high-income countries (66).

The incidence of SUDEP changes across population, in newly diagnosed patient group the incidence was found to be 0.1 per 1000, 2-5 per 1000 in chronic epilepsy patients and 9-10 per 1000 in candidates for epilepsy surgery (66). On the other hand, uncontrolled generalized tonic-clonic seizures is the main factor showing seven times increased risk (6). Other risk factors are early onset (<16 years of age) and long duration (>15 years) (67). When compared with general population, young people

show 16-24 times higher risk of sudden death associated with epilepsy (68). In childhood-onset epilepsy, SUDEP develops mainly in patients who are not in remission and in those with known cause of epilepsy, while it rarely happens before adulthood (69, 70).

Comorbidities increase the burden of epilepsy and complicates the drug selection and prognosis. Comorbidities include depression, learning disabilities, attention disorder and autistic features (6). Diagnosis of anxiety or depressive disorder is present in a third of people with epilepsy, twice the prevalence of general population (71), somatic comorbidities is also common (72). These comorbidities might be causative (e.g., epilepsy caused by a cerebrovascular disease) or resultant (e.g., depression or obesity caused by antiepileptic drug) or epilepsy and comorbidity can share a common underlying cause (72).

2.1.4.3. Pathophysiology

Transient abnormal synchronization leading to epileptic seizure disrupts normal patterns of neuronal communication which can produce several different symptoms and signs according to the site of origin of the seizure (epileptic focus) and the connections. Seizures are believed to originate from increased excitation or decreased inhibition within the epileptic focus (6). But the presence of neuronal networks such as cortico-thalamic and basal ganglia networks (72, 73) or hippocampal and neocortical networks (74,75) plays a significant role in pathophysiology. The connectivity between different networks is the basis for coordination of different tasks and behaviors which is responsible for wide-range comorbidities of epilepsy (6).

Different networks can be employed in the initiation, spread, or termination of seizures, these networks can also undergo plastic changes through development in region-specific, sex-specific and age-specific ways (6). Therefore, identification of specific subcortical networks has paramount importance in seizure modulation. Epigenetic factors (such as stress, seizures, inflammation, and drugs) can also affect dynamics of network in terms of signaling pathways and brain development (76,77).

2.1.5. Diagnosis

Correct diagnosis of epilepsy syndrome can be complex; medical history of family, age of the first occurrence of the disease, seizure type, neurological and cognitive condition, and an interictal EEG are compulsory (6). A brain MRI is often used except in the case of typical syndromes such as childhood absence epilepsy. Blood tests and lumbar puncture are applied when specific causes are suspected (6). It should carefully be evaluated, so that misdiagnose between epileptic seizures and other disorders such as convulsive syncope and psychogenic non-epileptic attacks is avoided (78).

Advanced imaging technology along with the application of epilepsy-aimed protocols for image acquisition and analysis (three-dimensional fluid-attenuated inversion recovery and voxel-based analyses of multiple contrasts) is highly useful.

Electroencephalograms and magnetoencephalography are used to observe the electrical energy bursts of seizures. These bursts are characterized by highly elevated electrical and magnetic signal frequencies and amplitudes (79). These neural oscillations require elevated energy (80). The interaction of GABAergic inhibitory mechanisms and glutamatergic excitatory mechanisms comprise the neuronal firing whose amplification and synchronization is the base of seizure (1).

2.1.6. Management

Overall, almost 70% of patients gain freedom from seizure with proper medical treatment with response rate dependent on the type of epilepsy syndrome, underlying cause, age (neonates, infants, elderly people), sex (issues related to contraception, childbearing potential and bone health), drug interaction potential, comorbidities and other factors (81,82). Management of epilepsy involves multidisciplinary teamwork involving trained neurologists, neurosurgeons, neuropsychologists, nurses and EEG technicians and radiology staff (8). Treatment modalities involve pharmacological treatment, hormonal therapies, surgical treatment and neurostimulation. The mechanisms of each therapeutic method vary and thus affect epilepsy to varying extents (1).

2.1.6.1. Pharmacological Treatment

The best treatment for epilepsy remains antiepileptic drugs (AED) (table 2). No single AED is ideal for first-line treatment in all patients (6) and the choice involves consideration of the type of epilepsy syndrome, seizure type, pharmacokinetic profile, interactions/other medical conditions, efficacy, possible side effects and cost (8). Current AEDs show their effects on abnormal neuronal networks mainly through blocking excitatory NMDA receptors, targeting neuronal voltage-gated sodium and calcium channels or enhancing GABA neurotransmission (83) (table 3). For instance, while Na_+ channel blockers such as carbamazepine reduce the frequency of neuronal action potential, GABA transaminase inhibitors such as vigabatrin increase GABA-mediated inhibition. These established antiepileptic drugs remain valuable first-line options while some newer antiepileptic drugs are increasingly used due to their improved tolerability and reduced tendency to cause drug interactions (6).

Table 2. Antiepileptic Drugs: Timeline of Introduction

1857	- Bromides	1974	- Carbamazepine	2000	- zonisamide (ZNS)
1912	- Phenobarbital	1975	- Clonazepam (CZP)	2005	- Pregabalin (PGB)
1937	- Phenytoin (PHT)	1978	- Valproate (VPA)	2008	- Lacosamide (LCM), rufinamide
1944	- Trimethadione	1993	- Felbamate (FBM),	2009	- Vigabatrin (VGB)
1954	- Primidone	1995	- Lamotrigine (LTG)	2011	- Retigabine
1958	- ACTH	1997	- Topiramate (TPM),	2011	- Clobazam
1960	- Ethosuximide	1999	- Levetiracetam	2012	- Perampanel
1963	- Diazepam	2000	- Oxcarbazepine	2013	- Eslicarbazepine

The treatment with current AEDs also has several major drawbacks such as cognitive impairment, including memory loss, mental deterioration and impaired learning (84) and these side effects become more severe in the case of polypharmacy. On the other hand, in patients who failed to respond to the initial antiepileptic drugs, the probability of their response to other pharmacological treatment decrease proportionally with the number of other antiepileptic drugs (6) and these patients become medically refractory. Increased risk of teratogenicity in woman who are receiving pharmacological therapy for epilepsy is also documented (85). Family planning might also be affected for woman who take the enzyme-inducing AEDs such as carbamazepine because hormonal forms of contraception are disturbed (86).

The two most common drugs used in absence epilepsy are ethosuximide and valproic acid. Ethosuximide is the first choice and seizures generally respond well to this drug. Possible side effects include nausea, vomiting, sleepiness, hyperactivity. Valproic acid on the other hand has been associated with higher risk of birth defects in babies. Furthermore, adverse effects such as mood alteration, suicidality, skin rash, hepatotoxic effects, decreased bone mineral density, weight management difficulties, severe mucocutaneous reactions, pseudo lymphoma might be seen after AED treatment leading to the failure of treatment (87).

2.1.6.2. Immunomodulation Therapies/hormonal therapies

Some epilepsy forms such as the ones developed due to autoimmune encephalitis (e.g., anti NMDA receptor encephalitis) do not respond to AED but may respond to immunomodulation. Corticosteroids and adrenocorticotropic hormone (ACTH) are the first-line therapy for many epileptic encephalopathies (88). It was demonstrated that when the comparison was done as hormonal treatment with an antiepileptic drug (vigabatrin) versus hormonal treatment alone, higher proportion of spasm-free children between 12-42 days was found in the hormonal treatment and vigabatrin given group (72%) compared to those given only hormonal treatment (57%) (89).

2.1.6.3. Surgical treatment

Ten to twenty percent of patients fail to obtain adequate seizure control with anti-epileptic drugs, and 50% meet the criteria for surgery with a clear focus via removal of the epileptic regions and thus blocking the abnormal cerebral network components (109).

Epilepsy surgery is performed in appropriately selected cases which involve: (a) presence of AED resistance; (b) epileptic zone delineation in the brain; and (c) risk estimation of the surgery that involve neurologic and/or cognitive deficits. Types of epilepsy surgery are (1) intracranial video-EEG monitoring (‘’Phase 2’’ monitoring with implantation of subdural grid, strip, and/or depth electrodes); (2) resective surgery (e.g., anterior temporal lobectomy, transsylvian selective amygdalo-hippocam-pectomy); (3) disconnective surgery (e.g., corpus callosotomy, multiple subpial

transection); and (4) neuromodulation (e.g., vagus nerve stimulator implantation, NeuroPace device implantation, deep brain stimulator implantation) (8).

Development of MRI-guided laser ablation also known as MRI-guided laser interstitial thermal therapy enables a greater degree of precision in ablation of targeted area without damaging neighboring areas and decrease mortality.

Table 3. Mechanisms of Action of Antiepileptic Drugs

Antiepileptic drug (AED)		Voltage-dependent channels		Neuromodulation		Carbonic Anhyrase	Novel Target
		Na^+	Ca^{2+}	GABAergic	Glutamatergic		
Established AEDs	Carbamazepine	inactivated					
	Ethosuximide		inactivated				
	Phenobarbital			activated			
	Phenytoin	inactivated					
	Valproic acid	inactivated	inactivated	activated			
Second-Generation AEDs	Felbamate	inactivated	inactivated	activated	inactivated (NMDA)		
	Gabapentin		inactivated				α2δ subunit
	Lacosamide	slow inactivation					
	Lamotrigine	inactivated					h-current
	Levetiracetam						SV2A
	Oxcarbazepine	inactivated					
	Pregabalin		inactivated				
	Retigabine						K_v7 potassium channels
	Rufinamide	inactivated					
	Tiagabine			activated (decreased reuptake)			
		inactivated	inactivated	activated	inactivated (AMPA/Kainate)	inactivated	
	Vigabatrin			activated (decreased metabolism)			
	Zonisamide	inactivated	inactivated	activated (?)		inactivated	

2.1.6.4. Neurostimulation

Neuromodulation therapy provide seizure control in a minority of cases and was developed as a palliative second line treatment for patients with drug-resistant epilepsies and not amenable for resection surgery.

Vagal nerve stimulation is mostly accepted and used approach with consistent data across countries showing 50% or more seizure reduction in half of the treated patients (3), although only 5% gain seizure freedom after this treatment. Main techniques involve transcutaneous stimulation of vagus (16) and trigeminal nerve (57) with encouraging results that is needed to be corroborated in further trials.

Other neuromodulation therapies include transcranial magnetic stimulation which is used rarely due to mixed results and deep brain stimulation that only used in severe epilepsies. Newer modalities of neuromodulation therapy include, closed loop systems as responsive neuro-stimulation and transcranial direct-current stimulation (7).

2.1.6.5. The ketogenic diet

The ketogenic diet (KD) is described as high fat, low-protein and very low carbohydrate and response rate to seizure reduction may vary from 38% to 76% for specific syndromes (7). Generally, KD can be beneficial for almost 50% of children suffers from epilepsy (7) and become the first-line treatment for children with GLUT-1 and pyruvate dehydrogenase deficiencies. Epilepsy syndromes such as myoclonic astatic epilepsy, infantile spasms, Dravet syndrome, Lennox Gastaut syndrome, Rett syndrome and tuberous sclerosis also are positively affected from ketogenic diet (90), although some contradictions may appear such as hyperlipidemia, pyruvate carboxylase deficiency, porphyria and fatty acid oxidation disorders (7).

2.2. Characteristic of human absence epilepsy

2.2.1. General

Clinical absences are classified as ‘’generalized nonmotor seizures, and defined as impairment of consciousness with mild clonic, atonic, tonic, or autonomic components by ILAE (91) with clinical behavioral hallmark of a ‘’blank state’’. As a result of the localization and the involved cortical networks, some motor symptoms may arise such as bilateral mild eyelid fluttering and myoclonic jerks of extremities (6).

The term absence seizure is used for absence of consciousness which itself compose of arousal (alertness) and awareness components (knowledge of self and

surroundings) (92). During absences, eyes are open, and patients retain an upright position. Differential diagnosis include sleep, unproper reaction to exogenous stimuli due to neurological problems such as dementia, chronic disturbances of consciousness like unreactive wakefulness (apallic) syndrome, and psychiatric conditions with hallucinations, fugue states, stupor, and other neurologic/psychiatric conditions (6).

2.2.2. Incidence

Absence seizures have prominent inheritance and the incidence changes among different populations. It is reported that of nearly 200 children with seizures <15 years of age, 19% showed absence epilepsy while in a study including 8285 children in Argentina, the incidence was found to be 4% (5). In the United States (94) the reported value was 10-12% whereas 6.5% incidence rate of absence epilepsy was documented in the Spanish study (5). From several studies, 10% is indicated as the average incidence of absence seizures in pediatric epilepsy (5, 6, 7, 10). The proportion of absence syndromes is higher in females when compared to male with the mean of age is 7.5 years (5).

2.2.3. Types

2.2.3.1. Childhood versus juvenile absence seizures

Childhood absence seizures bear the average ictal duration of 9 s, and with the clinical features of staring, activity arrest, loss of awareness, 3-Hz bilateral spike-and-wave discharges as well as the eyelid movements (9).

In juvenile form, female patients are more susceptible than male (95) with the mean age of 12 years and most of them have the family history. Generalized tonic-clonic seizures accompany in nearly one-half of juvenile epilepsy patients who usually become seizure free in about 10 years. Patients often show mild cognitive impairment especially verbal memory with a characteristic personality trait of neglect of physical need and reluctance to continue therapy (5). Juvenile form is more frequent than childhood form in patients of all ages with absence seizures (96), but the patients with childhood absence epilepsy show better prognosis (5).

2.2.3.2. Frequency and duration changes

Ictal bilateral SW complexes are differentiated according to their frequency and duration. The first group of complexes last >4 s with a quick decrease in frequency from 5 to 3.5, and then to 3 Hz while second group shows a shorter duration (<4 s), also decreased in frequency from a similar 5 to 2–2.5 Hz. It is concluded that the different types of changes correspond to different dynamics in the corticoreticular-cortical loop (97).

2.2.3.3. Typical versus atypical absences

Clinical and EEG characteristics of typical and atypical absences are similar but seizures in atypical type may be more resistant to control at the beginning (5). No differences are present between typical and atypical absences in regard to complex automatisms, incontinence, attention-deficit hyperactivity profile and learning disabilities (5). However, in the atypical form less abrupt onset and offset is emphasized while less developmental delay and a better response to medication is documented in typical form (98).

Two per second slow spike-and-waves (petit mal variant) represents ictal EEG pattern which is differentiated from typical 3/s SW discharges (SWD) (6). The loss of consciousness in frontal (99) and temporal lobe epilepsies (6) might be evaluated as absence seizures. Atypical absences in absence-like seizures in focal epilepsies can be distinguished from typical absence seizures by EEG and accompanying neurological symptoms (8).

Atypical absence seizures show less abrupt onset and offset but tend to last longer than typical absences. More pronounced changes occur in tone and different extent of impairment in consciousness (91). Atypical absences most likely occur during drowsiness and cannot be aggravated by hyperventilation or photic stimulation (100). Characteristic of atypical absence seizures is the interictal EEG pattern with generalized slow (1.5-2.5 Hz) spike and wave complexes which are irregular, asymmetrical, and lower amplitude while the ictal pattern is diffuse, irregular, slow spike and wave discharges (101). The ictal EEG discharges show irregular slow spikes and waves as well as waves and/or polyspikes (102).

Atypical absences develop through the course of symptomatic generalized epilepsies, such as the Lennox-Gastaut syndrome, in patients having severe neurological deficits (28). This type of absences also shows less pronounced impaired consciousness when compared with typical absence seizures.

2.2.3.4. Cognitive changes

Cognitive functioning in children with absence seizures, especially attention, fine-motor fluency, and visual memory show improvements after the children become seizure free (103). Longer duration of the generalized 3- Hz SW complexes is associated with poorer performance on the visual memory task. It is found in one study that 25% of children with ictal absences present with subtle cognitive deficits, 30% with behavioral disorders while 43 % with linguistic difficulties and 61% with psychiatric diagnosis (104). Jacquin et al., (105) stated that if absence seizures in children are not treated, the bilateral SWD can potentially lead to significant developmental problems in brain that interferes with information processing and contribute to mental status, eventually behavioral dysfunctions.

2.2.3.5. Absence epilepsy in infants

In one study, typical absence seizures in eight infants were documented with a mean age of onset of 20 months, and 3-4 Hz SWD on EEG. All responded to pharmaceutical treatment and in 2-7 years all become seizure free (106). Thus, the absence epilepsy before the age of 3 shows a favorable prognosis.

2.2.4. Relation of EEG and Clinical Symptoms

The onset of absence seizures is associated with the activation of distinct areas of dorsolateral frontal and orbital lobes (9). The negative slow waves are higher in frontal cortex, and the spike is dominantly represented in the frontopolar regions of the orbital frontal lobe. Ictal absence seizures therefore are not exactly generalized with global cortical concomitance, instead involves selective cortical networks (5) and when involved areas increase in number the behavioral symptoms also vary. It is stated by Holmes (107) that due to involvement of discrete networks, all seizures should essentially be classified as corticothalamic or corticolimbic in nature.

Clinical symptoms start to manifest when the duration of SWD excess minimum >5 seconds but with the shorter discharge there might be a slight impairment in cognition and motor functions (108). The degree of consciousness is strongly correlated with voltage and distribution of the SWD (81,110,111) without the effect of the duration (111).

Several clinical studies show that motor symptoms dependent on spikes, occur synchronously (65) to spikes. And types of motor symptoms such as eyelid fluttering, and mild myoclonic jerks are associated with the regions occupied by spikes that is the frontal eye field and frontal motor areas (112). In the case of juvenile myoclonic epilepsy, exaggerated jerks are represented to time-locked pattern to polyspikes (65).

There are no clear synchronous fluctuations, instead cortical function is suppressed for a short period of time. Slow waves are proposed to be inhibitory response to repress the development of poly-spikes (6). The ‘’staring spells’’ is considered to be the inhibitory seizures because of the inhibition of cortical activity (113).

MRI studies on the other hand shows the activated subcortical areas during ictogenesis, such as medial and posterior nucleus of thalamus, decrease activity in precuneus, caudate nucleus (5). It was also shown in the rodent models of spike-and-wave seizure that certain networks might be spared while SWD predominantly be observed in anterior frontal and parietal neocortical regions as well as corresponding thalamic nuclei with a little or no involvement of limbic and posterior thalamocortical networks (52,115).

2.2.5. Development of a seizure

Abnormal discharges of neurons which lead to the disruption of normal order of electrical patterns forms the basis of epileptic activity. Larger population of neurons are then subsequently synchronized which affect the long-range networks.

Once the cortical epileptic discharge is initiated, they are propagated from a small cortical area to larger areas and also to subcortical structures through cortico-cortical pathways and different brain circuits (145). Epilepsy shows more synchronized neuronal activity with depressed inhibition; thus, seizures are mediated via the decreased threshold for excitation or synchronization (32,146). This neuronal

activity is started and provoked by a positive feedback mechanism including cortical neurons with local axonal collaterals (147).

All-or-none principle is applied to the occurrence of epileptic bursts showing dependency to the firing frequency threshold that is in turn affected by the recovery from the former burst. Once the supra-threshold firing and firing rates are increased before a burst, population burst can be facilitated, on the other hand, reducing the cellular excitability or the activity of the excitatory transmission can suppress the burst onset.

The number of restrained depolarizing shifts cycles conversely affect the speed of the discharge propagation. This emphasis the importance of the numbers of recruited networks in epileptic seizures. With more discharges received from the neighboring area, the wave front progression needs more time, and the speed gets slower. Epileptic seizures propagate slower than action potentials and the mechanisms are structured by action potential conduction and recurrent excitation through excitatory synapses. This results in positive feedback, electrotonic coupling and non-synaptic interactions (146,148).

Epileptic seizure has a uniform hypersynchronous pattern, and largest synchronization is seen at ictal onset and at the termination with a gradual reduction throughout the seizure. At the midst of the seizure, networks show no synchronization.

After the onset of the epileptic seizure, the time of the field potentials propagation gradually decreases with an increase in the long-range synchrony. It extends the maximum level at the end of the seizure by desynchronization (149).

It is shown that the speed of the discharge propagation depends on the inhibitory mechanisms: GABA-antagonist elevated excitation reveals faster propagation at speeds of 10-100 mm/s while reduced inhibition show slowly propagating waves (0.1-20 mm/s). On the other hand, epileptic seizure activity is faster in elevated excitation models, conversely it propagates slower in decreased inhibition model. This shows that the energy produced during excitation sustain seizure propagation (1).

When there is enough energy and neurons to fire, the seizure can last for a longer period (150). And, either disrupted or mediated synchronization may lead facilitation of the termination of a seizure. The mechanisms to stop the seizures include

highly increased membrane conductance and disrupted synaptic integration which leads to reduced transmission efficacy, inhibitory transmission (151), and the changes in the abutting environment (149).

2.2.6. Origin of the Spike-and-Wave Discharges

The history of the search for mechanisms involved in SWD can go back to the first description by Hans Berger (1933) of a single EEG record obtained from a patient during absence attack (137). The incidence revealed a very rhythmic high voltage of 3-Hz discharges without ‘’spikes’’ and prominent generalized nature of the EEG pattern. It is also believed by the author that the seizures are caused by abrupt withdrawal of tonic inhibitory effects of thalamus upon cortex.

Four hypotheses have been suggested in explaining the origin of generalized spike-wave discharges seen in absence epilepsy so far. The first two theories give primary importance to the subcortical structures. ‘’Centrencephalic’’ theory was developed by Penfield and Jasper (1954) states that SWD originate from a deep subcortical structure in brainstem and midline thalamus that give rise to wide-spread projections to cortex on both hemispheres (138). Buzsaki et al., (1988) suggested the ‘’thalamic clock’’ theory which priorities the reticular thalamic nucleus for recruiting the pacemaker cells for SWD (139). On the other hand, ‘’cortical theory’’ proposed by Bancaud and Niedermeyer (1972) advocates that the discharges originate from a particular focus in the cortex with a passive involvement of subcortical structures (140,141). The ‘’corticoreticular’’ theory of Gloor et al., (1968,1969) covers both cortex and thalamus and suggests that a hyperexcitable cortex gives an abnormal respond to initially normal thalamocortical afferents leading to abnormal thalamocortical oscillations (142,143).

The development and persistence of widespread, synchronized SWD are correlated with highly synchronized oscillations of thalamo-cortical-thalamic circuit. The perioral region of the somatosensory cortex was consistently revealed as a cortical focus, and SWD at other cortical locations were lagged behind this site (Figure 2). Also, in a study comparing WAG-Rij rats with control rats, focal increase in sodium channel expression was found in the perioral somatosensory cortex of WAG-Rij before seizure onset (144). The intense focal seizure activity based on both

electrophysiological studies and fMRI was also showed in the previous studies (52,115,144). The cortical and thalamic sites show bidirectional interactions with a varying direction during a seizure. Nevertheless, in the first 500 msec, the cortical focus always precedes the thalamus, making it a dominant factor in initiating the paroxysmal oscillation in the corticothalamic network (52).

Basically, the cortex triggers the thalamus and the intact thalamus entrain the sustained character of the oscillations. Once the discharges have been set into motion, cortex and thalamus act as a unified network. This ‘’cortical-driven and thalamic-sustained’’ hypothesis for the origin of SWD make a synthesis between the ‘’cortical’’ and ‘’corticoreticular’’ theories and provides new concept in understanding of the SWDs (26).

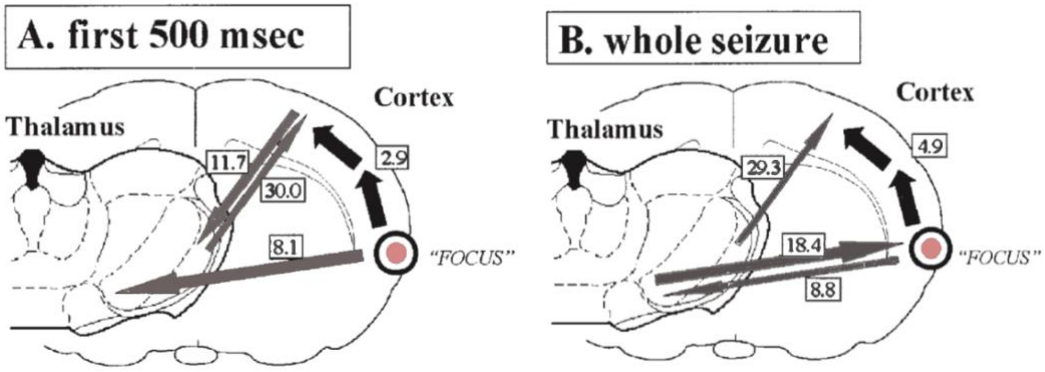


Figure 2. Representation of the corticocortical (black arrows), and cortico-thalamic (gray arrows) transmission during absence seizures in the WAG/Rij rat. The thickness of the arrows shows the association strength, and the direction indicates the direction of the lagging. A, In the first 500 msec of seizure, a cortical focus was found in the upper lip and nose area of the somatosensory cortex. B, Throughout the whole seizure, the same cortical focus as in A was found. Compared with A, the time delay from the seizure focus to other sites has increased. (Modified from Meeren, K. M.H. 2002 [52].)

2.2.7. Neural networks in seizures

Generalized seizures tend to start rapidly and engage into bilaterally involved networks with a different propagation pattern (179). The primary fundamental network in generalized seizures is the cortical-thalamic-cortical circuit. Due to a direct association between thalamic and seizure suppression, thalamic excitatory

neurotransmission mediate excitation (1). Thalamic engagement has been described as divergent-convergent excitatory amplification which employs two separate limbic pathways that arrive from the initiation point to the reuniting point. The first circuit involves connections from the subiculum to the prefrontal cortex and the second circuit employs connections from the piriform cortex to the entorhinal cortex. The circuits among this structure provide monosynaptic excitatory transmissions within the limbic circuit (1).

Action potential burst firing leads to an increase in neural synchrony, and further facilitates rhythmic activity in cortex and thalamus. As the onset of a seizure begin, enormous network activity occurs, which then crack into lesser subnetworks while spreading. These fractured networks are then united to form a dominant network component through the end of the seizure in focal seizures (178). Different orientation of subnetworks synchrony could lead to the termination of a seizure (1).

The key components in epileptic seizure are the cortical pyramidal neurons, GABAergic inhibitory neurons in thalamic reticular nucleus (TRN) and relay nuclei of thalamus. The TRN functions as a main inhibitory input source and receive excitatory inputs from corticothalamic (CT) and thalamocortical (TC) neurons (180). Inhibitory projections from TRN are then send only to thalamus. It was found that at seizure onset, CT-TRN-TC feed-forward inhibition is diminished while CT-TC connection is unaffected (181).

By providing simultaneous excitation to the relay nucleus and TRN, neocortical focus initiates the circuit, thus triggers the ictal discharges. The thalamus continuously affects the seizure propagation through the midline nuclei which connected to neocortical structures as well as the limbic system (32). Additionally, activated glutamatergic AMPA and NMDA receptors can also boost cortical/thalamic excitation of TRN leading to seizure enhancement (169).

Epileptic activity is believed to cause by abnormal dynamics within normal circuitry elements. Neuronal network employs various cell types that are (inter)connected through synapses whose activities are modulated by extracellular milieu. Neuronal networks are connected with synaptic- or non-synaptic interactions and the production, spread and termination of epileptic seizures depend on this transmission. This include the abnormal functioning of neurons and glial cells addition

to the excitatory and inhibitory mechanisms with feedback responses. The cortico-thalamo-cortical circuit plays an important role in ictal activities in which the imbalance between GABAergic inhibition and glutamatergic excitation is the main pathophysiological incidence. Neuronal networks can be affected by regulatable negative feedback via mediating inhibition and excitation through neurotransmission fluctuations, receptor and transmitter signaling, and corresponding influences on ion concentrations and field potentials (1). Neurons are highly sensitive to synaptic changes and show slow adaptations to input levels. The ratio of the adaptation rates of the excitatory and inhibitory populations affects the stability of the adapting network.

2.2.7.1. Neurons

Major constitutional elements of cerebral cortex are principal (dominated by pyramidal cells) neurons (approximately 80%). Abnormally discharging pyramidal neurons have the ability to recruit surrounding neurons resulting in a seizure initiation in the seizure focus. Cortico-cortical transmission include excitatory glutamatergic pyramidal neurons that can send projections to subcortical areas (152). GABAergic neurons on the other hand plays an important role in the excitation of the network by regulation on the multiple excitatory neurons (153).

Interneurons constitute 20% of the total neuronal population and form a connection between neighboring neurons. They can either be excitatory or inhibitory with no motor or sensory function. Their ability to connect with distant regions is limited compared to the projection neurons (154). Interneurons can suppress heterogeneous network activity (155) because they can fire asynchronously without any propagation properties (156). Interneurons that release GABA as a primary neurotransmitter rectify excitation over local circuitries in which excitatory and inhibitory neurons make synapses (1). The loss of inhibitory interneurons causes a decreased projection to the principal neurons in the cortex which results in epileptogenesis (157).

Direct effect of interneurons on the microenvironment of synapses contribute to the cell migration and axonal growth (158). Additionally, interneurons can shape and control the cortical circuits through gap junctions via electrical coupling (1).

Inhibitory interneurons on the other hand, can regulate the firing of pyramidal cells as well as the organization of sensory fields and cortical plasticity for they can synapse with each other as well as with cortical pyramidal neurons (152). In fact, it has been shown that these interneurons are activated before seizure onset and show a synchronous behavior leading to facilitation to the transition from normal to seizure-like oscillations. This synchronous activity is the hallmark of transition from interictal to ictal in hippocampus and intrinsic rebound excitation of pyramidal neurons from depolarization block is believed to be underlying mechanism. In detail, the end of the interneuron theta coherence is suggested to be the onset of the transition to a seizure that is in turn mediated by the beginning of sustained ictal spiking (159).

The chloride accumulation in inhibitory interneurons in the ictal state, results in a depolarization of more GABAergic interneurons which facilitate the principal neuronal activity (160). In fact, the decrease of spikes in case of GABA receptors blockage in interneurons suggests the primary role of inhibitory interneurons in propagation synchrony (161).

2.2.7.2. Glial cells

The transportation of ions and metabolites between brain vascular structures and neurons take place with the help of glial cells. These cells are also responsible from regulating the extracellular environment such as neurotransmitter and potassium spatial buffering which is important mediator in seizure dynamics (162).

Glial cells use gap junctions to communicate with each other, and dysfunctional astrocytes can cause an increase in [K^+] through gap junctions in epilepsy. The excess ions cause the spatial redistribution of K^+ and misplacement of water channels that act in impaired K^+ buffering (1). Astrocytes support feedback mechanism to counteract the transiently excessive coupling (164). They can play a role in seizure development by increasing neuronal excitability and inflammation (165). Astrocytes can promote seizure with their receptors for GABA and glutamate transporters despite being less sensitive to electrical stimulation than pyramidal neurons due to their random orientation (163).

Astrocytes can release GABA consequent to glutamate uptake to modulate the neuronal excitability. This is mediated via tonic GABAergic inhibition, which is in

turn affected by slowly desensitizing, high affinity extrasynaptic GABA receptors (1). When network activity decrease/increase, the tonic activity changes accordingly leading to a flexible negative feedback loop (166).

Astrocytic release of ATP and glutamate via exocytosis and presynaptic receptors or postsynaptically on extra-synaptic N-methyl-D-aspartate (NMDA) receptors contributes to the development of focal ictal discharges (1). ATP release facilitated by adenosine from ATP hydrolyzation cause a presynaptic inhibition of excitatory synaptic transmission which is dependent on the number of astrocytes involved and the speed of Ca^{2+} wave propagation (164).

2.2.7.3. Synapses

Axonal and dendritic synapses are essential in epileptic activity, fundamental component of this connections are the axo-axonal synaptic interactions primarily between principal neocortical cells. On the other hand, proper functioning of micro circuits is closely associated with the development, modulation, and elimination of the dendritic excitatory synapses that are the main excitatory postsynaptic elements in brain (167).

Chemical synaptic connections via neurotransmitters are the main way of communication both for excitatory and inhibitory synapses. Excitatory glutamatergic and inhibitory GABAergic interneurons are major modulators in the neuronal networks. The mechanisms of glutamatergic activity can be summarized as; the saturation of glutamate receptors, desensitization of AMPA receptors, activation of presynaptic metabotropic glutamate receptors and inactivation of presynaptic voltage-gated calcium channels (168). NMDA receptors give faster response to synaptic glutamate increase than AMPA receptors due to their high affinity for glutamate with also an increase in activity after higher levels of glutamate (169).

Major mechanism of synaptic depression is the depletion of synaptic vesicles from readily available pool (1). The GABAergic synapses on the other hand, function to inhibit synchronization causing to hypersynchrony (1). In the case of a selective loss of GABAergic synapses that cause a sparing in the excitatory synapses, significant reduction in GABA release could be observed which cause a shift in the excitation/inhibition balance (158). For example, in a kainic acid injected mice model

of epilepsy, the receptors of $GABA_B$ in principal and interneurons are down-regulated rapidly, followed by an increased activity of GABA, affecting the abnormal discharges in the epileptic network (170).

Other way of transmission is the electrical synapse which is mechanically occurring via gap junctions. Gap junctions can also play a significant role in the synchrony of neural networks since the modulators of gap junctions such as blockers and enhancers affect the inhibition and increase respectively, and also in the absence of chemical synaptic transmission, the spontaneous synchronized neuronal activity can be mediated by gap junctions.

The propagation of action potentials recruits appropriate number of axonal gap junctions. But increased gap junctions might cause difficulty in the network oscillation coherency (171).

It has been shown that high level inter-neuronal gap junctions occur after seizure with a controversial consequence such as the prevention of further escalations or the facilitation of seizures (172).

The major communication way in gap junctions is via NMDA receptors, and the network synchronization is facilitated by the amount of released presynaptic GABA and the $GABA_A$ receptors. The GABA release and the receptor turn-over are in turn strongly correlated with gap-junction mediated inter neuronal synchronization (173).

During epileptic seizure activity, the gap junctions and chemical synapses cooperate and regulate each other with tightly regulated interplay (1).

2.2.7.4. Non-synaptic interactions

Gap junctions are no longer classified as non-synaptic transmission in seizures, instead they are classified as electrical synapses. Non-synaptic way of communication includes ionic interactions and field effects, and extracellular changes in these elements can result in swelling resulting in cell death. Modulations of cell shrinkage and increase in extracellular space can block the seizure activity (174), showing the significance of extracellular space in synchronization of neuronal activity.

The non-synaptic communications also sustain electrical activity and modulate changes. Epileptic discharges can alter the ionic environment in neuronal circuits such

as the increased concentration of extracellular K^+ and reduced extracellular Ca^{2+} (175), which further facilitate epileptic activities. Cellular excitability can easily be elevated by an increase in K^+ levels which results in synchronized responses. The equilibrium of K^+ concentrations depend on K^+ uptake and spatial K^+ buffering, which is regulated either by Na-K-ATPase or Na-K-Cl cotransporters. Cell swelling and changes in glial membrane potential is consequently followed (176).

Electrical activity modulated by extracellular environment generates electrical field effects, that can either lead to seizure spread (159) or suppress the epileptiform activity depending on the orientation of dendritic-somatic axis of the neurons (177).

2.2.8. Balance between excitation and inhibition

It has been shown that both excitatory and inhibitory communication are diminished during acquired network discharges. The decrease in excitatory synaptic transmission is more pronounced than inhibition, leading the hypothesis of the existence of inhibitory barrier which restrict normal brain to develop into overexcited status (1).

The pyramidal activity results as a collective affect from distal and proximal inhibitions. Distal inhibition inhibits dendritic NMDA-mediated spikes whereas proximal inhibition controverts pyramidal firing (148). The inhibition density can be modulated throughout the ensuing depolarizations which then lead to a deficiency in blockage of the fire increase.

Density of inhibitory synapses compared to excitatory ones shifts from 50:1 in proximity to 1:50 in close epileptic zone of the trunk of hippocampal CA1 pyramidal cells. Thus, glutamatergic transmission of peripheral dendrites enhanced slowly, while inhibitory synapses evoked by proximal dendritic tree through GABAergic receptors are effectually immediate. It is proposed that strong excitation might not result neuronal firing by suggesting that inhibition in the distal parts could well be vitiated by powerful excitation, on the other hand proximal inhibition exhibits to counteract the excitatory drive (148).

Wide-range brain regions recruits abnormal synchronous activity in generalized epilepsy. The balance between excitatory and inhibitory neurotransmission plays significant role in epilepsy as can be seen in the neurotransmitter release in epileptic cell culture model (182). $GABA_A$ antagonist

manipulation could lead to the discharge of pyramidal neurons showing that GABAergic modulation is the primarily dysfunctional mechanism. The time of the reorganization of excitatory and inhibitory synapses might also influence when excitation is suppressed (1).

Glutamate and GABA transporters play significant role in the balance between excitatory and inhibitory mechanisms in the brain. The activity of glutamate transporters can affect the turnover the GABA transporters via changing the intracellular concentration of Na^+ in astrocytes. The discharges could trigger the exchange of Glutamate to GABA or vice versa and the overexpression of glial glutamate and GABA transporters. When the exchange is suppressed, GABA concentrations significantly decrease while the duration of seizure-like processed enhanced in vitro (1). The uptake of glutamate leads the extracellular GABA levels to increase as well as the increase in the tonic inhibition through reversal of glial GABA transporters. This transporter mediated exchange mechanism can be thought as an adjustable negative feedback to counteract the dense excitation in seizures (166).

In epilepsy, neuronal networks can go plastic changes during abnormal cortical development. There are several mechanisms which enhance the frequency rise and severity of chronic spontaneous seizure occurrence such as; synaptic reorganization, selective elimination of GABAergic interneurons and axonal sprouting, and the formation of new excitatory circuits (183.).

Adult neurons and immature neurons respond differently to GABA during development. The inhibitory transmitter GABA inhibits adult neurons while it causes immature neurons to excite because after the first depolarization by initial high intracellular concentrations of Cl^-, cells lead to hyperpolarization and inhibitory pattern enhanced. But with the help of K-CI cotransporters that is in turn dependent to the K^+ electrochemical gradient, Cl^- can be stabilized, and the inhibitory actions of GABA can be reinstated (184).

Synchronous pattern of cortex is modulated by regulating mechanisms that is able to increase inhibition with increased excitation. The mechanisms compose of (a) combinations of different networks that release both excitatory and inhibitory neurotransmitters, (b) modulation of ionic conductance, (c) the number of ion channels

in the cell membrane (148) and (d) coordination the synaptic connections and counterbalancing the inhibition and excitation (185).

Neurons tend to accommodate very slowly to input levels leading to sustain their susceptibility high for synaptic changes. The adapting network's strength is closely related with the ratio of adaptation flexibility of excitatory and inhibitory neurons as a population (186).

In a study evaluating the precisely timed excitatory and inhibitory conductances and their dynamic detection to explore the balance of neuronal network, it is showed that inhibition is more pronounced in the pyramidal cells at the initiation of seizures, which is then converted to excitation throughout the propagation and in the termination part balance shift again toward inhibition (187).

The mechanism of epileptic discharges might involve the elevated glutamatergic input and variation in the GABAergic effect, which is perhaps resulted from repressed presynaptic inhibition, GABAergic vesicular depletion, postsynaptic desensitization, and an elevation in postsynaptic chloride or extracellular K^+ that in turn affect the depolarization of the GABAergic turnover potential (148).

Additionally, since seizure propagation over-ride adaptation over excitation from the excitatory focus influence the inhibitory cells leading to a reduction in the factors affecting the inhibitory presynaptic depression (146). This is the possible explanation of the ineffectiveness of some of the abnormal discharges on seizure-like event occurrence, without any clinical symptoms (1).

2.3. Typical Absence Seizures

2.3.1. General Characteristics

Typical absences are characterized as an impairment of consciousness with mild clonic-tonic motor symptoms and developed in childhood, between 4-10 years of age, with a few reports of later age (117). Typical absences mostly accompanied with idiopathic generalized epileptic syndrome (10) which can be provoked by hyperventilation (4) and rarely by photic stimulation (118).

The neurophysiological characterization of typical absence epilepsy is the occurrence of generalized 3 Hertz (Hz) SWD on EEG with rapid onset and termination (115), sometimes with higher amplitude on frontal cortical areas (119). The interictal

EEG shows normal background activity but sometimes short bursts of SWD might occur. These characteristic electroclinical features enable to differentiate typical absence seizures from atypical absence seizures or other seizures with impaired consciousness. (4).

Brain MRI of typical absence epilepsy patients shows no abnormal representation, therefore in the diagnosis this imaging technique does not necessarily applied.

Generalized epilepsy involves abnormally synchronized activity in interconnected networks which are either among local microcircuits or more long-range network interactions. Burst firing mode of action potentials is a proposed way of spreading the increased neural synchrony and believed to boost the cortical/thalamic rhythmic network activity. Enhanced burst firing is thought to be crucial in the transition from normal brain state to epileptic form and in corticothalamic neurons, this firing mode enables $GABA_B$ activation in the thalamus which then cause a slower and more synchronous oscillations seen in spike-and-wave discharges.

The significance of interactions between/within cortical and subcortical structures for the development of generalized epilepsy was emphasized by Jasper and Penfield (1941) (120,121). The major leading factor for development of typical absence epilepsy is attributed to genetic factors (122,123), with no described structural lesion/damage as a possible cause (124). The networks can be manipulated on the molecular level, as perturbations in neurotransmitters, their receptors, or various ion channel can affect this network (13).

2.3.2. Neural networks in typical absence epilepsy

2.3.2.1. Communication between Cortex and Thalamus during absence seizures

Thalamocortical networks have the capability to generate different synchronized oscillations, such as 7- to 15-Hz sleep spindles and the 3- to 4-Hz generalized spike-wave discharges observed in absence epilepsy. The cellular elements are shown in figure 3. Thalamocortical relay cells receive efferents from variety of brain regions, then thalamocortical cells send excitatory (glutamatergic) afferent to cortical pyramidal neurons (layers III-IV) to convey information addition

to reciprocal excitatory and inhibitory pathways between cortical and TC neurons figure 3. Cortical pyramidal neurons from layer VI in consecutive make excitatory connections with thalamus reciprocally. Inhibitory GABAergic interneurons are present throughout the cortex and the thalamus while the specific thalamic nucleus in the lateral thalamus, reticular thalamic nucleus (TRN), has pure GABAergic interneurons (14). TRN receives collaterals from thalamocortical and corticothalamic axons which excites the TRN neurons. GABAergic neurons in the TRN project back to the thalamus while also make synapses with other GABAergic TRN neurons (13).

To generate both 6-to 10-Hz spontaneous spindle wave oscillations and a 3-to 4-Hz paroxysmal rhythm cyclical interaction between excitatory and inhibitory neurons in the intrinsic circuitry in thalamus is needed (125,126). Spindle waves are on the other hand develop through interactions between excitatory thalamo-cortical cells and inhibitory GABAergic TRN cells. Fast $GABA_A$ receptor-mediated inhibitory postsynaptic potentials (IPSPs) in thalamocortical cells are generated in thalamus. After recovery from a hyperpolarization IPSP large calcium spikes are developed by thalamocortical cells, with a superimposed burst of action potentials. Low-threshold calcium spikes play an important role in generating bursts of action potentials (127). Firing of action potential in thalamocortical cells, in consecutive generates excitatory postsynaptic potentials (EPSPs) in GABAergic thalamic neurons which cause calcium spikes with superimposed burst of action potentials. These action potentials set the next cycle of the network oscillation (128). It is therefore concluded that during spindle waves, $GABA_A$ receptor mediated IPSPs determine the network oscillation frequency at 6-10 Hz. Spontaneous 3- to 4- Hz activity also can be generated for example, by blockade of $GABA_A$ receptor and this transition is correlated with a large increase in the burst firing of thalamocortical and TRN neurons and increase in the duration of calcium spikes and EPSPs.

Various inhibition wave patterns can be produced in thalamus according to the manner of GABAergic cells firing (126). Brief firing in GABAergic PGN cells for example, generate fast (duration of 100 ms) $GABA_A$ receptor mediated IPSPs in thalamocortical neurons which can set the oscillation frequency to the spindle wave frequency of 10 Hz (126). On the other hand, strong firing is able to provoke slow (duration of 300 msec) $GABA_B$ receptor-mediated IPSPs in thalamocortical neurons,

leading to change the oscillation frequency to 3- to 4- Hz. Therefore, increased burst firing in thalamic networks plays an important role transition from sleep spindles to spike-wave complexes in cortex. Increased IPSP durations in thalamocortical cells and increased EPSPs in TRN neurons might be the modulators leading to enhancement in calcium spikes for both cases (13). Furthermore, there are several hypotheses about how the increased cortical firing interact with thalamic network. It is suggested that increased burst firing in long-range networks might be a possible mechanism for synchronization of the epileptic activity in widespread brain regions. According to this hypothesis, GABAergic neurons in thalamus increase their firing as a result of the increased firing of the cortex which releases high amount of GABA into thalamic synapses. The excess GABA activates $GABA_B$ receptors which in turn changes the oscillation frequency to 3 Hz instead of normal spindle-wave oscillations (13).

The slow IPSPs occurred during 3-to 4-Hz oscillations employ the $GABA_B$ receptors for in the case of blockade of these receptors, only fast 6- to 10- Hz network oscillations can be produced. The same is valid for the fast IPSPs, they are mediated by $GABA_A$ receptors because when $GABA_B$ receptors are blocked, slow IPSPs and slow 3- to 4- Hz oscillations are the only produced effects (13).

It can be said that by simple shifting of corticothalamic input from single bump to bursts, thalamic activity can be switched from $GABA_A$- mediated spindle wave form of decreased burst firing in thalamic neurons to 3- to 4-Hz $GABA_B$-mediated paroxysms with sustained burst firing in both thalamocortical and GABAergic neurons (129).

The crucial element for the transition from normal neuronal activity to spike-and-wave seizures is the increased burst firing of cortical and thalamic neurons. During absence activity, abnormally synchronous and high-frequency neuronal firing can be observed in widespread brain regions, with a need of specific nodes for propagation and physiological translation of seizures (130). Imaging study of human generalized tonic-clonic seizure revealed that focal regions of frontal and parietal association cortex get fully activated while other regions are relatively spared (131).

Several mechanisms involve in increased neuronal burst firing mode, which include alterations in action potential following depolarization facilitated by Na^+ or Ca^{2+} conductances (127). Abnormal expression of persistently activated Na^+ channels

affect primarily the enhanced burst firing of cortical neurons (132,133). This enhanced burst firing by Na^+ channel-mediated action potentials can be considered as the final pathway of electrical hyperexcitability in interictal and ictal activities in epilepsy (134).

Throughout the nervous system, there are more than eight voltage-gated Na^+ channel genes showing different expression patterns and functional properties. Specific combination of these channels in neuronal membrane determines the membrane's firing pattern and electrical conduction features (135). Nav 1.1 and Nav 1.6 sodium channels which is related with a persistent current underlie neuronal burst firing (136) which lead to hypothesis that increased expression of Nav 1.1 and Nav 1.6 may have a role in intense epileptic activity observed in anterior cortical regions during spike-and-wave seizures (13).

2.3.3. Molecular Mechanisms of Absence Seizure

Factors that have an impact on the way of development of normal brain function may also affect the absence seizures (epileptogenesis) such as including neurochemical, neuroanatomical and neurophysiological manifestations (5).

Absence seizures show bilaterally synchronous burst-firing of reciprocally connected neuronal populations found in the thalamus and neocortex. It has been shown that neurons in the reticular thalamic nucleus (TRN), thalamic relay neurons (RNs), and neocortical pyramidal cells comprise a circuit that sustains thalamocortical oscillatory burst-firing of absence seizures.

Three intrinsic neuronal mechanisms have been proposed to be responsible from the increased likelihood of thalamocortical oscillations. The first mechanism is the T-currents induced by activating the T-type calcium channel, which appear to trigger sustained burst-firing of thalamic neurons during absence seizures (The anti-absence agent ethosuximide suppressed T-currents). A second intrinsic mechanism is via $GABA_B$ receptors which can cause long standing hyperpolarization in thalamic neurons needed to 'prime' T- channels for sustained burst-firing. A third mechanism involves the ability of $GABA_A$ receptors, located on TRN neurons, to facilitate recurrent inhibition. Enhanced activation of this $GABA_A$ receptors decreases the

pacemaking capacity of the neurons they are expressed in, therefore decreasing the risk of absence seizures.

2.3.3.1. Gamma-Aminobutyric acid

The first report of GABAergic system dysfunction in children with absence seizures came in 2004 (188). Lower levels of GABA transaminase (GABA-T) has been reported in these patients than in controls. The capacity for GABA uptake is higher in patients taking the antiepileptic drug.

2.3.3.2. T-calcium channels

T-calcium channels can lead the further depolarization of the cell membrane via the entrance of its two positive charges with every calcium, therefore it is suggested that these channels have a significant pacemaker role in gating Na^{+} and Ca^{2+} channels as a part of the ictogenesis in absence seizures (189). High level expression of these channels in the dendrites shows their importance in synaptic integration. More specifically, ictogenic burst firing of thalamic neurons is driven by Ca^{2+} influx through T-calcium channels making these channels an important target in absence epilepsy (190). These channels are also activated by membrane hyperpolarization as a result of inhibitory inputs. Thalamocortical neurons receive these inputs from GABAergic neurons in the thalamic reticular nucleus (5).

2.3.3.3 Typical absence epilepsy and thalamus

Thalamic activation is profoundly increased in ictal absence seizures and the cortical changes are likely associated to a thalamocortical network, while less pronounced activation of cortex is present apart from the deactivation in some cortical areas (192). The 3-Hz SWD are correlated with decreased signal detection in the caudate nucleus, precuneus, and parietal areas, while in the medial thalamus there is a bilateral increase (193) which are part of the ‘’striato-thalamo cortical network’’.

Anterior thalamic volumes are increased in patients with absence seizures, contributing the structural difference in absence patients (191). Significant lower ratio of thalamic N-acetyl aspartate (NAA)/creatine level determined by magnetic resonance spectroscopy is also apparent in patients with absence seizures suggesting neuronal dysfunction in the thalamus in the epileptogenesis of absence epilepsy.

2.3.3.3.1. Thalamocortical mechanisms that regulate the synchronized burst-firing

The main function of thalamus is to convey and control input from periphery to cortex. Thalamus is comprised of different nuclei and each has the ability to produce and sustain certain natural rhythms including synchronized EEG patterns. The properties of the thalamus that contribute to generate rhythmic activity are the intrinsic behaviors of its neurons that produce burst firing patterns and the synaptic connections constitute the thalamocortical network (194).

2.3.3.3.2. Intrinsic properties of thalamic neurons

Thalamic neurons are able to generate ‘’burst’’ of action potentials, as well as Ca^{2+}-dependent low-threshold spikes (LTS). The LTS are produced at lower firing threshold than normal and they are identified as slow depolarizing oscillations that are able to produce several fast Na^{+}-dependent action potentials. The LTS is strongly related with resting membrane potential (RTS).

Thalamic neurons do not get activated under normal RTS (approximately -60 mV) but when the membrane is polarized to 7-15 mV, their activation can easily be facilitated (2). In the case of depolarization, tonic firing is promoted without LTS, which is strongly related to stimulus intensity (127,195). At the hyperpolarization state, on the other hand, LTS is generated by synaptic transmission or by way of polarization following phasic or burst firing as a response to normal threshold excitation.

As a result, there is a strong relationship between RTSs and membrane potential. With hyperpolarization, Ca^{2+} conductance that is the basis for LTS gets deactivated, leading to a burst discharge. The prolonged/cyclical hyperpolarization on the other hand is needed for phasic firing rhythms to continue, perhaps via thalamocortical circuitry (196).

2.3.3.3.2.1. Rhythmicity provoked by thalamocortical activity

Relay cells in thalamus via their efferents projections activate the reciprocal inhibitory feedback mechanism which is facilitated by inhibitory neurons of TRN. The inhibition from TRN is mediated by activation of postsynaptic GABA receptors as a consequence of hyperpolarization at the voltage range that is appropriate for LTS to

get activated. Therefore, TRN inhibition in turn mediates rhythmic firing via deactivation of the LTS (194). The rhythmicity may be generated and sustained via the hyperpolarization mediated RNs inhibition as a result of TRN cells activation; the release of hyperpolarization in RNs since without any inhibitory affect or direct excitatory input, LTS activation occurs with several Na^{+}-dependent action potentials; TRN and cortical neuronal excitation evoked by the burst output from RNs; and finally activated TRN cells could restart the entire cycle (Figure 3).

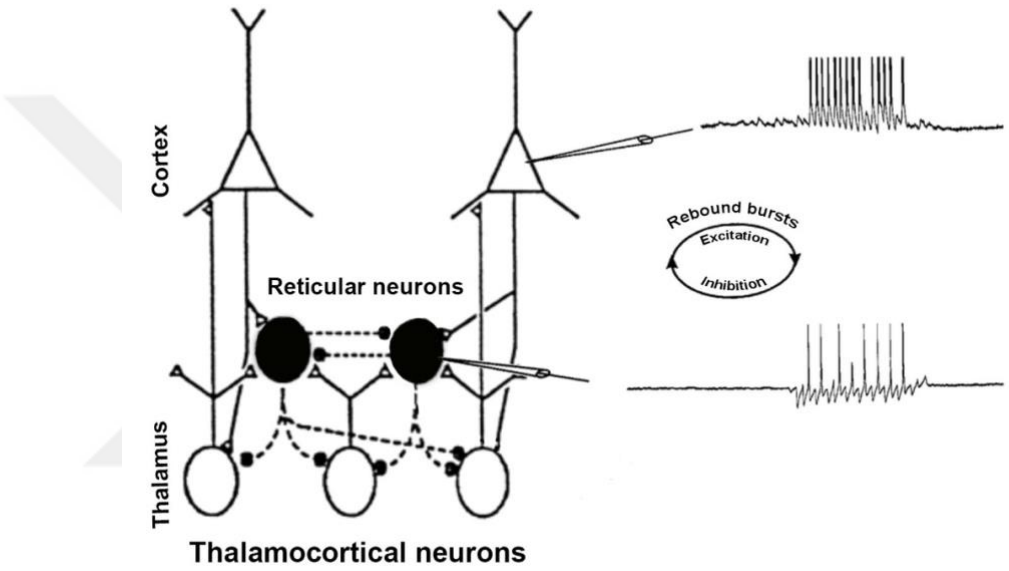


Figure 3. Once reticular neurons are activated, thalamocortical relay neurons consequently get inhibited which propagate Ca^{2+}- mediated action potentials that in turn excite the reticular neurons again. Retrieved from Futatsugi, Y. et al. 1998 [2].

2.3.3.3.2.2. Calcium flow in thalamic neurons

Thalamic neurons have three distinctive voltage-dependent activation patterns at their Ca^{2+} currents: (i) small depolarizations (low-threshold, -60 to -40 mV) activates inward current that is on the contrary gets inactivated very fast; (ii) stronger (higher-threshold, -30 to +20 mV) depolarizations lead to persistant current through the end of the depolarization; (iii) the strongest (>+10mV) depolarization noninactivates the current.

TRN cells represent comparatively large low-threshold transient currents. The large inactivating current is activated in the appropriate membrane potential range to promote the LTS (197).

2.3.3.3.2.3. $GABA_B$ receptors on thalamic neurons

Absence seizures can be exacerbated by drugs affecting the $GABA_A$ and $GABA_B$ receptors. The activation of these receptors shows different effects; $GABA_A$ receptor activation on thalamic neurons leads to short-lasting, chloride-dependent IPSPs while $GABA_B$ receptor activation generates long-lasting, potassium-dependent IPSPs. LTS is shown to be elicited by late $GABA_B$ dependent IPSPs (198). Increased number of $GABA_B$ receptor binding site has been found in mice model of absence seizures with a significant correlation between the magnitude of increased number with the frequency of seizures (199).

The post synaptic $GABA_B$ receptors on TRN can provide the lengthy, intense hyperpolarization needed to remove the inactivation (deactivation) of T-type calcium channels which are easily inactivated after activation.

2.3.3.3.2.4. $GABA_A$ receptors in nucleus reticularis thalami

It is shown that intra-TRN $GABA_A$ mediated inhibition plays a significant role in controlling the thalamic excitability (194). The mostly used drug clonazepam ($GABA_A$ agonist) facilitate $GABA_A$-mediated recurrent inhibition at TRN neurons while decrease its $GABA_B$-mediated inhibitory output into TRN. Benzodiazepines also enhance postsynaptic GABAergic inhibition in TRN leading to increase LTS that in turn results in more oscillations and exacerbation of absence seizures such as $GABA_A$ agonists. On the other hand, the $GABA_A$ antagonist bicuculline methiodide (BMI) when applied in RNs can enhance both $GABA_A$ and $GABA_B$ mediated IPSPs in TRN (2). Focal applications of BMI in TRN also could block the $GABA_A$ mediated IPSP without any effect on $GABA_B$ inhibition (2).

2.3.3.3.3. Ventrobasal thalamus and absence epilepsy

2.3.3.3.3.1. Thalamic communication with cortex

The thalamus is a nuclear complex found in the diencephalon and broadly divided into three subgroups as lateral, medial and anterior groups (61). It processes the sensory input subserving both sensory and motor mechanisms and filter in conjunction with the neocortex. The thalamus comprises over 50 different nuclear groups, each with different relaying characteristic signal to a functionally disparate cortical area, making the thalamus as a functional modality (59). Multiple cortical areas receive afferents from a single thalamic nucleus and send projections back to different thalamic nuclei.

Based on their morphology, synaptic location and synaptic pharmacology, excitatory afferents of thalamus are classified as either driver or modulator inputs (264). Driver inputs make synapses with relay neurons close to the soma, while modulator inputs synapses more distally in the dendritic tree (265). According to this input differences, thalamic nuclei is categorized as either first-order or higher-order nuclei; based on the origin of their driver input, the first-order nuclei make synapses with driver inputs from the periphery (e.g., auditory, visual, somatosensory), whereas higher-order nuclei synapses with driver inputs from cortical layer V (266) (Figure 4).

Thalamocortical neurons, of all thalamic nuclei, project to layer IV of the cortex and in turn get reciprocal modulatory corticothalamic inputs from layer VI which modulate the transmission of the information from driver inputs (267). Both thalamocortical and corticothalamic afferents send collaterals to associated thalamic reticular nucleus, which consequent to TC and CT innervation provide feedback and feedforward inhibition to thalamic nuclei, respectively (268,269,270).

Thalamocortical neurons (TC) of the ventrobasal nucleus of (VB) thalamus receives sensory inputs and send projections to layer IV in cortex. The neurons in the layer IV are then reciprocally send excitatory efferents to thalamus, making a thalamocortical loop. The sensory and corticothalamic (CT) afferents to VB are glutamatergic with a varying difference in the synaptic structures, postsynaptic receptor complement and functional properties of the two (Figure 4).

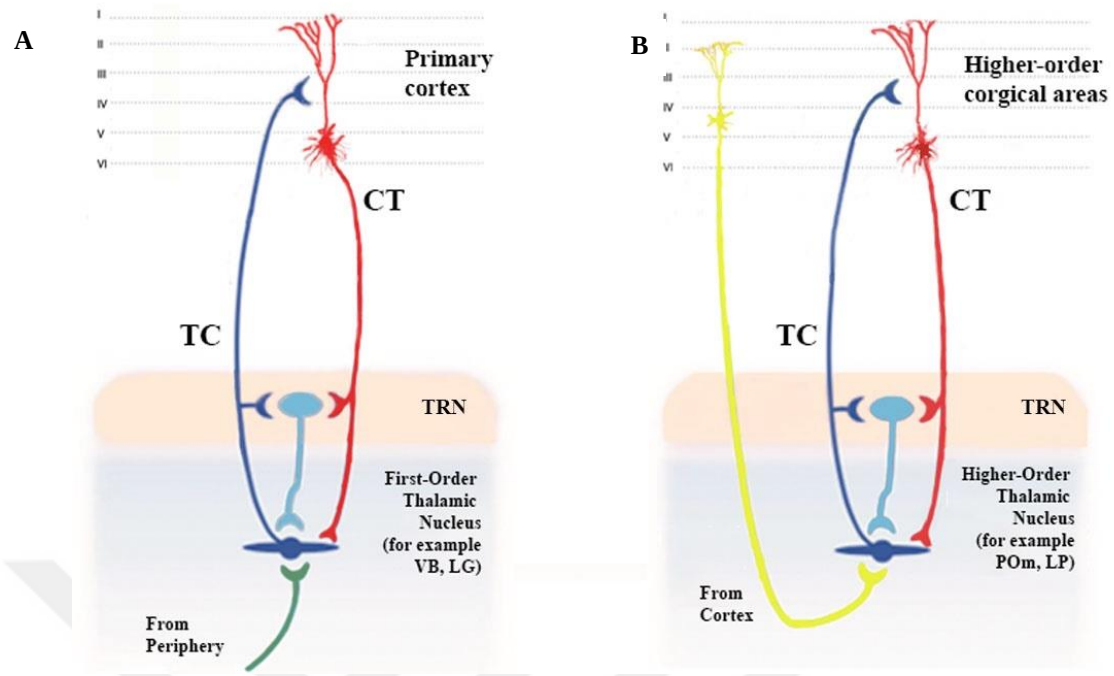


Figure 4. Thalamocortical circuits. A, Specific sensory or motor nuclei project to cortical layer IV, with collaterals producing thalamic feedback inhibition by reticular nucleus. The return pathway from layer-VI pyramidal cells synapses with specific- and reticularis–thalamic nuclei. B, Nonspecific intralaminary nuclei project to the most superficial layer of the cortex and layer-V cortical pyramidal cells return oscillation to the reticular- and the nonspecific-thalamic nuclei, making another resonant loop. Modified from Salt, T. E. et al. 2017 [59].

As in other thalamic nuclei (271), the increased CT stimulus intensity lead to a graded postsynaptic response in TC neurons of VB thalamus, also in multiple afferent recruitments (46). Additionally, ionotropic glutamate receptors relatively contribute to postsynaptic responses at the two synapses in which the ratio of NMDA to AMPA/kainate receptors are greater in the CT synapses (272). Preferential postsynaptic location of metabotropic glutamate receptors subtype 1 (mGlur1) to CT afferents in TC neuron dendrites (273), makes them a modulatory element in the firing activity of TC neurons (274). These receptors can therefore be employed in the expression of thalamocortical network oscillations in different behavioral states (9,275).

Dysfunctional cortico-thalamo-cortical circuitry has been reported by several researchers for absence epilepsy (52,129,142,276,277). It has been stated that for the occurrence SWDs both properly functioning thalamus and cortex are essential (278,279,280). Thalamus is involved in synchronization of thalamic neurons and spreading SWDs over the thalamus and cortex as well as their maintenance (281,282).

The typical absence epilepsy in rodent is initiated in layer 5/6 of the perioral region of the somatosensory cortex and then transmitted to the ventrobasal thalamus, primarily ventromedial nucleus, and the caudal TRN. The circuitry within thalamus behaves as an intrinsic oscillatory unit which is modulated by reciprocal synaptic connectivity between thalamocortical relay neurons (excitatory) and thalamic reticular neurons (inhibitory) as well as by post-inhibitory mechanism in relay neurons (283) (Fig 2). As a result, cortex primarily drive the excitatory impulse while thalamus organizes, amplifies and synchronizes the seizure behavior and the thalamocortical circuitry become the responsible element for all the electrographic, behavioral and pharmacological features of typical absence seizures (51).

The generation and maintenance of absence seizures are the results of abnormal firing activity among cortical cells as well as the neurons in the thalamic reticular and sensory thalamic nuclei. Enhanced GABA activity occurs as a consequence of increased firing in GABAergic neurons of TRN which is in turn drive by the synchronized cortical input, in sensory thalamic nuclei (Figure 5). The diminished GAT-1 transporter function additionally causes an increase in tonic $GABA_A$ current in thalamocortical neurons. This increase results a drastic reduction in the firing of thalamocortical neurons meanwhile prevents sensory input transmission and reduces the effect of possible glutamate increase by way of astrocytes on these neurons (19).

On the other hand, conjectural increase in glutamate through astrocytes leads to a synchronized firing in TRN. Since no tonic $GABA_A$ current is found in TRN, released GABA from astrocytes does not have any effect on the predicted underlying mechanisms of typical absence epilepsy (19).

TRN also shows changes in increased expression of T-type Ca^{2+} and P/Q-type Ca^{2+} channels of presynaptic terminals, decrease in alpha3 GABA-subunits, an enhanced expression of mGlu4 receptors, and down-regulated CB1 receptors (284,285,286). Morphological changes in cortical pyramidal cells leading to increased

excitability and/or decreased inhibition in the somatosensory cortex have been also described by Karpova et al., (287) in addition to GABA$_B$ receptor dysfunctions (288), changes in NMDA receptors (289) and deficit in layer 2/3 pyramidal neurons in cortical inhibition (290).

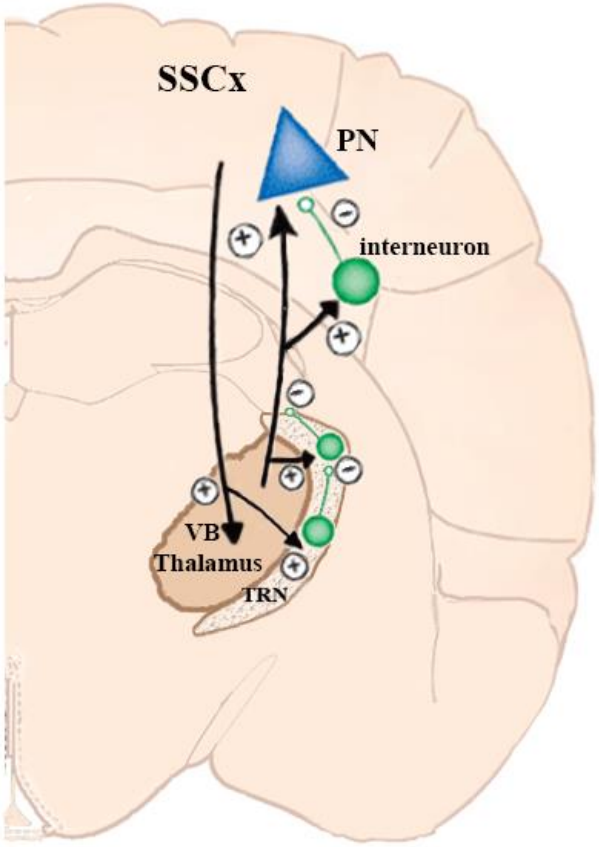


Figure 5. Schematic representation of the neuronal circuitry of typical absence seizures which includes the involvement of reciprocal connections between layer 5/6 of the perioral region of somatosensory cortex where the seizures are initiated, and the thalamus. This reverberating circuit is modulated and driven by reciprocal intrathalamic connections between the VB and rostral TRN (13). SSCx represents somatosensorial cortex; PN: Pyramidal neuron; VB, ventrobasal thalamus; TRN represent thalamic reticular nucleus; (−) indicates inhibition; (+) indicates excitation.

2.3.6.2. Ventrobasal glial cells

The glial cells in VB thalamus can be subdivided into two groups according to their [Ca^{2+}]i and electrophysiological responses to sensory and corticothalamic stimulation. The first group of astrocytes characterized with positive staining for S100B and ability to load with SR101. They have linear current-voltage relations with low input resistance, and show no voltage-dependent [Ca^{2+}]i responses, instead they develop mGluR5-dependent [Ca^{2+}]i transients as a consequence of stimulation of the sensory and/or corticothalamic excitatory afferent pathways. Other group of glial cells on the other hand stain positively for neuron-glia antigen-2 (NG-2) and represent with high input resistance and the presence of voltage-dependent [Ca^{2+}]i elevations as well as voltage-gated inward current (46).

Glial cells respond to synaptic stimulation with elevations in intracellular calcium [Ca^{2+}]i (291,292) which lead to release of gliotransmitters such as glutamate (293), therefore impact the local neuronal excitability as well as synaptic transmission (294). It has earlier been shown that astrocytes in VB thalamus behave as an independent glutamate supplier, leading to long-lasting NMDA-mediated activation of TC neurons both spontaneously (295) or in a response to mGluR activation (Figure 6) (296). Thalamic glial cells response to synaptic afferents differs from those of thalamocortical neurons, these cells are therefore likely to form a distinct level of cellular synaptic integration within the VB thalamus.

As VB astrocytes can respond to synaptic stimulation and signal to neighboring neurons, this glial cell organization might play a functional role in the processing of somatosensory information and modulation of thalamo-cortical network activities. Functions of astrocytes is summarized in table 4.

2.3.3.4. Factors external to thalamocortical circuitry

2.3.3.4.1. Cholinergic mechanisms

Cholinergic mechanisms may also play a role in absence seizure by modulating the excitability in cortex and glutamate-mediated mechanisms through depolarizing the thalamic neurons.

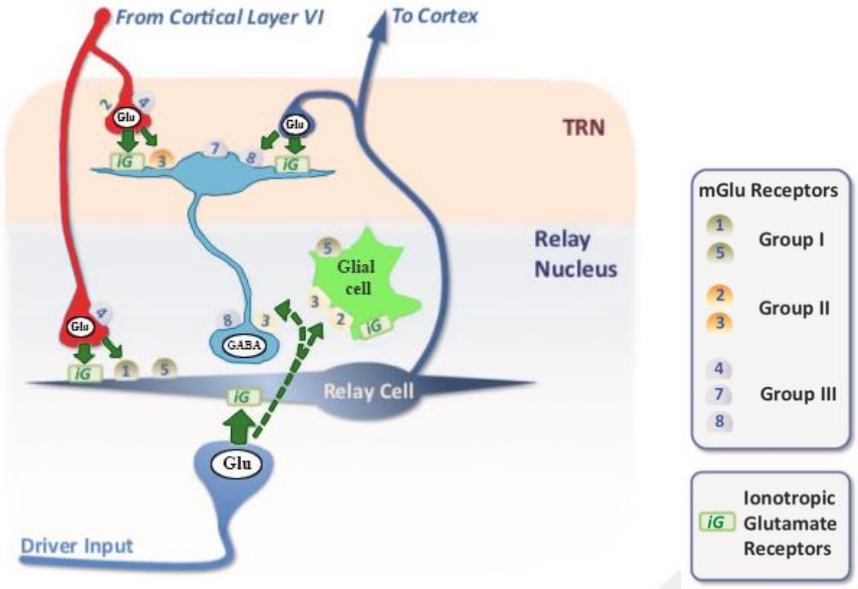


Figure 6. Synaptic connections among thalamic nuclei, with major afferents and efferents. Ionotropic and metabotropic glutamate receptors locations are depicted. Glutamate release is indicated by green arrows while dashed arrows show the possible synaptic spillover. Retrieved from Salt, T. E. et al. 2017 [59].

The level of vigilance is correlated with generation of absence seizure. The characteristic SWD are seen with low vigilance such as in passive wakefulness, drowsiness and light slow-wave sleep (200). The involvement of ascending cholinergic transmission in modulating the vigilance is known (201) with a significant effect of cholinergic efferent on SWDs (202). And also, the drug affecting the muscarinic and nicotinic receptor agonists, and anticholinesterases all suppress absence seizures (203). The threshold of thalamic and cortical excitability is enhanced by ascending cholinergic pathways that project to thalamus and cortex (2).

2.3.3.4.2. Excitatory amino acid-mediated mechanisms

Thalamocortical rhythmicity is modulated by glutamate mediated corticothalamic efferents. The descending input from cortex lead to a slow depolarization on thalamic relay cells with a decreased potassium current. The slow depolarization in turn blocks rebound burst firing. (204). Activation of glutamate receptors depolarizes the thalamic neurons and thereby decreases the likelihood of absence seizures (2).

2.3.4. Astrocytes Role in CNS and in the pathology of typical absence epilepsy

2.3.4.1. Classification of Neuroglia and Astrocyte Types in CNS

Neuroglia are subdivided into peripheral nervous system (PNS) and central nervous system (CNS) glia. The PNS glia are Schwann cells (myelinates and surrounds nerve axons), olfactory ensheating cells, satellite glia (present in peripheral glia), and enteric glia. On the other hand, CNS glia divides into two types as macroglia that is derived from ectoderm and microglia which is derived from mesoderm. Macroglia in turn contain several types including astrocytes, NG2-glia, and oligodendrocytes (15).

Astrocytes are star-like cells with a great heterogeneity among different astrocytes types in terms of structural and functional behaviors. The differences are also present between and among distinct brain regions (297,298). Astrocytes are divided into two different classes according to their morphology as protoplasmic and fibrous astrocytes. Protoplasmic astrocytes are localized in brain and spinal cord gray matter, own multiple primary processes expanding into complex arborization which do not overlap between astrocytes (299). These astrocytes send projections to surround synapses (perisynaptic process), blood vessels (perivascular end foot), and the pial area (subpial end foot, glia limitans). A single protoplasmic rodent astrocyte can contact around 1000.000 synapses (300).

Fibrous astrocytes present in the gray matter of brain and spinal cord as well as in the optic nerve and nerve fibers in retina. They show less arborization than protoplasmic ones, and cell bodies can be found between axon bundles with a regular pattern. Other than synapses at perivascular or subpial areas, fibrous astrocytes also send perinodal processes to synapse with axons at Ranvier nodes (301).

2.3.4.2. Tripartite synapse

The concept of tripartite synapse reveals the close relationship on synaptic transmission between astrocytes and neurons both structurally and functionally (Figure 7). Pre- and postsynaptic terminals are in many cases partially or completely ‘’ wrapped’’ by astrocytes in synaptic surroundings (Figure 8). For instance, perisynaptic astrocytes membrane is about 1 um away from synapse in hippocampus and cerebellum. And in the case of decreased glial encapsulation at synapses a prolonged EPSPs in the cerebellum (302); increased activation of mGlu receptors in

the supraoptic nucleus (303); and synaptic spillover of glutamate during stimulation in the hippocampus (304) have been observed. Therefore, the main function of perisynaptic glial processes at excitatory glutamatergic synapses is the uptake of glutamate. Moreover, activity-dependent perisynaptic membrane diffusion of the main astrocyte glutamate transporter, GLT1, also helps to shape synaptic transmission through glial mechanism (305).

Table 4. Functions of Astrocytes

Development of the CNS	Neurogenesis Neural cell migration and formation of layered gray matter Synaptogenesis
Structural support	Parcellation of the gray matter via ‘’tiling’’ Delineation of *pia mater* and the vessels by perivascular glia Formation of neurovascular unit
Barrier function	Regulation of formation and permeability of blood-brain and CSF-brain barriers Formation of glial-vascular interface (astrocyte end feet)
Homeostatic function	Control over extracellular K^+ homeostasis via local and spatial buffering Control over extracellular pH Regulation of water transport Removal of neurotransmitters from extracellular space Homeostasis of pH, CO_2, Na^+, and glucose
Metabolic function	Uptake of glucose; deposition of glycogen Provision of lactate to neurons in activity-dependent manner
Synaptic transmission	Regulation of synapse maintenance and assisting in synaptic pruning Provision of glutamate for glutamatergic transmission Regulation of synaptic plasticity Integration of synaptic fields Humoral regulation of neuronal networks via secretion of neurotransmitters and modulators
Regulation of blood flow	Regulation of local blood supply via secretion of vasoconstrictors of vasodilators
Higher brain function	Respiration Sleep and circadian rhythms Learning and memory
Brain defense	Reactive astrogliosis and scar formation Modulation of immune response and secretion of cytokines and chemokines
Neuronal activity	Ca^{2+}-dependent release of gliotransmitters

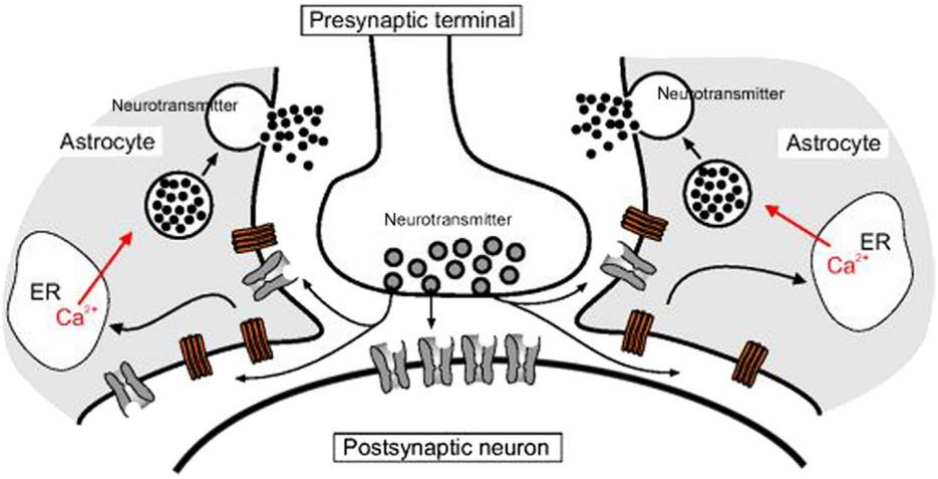


Figure 7. The tripartite synapse assumes that the presynaptic neurotransmitter once released, interacts with specific receptors on both the postsynaptic neuronal membrane and on the astrocyte membrane. The mechanisms lead to increase the Ca^{2+} signals in astrocytes which results neurotransmitters release from astrocytes that signal back to neurons. Retrieved from Hubbard, J.A. et al. 2016 [15].

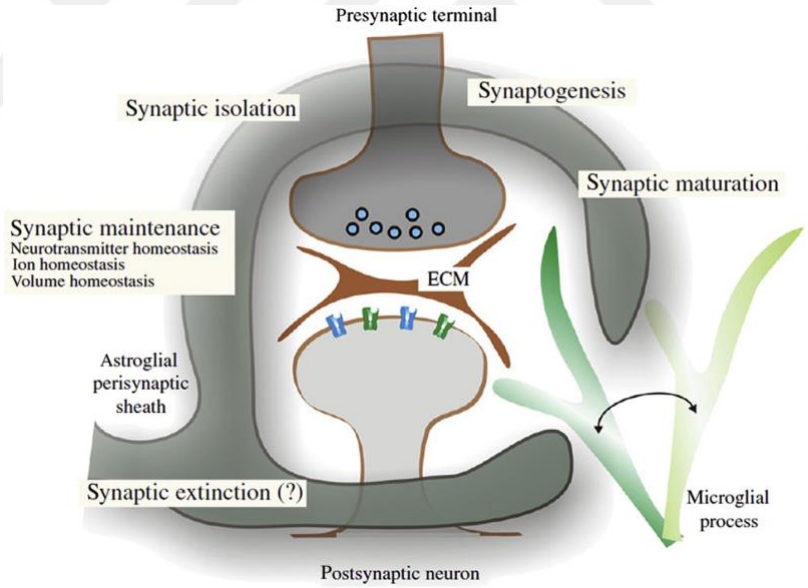


Figure 8. Astroglia wrapping. Several components of synapses era can be seen in the image which include: the presynaptic and postsynaptic terminals, the perisynaptic process of the astrocyte, the process of an adjacent microglial cell that periodically contacts the synaptic structure, and the ECM present in the synaptic cleft. Astroglial perisynaptic sheaths cover synaptic structures and regulate, influence and assist synaptogenesis, synaptic maturation, synaptic maintenance, and synaptic extinction. Retrieved from Hubbard, J.A. et al. 2016 [15].

2.3.4.3. Calcium Release

Synaptically engaged astrocytes has integral modulatory function (18) as proposed by tripartite synapse concept (311). Released neurotransmitters cause intracellular Ca^{2+} elevations in astrocytes (312). Consequently, these elevations can lead to the release of gliotransmitter including glutamate (313), ATP (294), and D-serine (314).

Astrocytes in the ventrobasal (VB) can show spontaneous Ca^{2+} oscillations which are facilitated by metabotropic glutamate receptors as a consequence of glutamatergic synaptic input. Astrocytes can also release glutamate in a Ca^{2+} dependent manner (18). The released glutamate then feedback to modify the synapse (18). As a consequence, N-methyl-D-aspartate (NMDA) receptor-mediated slow inward current (SIC) in thalamic neurons (295) can be generated. But SIC emergence is more correlated to the duration of afferent input (18), rather than the acute synaptic transmission. Therefore, the response of astrocytes in the ventrobasal (VB) thalamus to afferent activity was not the direct release of glutamate, instead they display integrative properties that induce long-lasting changes to astrocyte-neuron signaling. (18).

These slow inward currents can be found in several brain regions and can occur spontaneously or can be evoked with different methods to induce astrocytic Ca^{2+} increases (315,316).

2.3.4.4. Astrocytes Carrier Molecules

Astrocytes host several transporter molecules on their cell membrane to exchange different molecules with the extracellular space. Astrocytes play a significant role in the glutamate clearance from the extracellular space with the help of two glutamate transporter molecules, namely excitatory aminoacid transporter 1 (EATT1) and 2 (EATT2). In particular, increased level of cortical glutamate receptors was found in an animal model of absence epilepsy (363) perhaps as a compensatory mechanism to a significantly decreased glutamate uptake.

The expression of gamma-amino butyric acid transporter (GAT)3 is only weak, if at all, on astrocytes. The expression of GAT3 on astrocytes in the sclerotic hippocampus is increased (364). It is suggested that this increased expression might

contribute to the removal of excess GABA and thus decreased level of extracellular GABA during ictal state.

In sclerotic hippocampi the expression of water transporter molecule, AQP4, on the perivascular membrane of the astrocyte is reduced, whereas its expression remains unchanged, thus suggesting a probable decrease in the expulsion of water from the neuropil out to the blood vessel lumen (365).

2.3.4.5. Membrane Ion Channels of astrocytes

The presence of voltage-dependent Na^+, K^+, and Ca^{2+} ion and anion channels have been documented on astrocytes (366,367,368). It is found that Na^+ current densities (16, 369) are increased along with the significantly up-regulated expression of voltage-dependent calcium channel a1 subunits (371) in astrocytes obtained from epileptic human tissue. The data suggest a functional change in current characteristics of astrocytes and Ca^{2+} uptake.

Astrocytes play also a significant role in K^+ homeostasis in the brain in terms of removal the excess K^+ away from regions of high concentration and $[K^+]_o$ significantly increases during seizure activity. The inwardly rectifying potassium ion (Kir) channels on astrocytes play a major role in the removal of K^+ from the extracellular space. Impaired K^+ buffering in astrocytes is documented in epileptic patients due to defective Kir channels (370,372).

2.3.4.6. Astrocyte Specialized Enzymes

Astrocytes can degrade both glucose and glutamate (373) with their unique property of having key enzymes not normally found in neurons (16) (Figure 9). In epileptic tissue, the significant loss of glutamine synthetase immunoreactivity is documented (374). Glutamine synthetase catalyzes the conversion of glutamate to glutamine in astrocytes, and reduced levels of extracellular and cellular glutamine levels in the epileptic tissue lead to hypothesis of decreased capacity for glutamine synthesis in astrocytes (375).

Glutamate dehydrogenase (GDH) activity is found to be increased while aspartate amino transferase activity is reduced in the absence epilepsy (376). GDH is mainly expressed in astrocytes while some neuron-specific isoforms are also available in neurons. GDH functions in the conversion of glutamate to a-ketoglutarate with a

production of ammonia. GDH can also synthesize glutamate, thereby removing toxic ammonia at the expense of alpha ketoglutarate from the tricarboxylic acid cycle (TCA), that later could be replaced from astrocytes by anaplerosis (377). Astrocytes utilize glutamate as a substrate for energy metabolism when extracellular glutamate levels increase. Aspartate amino transferase facilitates the conversion of aspartate and alpha ketoglutarate to oxaloacetate and glutamate and vice versa.

2.3.4.7. Glutamate Glutamine turnover

Absence epilepsy model in immature and mature animals revealed altered astrocytic functions as the cause of epileptic seizures (17) with a normal interaction between glutamatergic neurons and astrocytes. It is also reported that glutamate metabolism in neurons and astrocytes are facilitated in cortex, while GABA levels decrease in cortex as well as in thalamus in animal models of absence seizures (306).

Neurons transmit electrical signals, whereas astrocytes affect the transmission in terms of modulating the threshold of neuronal firing and by the synchronization by regulating the extracellular environment within the synaptic cleft (307). Glutamate is released from glutamatergic neurons during neurotransmission and taken away from the synaptic cleft by glutamate transporters mainly into abutting astrocytes (308). In astrocytes, glutamate is either converted (i) to α-ketoglutarate a reaction catalyzed by glutamic acid dehydrogenase (GDH) or (ii) to glutamine by the astrocyte specific enzyme glutamine synthetase (GS) (309). After that, glutamine is transported into neurons where it is again converted to glutamate by the enzyme called phosphate-activated glutaminase (PAG). Finally, glutamate is converted to α-ketoglutarate and either join the TCA cycle or be reloaded into synaptic vesicles to be released. On the other hand, in GABAergic neurons glutamate is converted to GABA by glutamic acid decarboxylase (GAD). This circuit between neurons and astrocytes are defined as glutamine–glutamate–(GABA) cycle (Figure 9) and has significant importance in normal and pathological conditions because of the different degree in the distribution of enzymes and transporters in neurons and astrocytes (310).

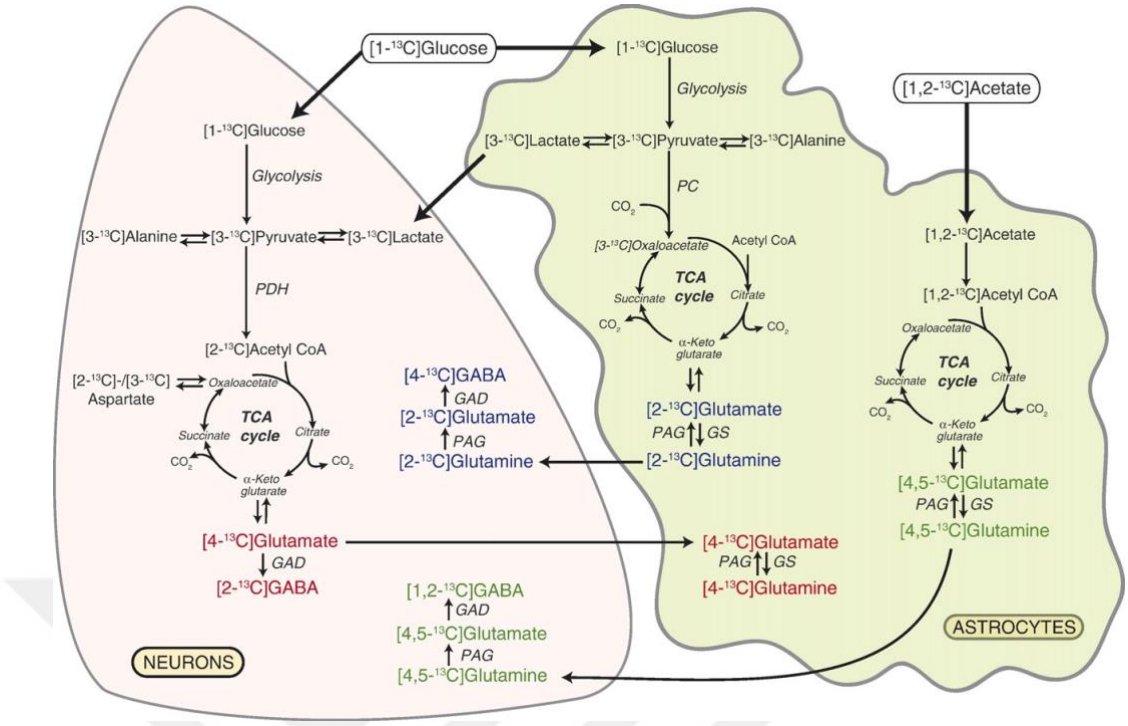


Figure 9. Representation of cerebral metabolism of glucose in neurons and astrocytes in TCA cycle. Neurons depicted here might be either glutamatergic or GABAergic. Glucose once transported from blood to brain, it can either be taken up by neurons or astrocytes. Glucose is converted to pyruvate (glycolysis) which then either be converted to lactate or alanine or acetyl CoA. If acetyl CoA condenses with oxaloacetate it gives rise to glutamate and subsequently GABA in neurons. In case of glutamate release from neuron, glutamate is then taken up by astrocytes and is converted to glutamine. Alternatively, in astrocytes pyruvate is carboxylated to yield oxaloacatete (pyruvate carboxylation) resulting glutamate or glutamine. In the case of glutamine uptake by neurons, it is converted to glutamate or GABA. Acetate is preferentially transported to astrocytes where it is converted to acetyl CoA, which can condense with oxaloacetate yielding glutamate or glutamine (green). If glutamine is taken up by a glutamatergic or GABAergic neuron it can be converted to glutamate and subsequently GABA. Abbreviations: GAD; glutamic acid decarboxylase, GS; glutamine synthetase, PAG; phosphate-activated glutaminase, PC; pyruvate carboxylase, PDH; pyruvate dehydrogenase, TCA;t ricarboxylic acid. Retrieved from Melo, T.M. et al. 2007 [17].

2.3.4.8. Gene Expression Profile in astrocytes

Increased expression level of several genes associated with astrocytes have been documented (378) such as glial fibrillary acidic protein (379,380), vimentin demoxytocin, CD44 antigen, AQP4, paladin, crystallin alpha B, calpain 2, chondroitin sulfate proteoglycan 2, plectin 1, presenilin 1, radixin (16). Some of these genes are responsible from changing the morphology of astrocytes.

Other up-regulated genes are associated with immune and inflammatory responses (378) including regulating chemokines with their receptors such as chemokine C-C motif ligand 2, chemokine C-X-C motif receptor 4, cytokines with their receptors (fibroblast growth factor-1) (FGF1), FGF2, FGF3, tumor necrosis factor ligand superfamily member 7 (TNFSF7), signal transduction protein (calmodulin-1 [CALM1], CALM3, protein phosphatase 3, catalytic subunit alpha isoform (calcineurin A alpha), PPP3CA, PPP3R1, protein ty- rosine phosphatase receptor type D (PTPRD), PTPRG, PTPRN, PTPRO, transcription factors (FK506 binding protein 1B, 12.6 kDa-FKBP1B, FKPB1A), transcription factor-related genes (AGT, COL1A1, COL21A1, NCAM1, VCAM1, CD44, IL11RA, IL13RA, IL15), complement (C1QB, C3, C4), and class II major histo- compatibility complex antigen genes (HLA-DPA1, HLA-DQA1, HLA-DRB1, and HLA-DRB3).

Astrocytes are shown to influence the inflammatory response, and can produce a variety of immunologically relevant molecules, including class II major histocompatibility complex antigens, many cytokines, and chemokines (16). Increased expression of NFkB-p65 subunit which in turn regulates several genes are documented in astrocytes (381).

2.3.4.9. Astrocytes and Vascular Changes in Seizures

Astrocytes make a close contact with the microvasculature in the normal brain. The blood-brain barrier formed by the endothelial cells is wrapped by astrocyte end feet which releases the signals to support the formation and maintenance of tight junctions among endothelial cells, and also the expression of transport molecules from endothelial cells. Additionally, astrocytes also are important in dislocation of the water and other molecules between brain parenchyma and blood. The proliferation of microvasculature has been reported in epileptic tissue (382,383). Moreover, it has been reported that the blood-brain barrier may become leaky during seizures, leading the

substance to pass from blood to brain such as albumin. The released albumin by way of vascular permeability into the brain is then transported to astrocytes via transforming growth factor b receptors, consequently transcriptional activation of downstream pathways (384) leading to a down regulation of inward rectifying potassium (Kir) channels in astrocytes, astrocytic activation, increased inflammation, and reduced inhibitory transmission (385).

2.3.4.10. Astrocytes Role in Typical Absence Epilepsy

Glia are involved in different physiological functions including the role of astrocytes in removal of glutamate from synapses, neuronal pathfinding, and the redistribution of K^+ during action potential (351). Microglia, on the other hand has immune functions such as initiating the inflammatory response to injury or infection (352). Glial cells release neuroactive molecules, and control synaptic neurotransmission by modifying the ion channels, gap junctions, transporters as well as receptors (327,354,355,356). Recently, it is suggested that glial cells are also effectively functioning in seizure susceptibility and the development of epilepsy (307,327,328). Moreover, significant changes in the shape and functions of glial cells are documented in different types of epilepsy that can affect the excitability of network as well as the development of epileptic seizures (8). Among these changes are the proliferation of astrocytes, dysregulated expression of water and ion channels, altered secretion of neuroactive molecules, and increased activation of inflammatory pathways (328,357).

Astrocytes give critical contribution to CNS development, structural support, barrier function, homeostatic function, metabolic support, synaptic transmission, regulation of blood flow, higher/integrative brain functions, brain defense, neuroprotection, and response to injury (Table 2). In addition, astrocytes get activated to become reactive in the epileptic brain (326). The reactive astrocytes show several changes in expression of different enzymes such as glutamine synthetase (353) which in turn can contribute to the increased neuronal excitability (8). Other functional changes involve astrocytic calcium signaling, potassium channels, water channels, glutamate metabolism, adenosine metabolism, gap junctions, blood-brain barrier disruption and inflammation (8).

There are three possible ways of how astrocytes can contribute to epileptogenesis: previously normal neurons can get hyperactivated, abnormal neurons might lead to show epileptic bursting activity or hyperactivity cannot be neutralized. Astrocytes express same receptors as neurons, but with different strength, whose activation is dependent to synaptically released neurotransmitters, gliotransmitters or some molecules escaped into extracellular space (358). Among these, subunits of AMPA type of ionotropic glutamate receptors, mGluR1 shows elevated mRNA level in their splice variants in astrocytes of epileptic tissue (359,360). The up regulation of mGLuR 3, 5 and 8 also suggest that astrocytes increase their response to the released glutamate (361). As a consequence of activated receptors, intracellular Ca^{2+} and Ca^{2+} wave propagation increases which in turn cause glutamate release from astrocytes (362).

Brain functions both under normal and disease conditions are finely tuned and modulated by continuous communication between astrocytes and neurons. In epilepsy addition to the astrocytic dysfunctions of abnormal extracellular release and potassium homeostasis (326), the reciprocal signaling between astrocytes and neuron also influence the generation of a hyper-excitable networks (327,328). It was shown that when glutamate is added to cell cultures and brain slices, intracellular Ca^{2+} transients in astrocytes were elicited which propagate to neighboring astrocytes as Ca^{2+} waves. It revealed that astrocytes have various receptor for glutamate, GABA, ATP, adenosine, noradrenaline, acetylcholine, and dopamine (317); they can release glutamate (318,319), GABA (315,320), D-serine (314) and ATP (321) by way of intracellular Ca^{2+} changes and vesicle fusion events; astrocytic glutamate and GABA activate neurons with a specific electrical feature, the slow inward (SICs) (295) and outward currents (SOCs) (322); and gliotransmitters exert a fine tuning on synaptic efficacy in both the short- and the long-time domain (323). All these modalities affect neuronal excitability (324) and enhance neuronal synchronies (292). Besides the above-mentioned neurotransmitters, astrocytes can also release interleukin-1β (IL-1β), BDNF, GDNF, neurosteroids, nitric oxide, TNFα and TGFβ, which can all affect neuronal network activity (325).

The presence of expression of astrocytic IL-1b in the seizure initiation side in cortex (53), the sensitivity of absence seizures to IL-1b (329) and gain-of function of

thalamic astrocytic $GABA_A$ receptors that is the basis for tonic $GABA_A$ inhibition open new perspectives in the evaluation of absence epilepsy pathology. There seems to be growing need for the development of novel astrocyte-centered therapeutic applications for epilepsy treatment.

Absence seizures can be represented alone or most commonly are accompanied with other convulsive seizures. The occurrence of idiopathic generalized epilepsies with absence seizures is enhanced by paroxysmal activity within cortical and thalamic networks with little or no involvement of other brain regions (330). Genetic analysis of the families affected from this disease complexes have revealed a variety of mutations in several voltage-dependent and transmitter-gated channels such as Ca^{2+} channels and $GABA_A$ receptors (331,332).

Traditional view of the pathogenesis of epilepsy is based on either the enhanced excitatory transmission in glutamatergic neurons or the reduced GABAergic inhibitory mechanisms, or both. In addition, widespread loss of functions in $GABA_A$ receptor-mediated synaptic transmission as a result of the mutation of $GABA_A$ receptors genes (i.e. the R43Q in the γ subunit) (331), has been speculated as a leading pathophysiological mechanism in absence epilepsy. Although, the abnormalities in GABAergic transmission (i.e. decreased IPSC frequency) is prominently found only in cortex without prominent effect in thalamic reticular or thalamocortical neurons (290). A potential mechanistic explanation including astrocytic contribution of absence epilepsy is depicted in Figure 10.

It has been shown in several absence epilepsy models that the GABAergic inhibitory transmission in thalamocortical neurons of sensory thalamic nuclei is either increased or unchanged rather than pronounced decrease. These studies reveal that: (1) the majority of thalamocortical neurons shows rhythmic burst of $GABA_A$ IPSPs in ictal state in an animal of genetic absence seizure model (GAERS) (28). (2) there is either no change or an increase in phasic $GABA_A$ receptor-mediated inhibition (i.e. IPSPs or IPSCs) in thalamocortical neurons in in vivo models (334,335); in a absence epilepsy mice model of $GABA_A$ receptor -β3 subunit KO, $GABA_A$ IPSPs in thalamocortical neurons were unchanged compared to wild-type littermates (336); (4) GABA levels in thalamus is relatively high in GAERS compared to non-epileptic control animals (337);

(5) Absence seizure-like activities can be provoked by $GABA_B$ receptor agonists in na¨ive animals (338,28).

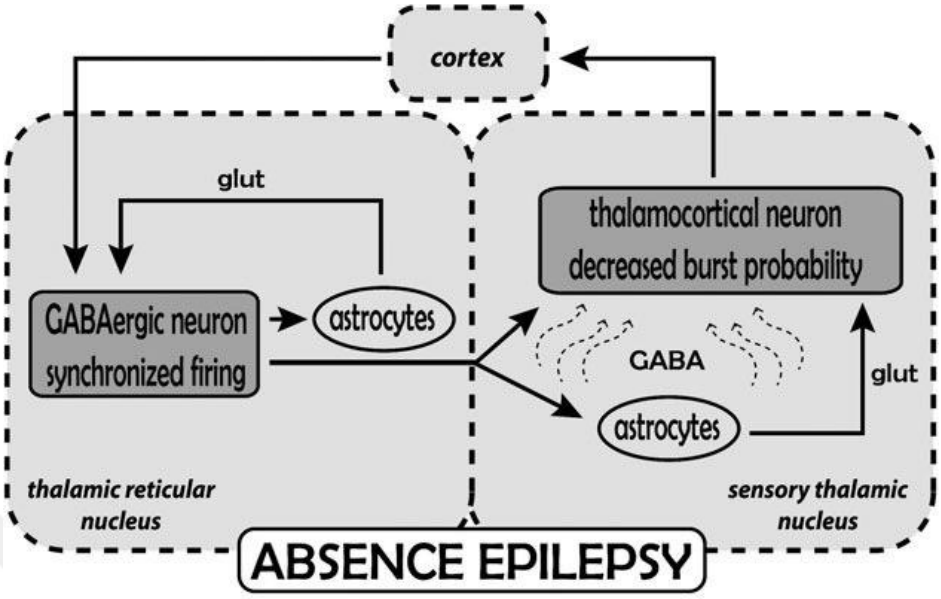


Figure 10. Astrocytic role in absence epilepsy and a potential mechanistic explanation of how the loss of function of astrocytic GAT-1 and the resulting increase in tonic $GABA_A$ inhibition lead to the expression of absence seizures. The generation and maintenance of absence seizures are the results of abnormal firing activity among cortical cells as well as the neurons in the thalamic reticular and sensory thalamic nuclei. Enhanced GABA (curved arrows) activity occurs as a consequence of increased firing in GABAergic neurons of TRN which is in turn drive by the synchronized cortical input, in sensory thalamic nuclei. The diminished GAT-1 transporter function additionally causes an increase in tonic $GABA_A$ current in thalamocortical neurons. This increase causes a drastic reduction in the firing of thalamocortical neurons meanwhile prevents sensory input transmission and reduces the effect of possible glutamate increase by way of astrocytes on these neurons. On the other hand, conjectural increase in glutamate through astrocytes leads synchronized firing in TRN. Due to no tonic $GABA_A$ current in TRN, one can not expect any effect of astrocytically released GABA in the genesis of absence seizures. Retrieved from Crunelli, V. et al. 2007 [19].

Also, when GABA levels are increased by drugs such as vigabatrin or tiagabine, absence seizures can easily be induced in animals and humans as well as aggravated in absence epilepsy patients (339,340). In particular, the increased tonic $GABA_A$ receptor-mediated inhibition in thalamocortical neurons is a common phenomenon in several animal models of absence seizure including GAERS, stargazer, lethargic and γ-hydroxybutyric acid (GHB), 4,5,6,7-tetrahydroisoxazolo-[5,4-*C*]pyridine-3-ol as well as succinic semialdehyde dehydrogenase-KO animals models (335,341) with a epileptogenic significance due to the presence of this increase before the onset of seizures (335).

It is further showed that the increased thalamic tonic $GABA_A$ current is not related with an increase GABA release or from the increased expression of GABA receptors or from the peri/extrasynaptic δ subunit containing $GABA_A$ receptors activity. The increased $GABA_A$ current is more associated with absence epilepsy and the loss-of-function in one of the GABA transporters, GAT-1 which is exclusively expressed in thalamic astrocytes (342,343) (Figure 11).

As shown by the measurements of the tonic $GABA_A$ current in animal models of absence seizure (19) and direct evidence from patch-clamp investigations in astrocytes from thalamic slices of GAERS that GABA transporter current is not affected by selective GAT-1 blocker (NO-711) but abolished by selective GAT-3 blocker (SNAP5114) (344,345) while in non-epileptic control rats the GABA transporter current is reduced by half in thalamic astrocytes when the blockers applied chemically (344,345). Since GAT-1 expression levels in absence models and non-epileptic strains are similar with no detected mutation in the GAT-1 gene in the genetic animal models (335), it is suggested that abnormality of GAT-1 may lie on its phosphorylation or the transporter might remain as an immature intracellular protein. On the other hand, astrocytic GAT-3, the only other GABA transporter available in thalamus (342,343,346), is not able to compensate due to its close location in synaptic sites which is far away from the tonic $GABA_A$ current activity in peri/extrasynaptic area (19). Although, GAT-1 is mainly localized close to the GABAergic synapses while GAT-3 is present both near and far away from synapses in thalamocortical neurons (347).

This drastic reduction in the activity of GAT-1 may cause the change in properties of the slow inward currents (SOCs) (the evidence of GABAergic astrocyte-to-neuron signaling) (315) such as changes in amplitude, rise and decap time (344,345) in GAERS. In contrast, in the case of the slow inward currents (SICs) (the characteristic signature of the glutamatergic astrocyte-to-neuron signaling (293,348)), there is no difference in the thalamic SICs properties between GAERSs and non-epileptic rats before seizure onset (344,345) as well as no change in glutamate transporters in thalamic astrocytes (349).

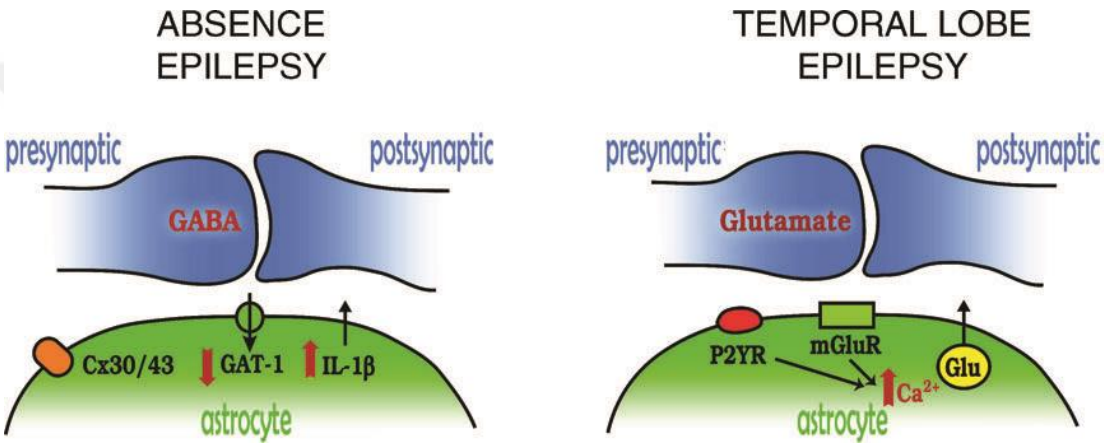


Figure 11. Astrocytic molecular components in absence and temporal lobe epilepsy. The function of the astrocytic GABA transporter GAT-1 is reduced in GABAergic synapses of the sensory thalamic nuclei in absence epilepsy. In the somatosensory cortex, on the other hand, interleukin-1β (IL-1 β) levels are increased. Astrocytic thalamic gap junctions, i.e. connexin (Cx) 30 and 43, has also contribution to the generation of absence seizures. Hyperactive glutamatergic neurons in cortex release ATP and glutamate acting on metabotropic purinergic receptors (P2YR) and glutamate receptors (mGluR) in astrocytes in temporal lobe epilepsy. Once these receptors on astrocytes are activated, they lead to intracellular Ca^{2+} increases, with a subsequent release of glutamate thus engaging an excitatory circuit with neurons. Retrieved from Crunelli, V. et al. 2007 [19].

On the other hand, as a consequence of reduced expression of the astrocytic glutamate transporters GLT-1 and GLAST in the cortical neurons in GAERS (349), glutamate uptake is decreased with a change in SICs in this region prior to seizure (350).

Other evidence for the involvement of astrocyte in epileptogenesis of absence seizure is the increased expression levels of glial fibrillary protein levels in cortical and thalamic astrocytes of GAERS with an selective induction of IL-1β in activated astrocytes in the peri-oral region of the somatosensory cortex (cortical initiation site of absence seizures) but not in other cortical regions or in the thalamus of GAERSs before seizure onset. It is also demonstrated that absence seizures can effectively be reduced by systemic injection of a IL-1β blocker (53), while the injection of an inducer of IL-1β such as lipopolysaccharide is able to increase the seizures in absence epilepsy (329) (For a clear understanding, the mechanisms involved in absence epilepsy were depicted in comparison to temporal lobe epilepsy in figure 11).

2.4. Animal Models of Typical Absence Epilepsy

Typical absence epilepsy affects children and adolescent which makes it hard to study the etiology in human for ethical reasons (28). In this regard, animal models are of paramount importance in revealing the information about the pathophysiological mechanisms of the disease. The demands for valid typical absence epilepsy model are listed in table 5.

Table 5. Criteria for experimental typical absence epilepsy

1.	EEG and behavior similar to the human condition
2.	Reproducibility and predictability
3.	Quantifiable
4.	Appropriate pharmacology
5.	Unique development profile
6.	Exacerbated by GABAergic drugs
7.	Blocked by GABAB receptor antagonists
8.	Involvement of thalamocortical mechanisms
9.	Absence of GSWD from hippocampus

Models that can mimic the clinical and pharmacological characteristics of absence seizures can either be experimentally induced or genetically determined (28). The injection of pharmacological agents including pentylenetetrazol, penicillin, gamma-hydroxybutyrate or GABA agonists can induce SWDs in rodents, cats or primates (205,206,207,208). On the other hand, the spontaneous occurrence of high voltage rhythmic activities on cortical EEG of laboratory rodents, mainly from Wistar strain, has been identified and extensively used by many researchers (206,209,210,211,212, 213,214) Such genetic models include GAERS (210, 215), the WAG-Rij rats (213) and several strains of rats and mice as can be seen in table 6.

The major advantage of genetically determined rat strains over pharmacological models is the resemblance of chronicity of the spontaneous SWDs in human. Additionally, genetic models avoid the methodological bias in which externally manipulated neurotransmission either with electrical stimulation or with pharmacological interventions might lead to misevaluation.

It has been shown that genetic factors have a prominent role in the idiopathic generalized epilepsies, including absence epilepsy. Twin studies revealed the genetic predisposition in which monozygotic twins suffered more frequently than dizygotic twins (216). Therefore, it is plausible to favor genetic animal models over the absence models that are induced experimentally. Genetic animal models offer a unique opportunity to study the natural course and the pathophysiology of the absence epilepsy. Different genetic models have been identified according to the electroencephalography recordings and concomitant behavioral arrest (218) can be seen table 6.

An ideal model should have ‘’face validity’’ that means the structural similarities between the animal model and the disease modelled; ‘’predictive validity’’ which is the prognosis in the model should predict the performance in patients; and ‘’construct validity’’ that is the model should represent a legitimate theoretical rationale and construct (217).

Table 6. Genetic Models of Absence Epilepsy in Mice and Rats and Their Epileptic Features

Species	Strains	Age of Onset	SWD Duration	Rhythmicity	Reported Mutations	Phenotypes (Seizures and Associated Phenotypes
Mouse	Tottering	Around 4 weeks	0.3-10	6-7	*Cacna1a*	Absence, focal motor seizure, ataxia
	Lethargic	Few days	0.6-5	5-7	*Cacnb4*	Absence, lethargic, dyskinesia, ataxia, focal motor seizure
	Ducky	-	0.6-5	5-7	*Cacna2d2*	Absence, ataxia, dyskinesia
	Stargazer	-	6	6-7	*Cacng2*	Absence, ataxia, head-tossing
	C3H/Hej	Irregular SWD: P5-15 Transitional SWD:P16-P25 Mature SWD: >P26	1.75 - 3.4	5-6 6-7 7-8	Natural IAP Retrotransposon insertion in Gria4	Absence
	Rocker	-	1-1.7	6-7	*Cacna1a*	Absence, ataxia, dyskinesia, tremor
Rat	WAG-Rij	P75-140 3 months	130	7-8	-	Absence
	GAERS	Oscillation: P15-25 Mixed: P26-P40 SWD: > P60	2-3 5-9 7-9	5-6 5-6 7-9	*Cacna1h*	Absence
	GRY	6-8 weeks	8-10	7-8	*Cacna1a*	Absence, ataxia
	SER	7-8 weeks	1-17	5-7	*tm* and *zi*	Absence, tonic, tonic-clonic, seizure, Spongiform degeneration
	TRM/Kyo	8-26 weeks	1-17	5-7	*tm*	Absence, spongiform degeneration, tremor- sterility
	WER	P25-P70	1-17	4-6	-	Absence, tonic-clonic seizure

2.4.1. Genetic Absence Epileptic Rats from Strasbourg (GAERS)

Researchers have observed that Wistar rats of the Strasbourg stock presented with EEG discharges of the spike-and-wave type in 31% in their breeding colony when the rats were 6-12 months old (215). These Wistar rats were then selected and cross-bred, leading the increased number of SWDs as well as the incidence, later named as the Genetic Absence Epileptic Rats of Strasbourg, GAERS. Breeding of selected parents over 3-4 generations produced a strain in which 100% of the rats were affected (29). These animals develop generalized, non-convulsive seizures at 40-120 days of age that last throughout their lifetime with an age-dependent increase in the number and mean duration of discharges (Figure 12).

The 4-month-old GAERS represents spike and wave discharges with 9 ± 0.5 c/s mean frequency along with varying voltage rates between 300 to 1000 µV. In a quiet wakefulness state, SWDs develop 1.3 times per minute and last for 17 ± 10 s with a 25 ± 8 s mean cumulated duration depending on the time of the day and recording conditions (210, 215, 220).

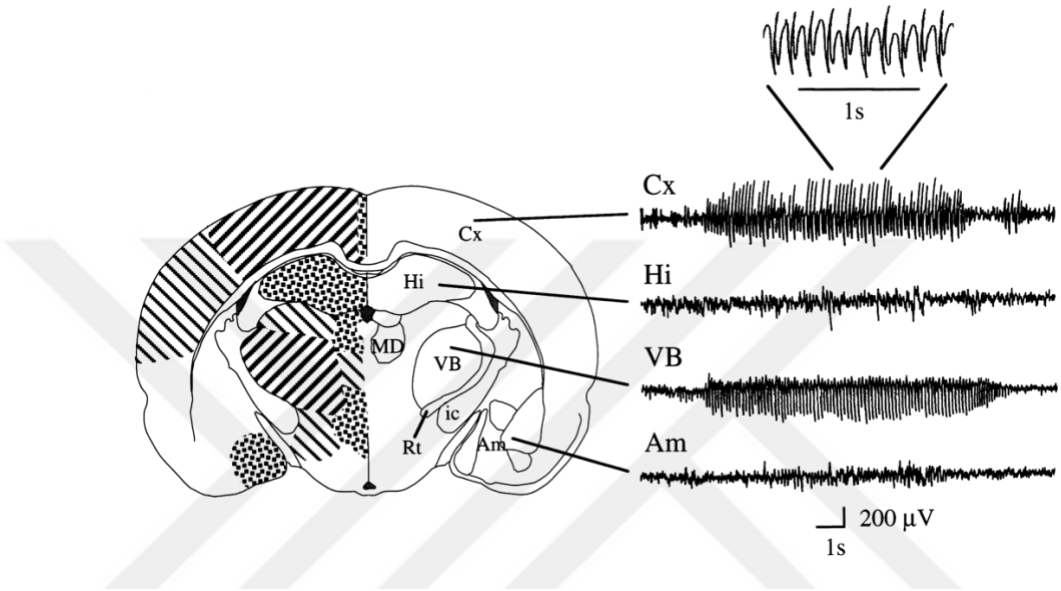


Figure 12. Spike and wave discharges in GAERS. *Left panel*: schematic of SWDs on a rat coronal brain section. Left hatched areas: high amplitude SWDs; right hatched areas: small amplitude SWDs; dots: no SWDs recorded; white areas: no recording done. *Right panel*: simultaneous EEG recordings from the cortex, dorsal hippocampus, ventrobasal thalamus and amygdala. On the top of the figure, SWDs were represented at 1s. Am: amygdala; Cx: cortex; ic: in- ternal capsule; MD mediodorsal thalamic nucleus, Rt: reticular thalamic nucleus; VB: ventrobasal thalamic nucleus. Retrieved from Danober, L. et al. 1998 [52].

SWDs mainly occur in a quiet wakeful state and disappear in the active arousal, slow-wave sleep and rapid-eye-movement (REM) sleep (28).

Behavioral manifestations accompanying SWDs in GAERS are immobility and rhythmic twitching of the vibrissae along the jaw muscles (28). Muscles relaxation in the neck might lead to a gradual and slight tilting of the head. Some rats might also exhibit light chewing movements with occasional tongue protrusions (29).

2.4.2. Wistar Albino Glaxo from Rijswijk (WAG/Rij)

WAG-Rij rats were derived from WAG strain which was also generated from Wistar stock by A.L. Bacharach at the Glaxo Laboratories in London in 1924. The WAG-Rij is an inbred strain in which brother-sister mating has been conducted for more than 130 generations (26). Apart from this line, other sublines were also created such as the WAG-Cpb, WAG-Kyo, WAG-Mbl WAG-Orl strains (219).

In 1986, it was discovered that all adult WAG-Rij rats develop SWD with concomitant mild clinical symptoms (213). In 6 months of age, both in female and male 300-400 discharges per day occur, mostly at the time of passive wakefulness, inattention, and in the transition between sleep and awakening. They can rarely occur during active wakefulness, deep slow-wave sleep and REM sleep (200, 221). These cortical EEG manifestations can not be observed before 2 to 3 months of age. SWD activity consists of bursts lasting from durations of 1-30 s and a spike-wave frequency of 7-10 Hz (221). Bilaterally symmetrical spike waves generalize over entire cortex (52,222) with a fronto-parietal dominance. Seizures are accompanied by phenomenological symptoms such as facial myoclonic jerks, twitching of the vibrissae, accelerating breathing, head tilting, and, eye twitching (26).

WAG-Rij model shows ‘’ face validity’’ in terms of the simultaneous occurrence of clinical and electroencephalographic symptoms of absence in rat, and the decrease in responsiveness during the presence of SWD. This model also has predictive validity showing the specificity at drug effectiveness in convulsive epilepsies but not in absence epilepsy. And the drugs affect spike-wave activity in human, also show the same effect in rats. The relationship between sleep spindles and spike-and-wave discharges as well as the origin of seizure activity in both human and rats lead to the construction validity of the model. The WAG-Rij model can therefore used in the absence epilepsy research (26).

Other characteristics of WAG-Rij rats include short latency to move out from the home cage to familiar surroundings; low open-field defecation/high open-field ambulation; nondistinctive sleep percentages; a low apomorphine-induced gnawing score; a high running-wheel activity; good 2-way and active shock-avoidance

acquisition; and mild deficiency in working memory with normal reference and spatial memory tasks (219,223,224).

Both the WAG-Rij and the GAERS are well-validated isomorphic, most predicted genetic rat models of typical absence epilepsy (28,225). Both the electrophysiological (spike-and-wave discharges) and behavioral (behavioral arrest) traits fit well with those observed in human patients with typical absence epilepsy (214). The similarities in the genetic inheritance and in the cerebral structures involved in the genesis of SWD to the human disorders suggest that GAERS could be a homologous model of absence epilepsy. The model is also helpful to explore simultaneously the neural circuits involved in the generation of seizures at different level of integration due to its easily detectable spontaneous recurrent seizures on electro encephalic recordings. Electroencephalographic recordings of absence seizures of epileptic rats show parallel characteristics with human epileptic patients (Table 7). The SWDs in GAERS and WAG-Rij are very similar, high-amplitude asymmetric synchronized rhythmic activity with fundamental frequency of 7-11 Hz. WAG-Rij rat model enables predictions about pharmacotherapy of epileptic patients and therefore this strain is considered as a valid model of human absence epilepsy (214).

Table 7. Comparison of features of typical absence seizures in rodent models and

		Rat/mouse	Human
EEG	Bilaterally synchronous GSWD	+	+
	GSWD frequency	7-11 Hz	2.5-4 Hz
	GSWD from thalamus & cortex	+	+
	GSWD from hippocampus	-	-
Ictal behavior	Staring: myoclonus	+	+
	Move during GSWD	-	-
	Precise EEG/behavioral correlation	+	+
Pharmacology	ETO, VPA, TMD	+	+
	$GABA_{A\&B}$ receptors	+	+
	$GABA_{B}$ receptor	+	No data
	Severe cognitive disability	-	-

These models offer a unique opportunity to study the natural history of absence seizures close to clinical situations and therefore provide ideal conditions to understand the pathophysiology of human absence epilepsy. There are several features of these models making them very valuable in understanding of the absence epilepsy

such as the possibility to record SWDs regularly in either freely moving or immobilized animals; the comparison of data between epileptic and non-epileptic lines; and the possibility to explore epileptic animals before the onset of seizures (25).

2.5. Optogenetics and the control of astrocyte-related epilepsy

Modulation of nervous system has so far been achieved with electrical or chemical (pharmacological) stimulation. Electrical stimulation affects cellular elements of the tissue exposed with temporal precision, whereas pharmacological modulation can be aimed to specific receptor subtypes, but without time precision that is so important in neural activity (60). Optogenetic approach on the other hand with emplacing the light-sensitive transmembrane channels and desired delivery of light of specific wavelength, provide specific control of neuronal populations (60). This technique has several advantages over other modulation modules such as being less invasive and allowing spatiotemporal control while pharmacological therapy is mostly accompanied by side effects (225) and current drugs fail to be efficient in around a third of patients.

2.5.1. Overview of optogenetics

With the discovery of channelrhodopsins (ChRs), a novel method of optogenetics was developed (226) giving a wide possibility to study both neural and non-neural elements (227). The technique allows either gain or loss of function to the cell population of interest, of which these functions can be manipulated by light (226,228). Optogenetic manipulation needs three requirements which include the selection of optogenetic rhodopsins (actuator), the targeted expression of this actuator and the light delivery system.

Generally, optogenetic actuators can be classified into two subtypes; one depolarizes the cell membrane, whilst the other make the cell membrane hyperpolarize respectively named as the depolarizing and hyperpolarizing actuators or rhodopsins (Figure 13).

Channelrhodopsin-2 ChR2 is a typical depolarizing optogenetic rhodopsin. It is a light-sensitive ion channel from the eyespot of *Chlamydomonas reinhardtii* and is responsible from the phototaxis of the microorganism. ChR2 contains a seven-pass transmembrane apoprotein which make covalent binds to a retinal molecule. With the

blue light illumination, the photoisomerization of all trans-retinal to 13-cis configuration is coupled to conformational changes in the protein which then lead to the permeation of ions such as H^+, Na^+ and Ca^{2+}. Thus, the single ChR2 molecule enables the light energy to be converted into an electrical signal (229). As so, ChR-2-opens in response to blue light (230) and the neural activity can be manipulated in the direction of depolarization (231,232). In detail, blue light evokes inward currents in ChR2 expressed neurons, which depolarize the membrane, leading to the opening of voltage-gated sodium and calcium channels, and finally to the generation of an action potential (231,233). Several depolarizing actuators were created such as the ChR variants including ChRWR, ChEF/ChIEF, ChRGR, and ChR2-T159C that are optimized for specific aims (51,234,235) as well as actuators from other microorganisms like *Volvox carteri and Mesostigma viride*.

Halorhodopsin (NpHR) is a representative of hyperpolarizing optogenetic rhodopsins isolated from *Natronomonas pharaonis*. It is a light-activated Cl^- pump which when illuminated by yellow light, allows chloride ions to be transported into cell thus leading to hyperpolarization of the cell membrane. Therefore, NpHR can be used to inhibit the neuronal activity. The use of hyperpolarizing optogenetic actuators helped to reveal many functions of neurons as well as neural circuits so far (236,237).

2.5.2. Optogenetics in epilepsy

Dysfunctional excitatory or/and inhibitory transmission lead to an imbalance that is the main pathological hallmark of epilepsy in which the main responsible elements are principle or interneurons (20). It theoretically became possible to control individual neurons to stop epileptic seizures. Also, the research of Tonnesen et al., showed that by targeting principal neurons of hippocampus with NpHR, light induced NpHR activity caused a hyperpolarization of targeted neurons and suppressed epileptiform activity (236). Two possible ways exist to stop hyperexcitation by way of optogenetic interventions. Hyperactivation of targeted cells can directly be inhibited with hyperpolarizing actuators or depolarizing actuators can be targeted to inhibit interneurons or non-neuronal elements. It is possible to use different mechanism to target the expression of ChR2 either to one specific or to mixed cell types such as using the Cre-loxp approach, tTA-tetO system or virus injection with specific promoters (238).

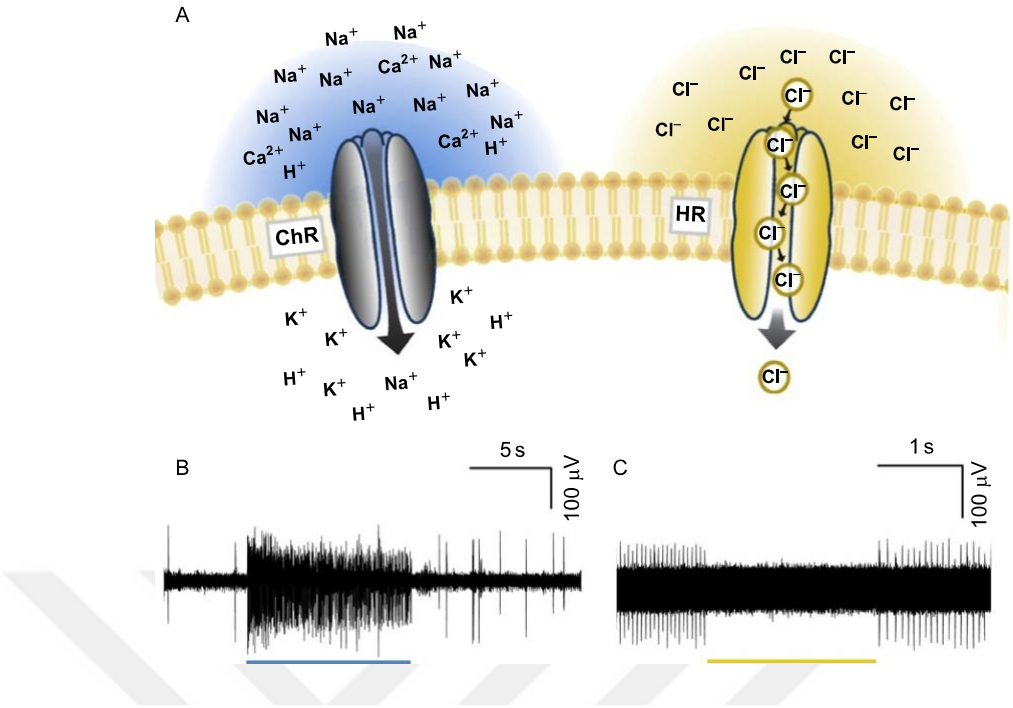


Figure 13. Excitatory and inhibitory approaches by optogenetics. (A)Two classes of opsins are excitatory ion channels (the channelrhodopsins) and inhibitory ion pumps (chloride pumps, pictured, include halorhodopsins, and proton pumps, such as bacteriorhodopsins). B) Sample recording from a neuron transduced with ChR2 in a wild-type rat. (C) Sample recording from a neuron transduced with eNpHR2.0 in a wild-type rat. Retrieved from Kalanithi, P.S.A et al. 2012 [52].

2.5.3. Optogenetic control of astrocytes

Optogenetic approach could be used to control the release of gliotransmitters such as glutamate and K_+ uptake and improve astrocyte function. For this, either ChR2, NpHR or Arch-T can be employed with different approaches as explained below (Figure 14).

2.5.3.1. Depolarizing Rhodopsins affect on astrocytic release of glutamate/ATP

When activated, ChR2 allows the entrance of Na_+, Ca_{2+} and H+ into the cell, and as a consequence of ChR2 expression in astrocytes would be the depolarization of membrane potential, decrease in intracellular pH+ or the elevation of Ca_{2+} levels (20). Each of this effect can change the astrocytes activity as well as the gliotransmitter release. In this regard, Gourine et al., (2010) showed that ChR2 expressing astrocytes release ATP as a consequence of ChR2 stimulation (239).

The release of glutamate by astrocytes can both be Ca^{2+} dependent with intracellular Ca^{2+} elevations (240), or Ca^{2+}- independent by reversal of uptake via glutamate transporters and anion channels (241). The reversal of glutamate transporters in turn can be achieved by membrane depolarization (242). In particular, with blue light illumination researchers achieved to evoke inward current in cerebellar astrocytes followed by a glutamate release that is confirmed by whole cell recording (243). The glutamate release upon blue light was shown to be triggered by the depolarization of astrocytes alone.

2.5.3.2. Hyperpolarizing rhodopsins affect on glutamate uptake

It is crucial to remove excess glutamate, the main excitatory neurotransmitter, in the synaptic cleft to prevent the increase in neuronal excitability. High glutamate concentration is eliminated mostly by quick removal with the help of astrocytes. The glutamate transporters in astrocytes are primary elements of the removal, whose activation is voltage-dependent and highly correlated to hyperpolarizing potential (244). In fact, hyperpolarization of astrocytes by ArchT lead to decrease in the extracellular glutamate (245) and thus suppress the hyperexcitation of nearby neurons (20). And loss-of function of glutamate transporters causes epileptic seizures (246). As a result, it is stated that hyperpolarized membrane potential of astrocytes plays a prominent role in glutamate uptake and when optogenetically manipulated with actuators like NpHR or Arch T, astrocytes can be hyperpolarized resulted in an increase of their glutamate uptake.

2.5.3.3. Hyperpolarizing rhodopsin affect on K^+

With neuronal activity, K^+ is released into extracellular space which can then cause an increase activity in the abutting neurons which also if not cleared on time leading to a pathological condition. Astrocytes due to the expression of several membrane proteins such as Na/K pump and K-transporter or K^+ channels are responsible in maintaining the K^{2+} balance. The dysfunctions of any of these channels might result in epilepsy (20). For example, the knockout of Kir4.1 channel results a depolarization in astrocytes membranes, which then inhibits the uptake of K^+ finally leading to a seizure (247). Hyperpolarizing actuators such as Arch T when expressed in astrocytes can possibly improve the uptake of K^+ by increasing the activity of Kir

channels. Upon yellow light stimulation, this rhodopsin open, leading to efflux of protons, thus increases the pH while hyperpolarizing the membrane potential of astrocytes. This aftereffects of the alkalization and hyperpolarization of astrocytes lead to the increase uptake of K^+ via Kir channels. Thus, hyperpolarizing actuators offer a possible way to avoid astrocyte-related epilepsy through increasing the uptake of glutamate or and/ K^+ (20).

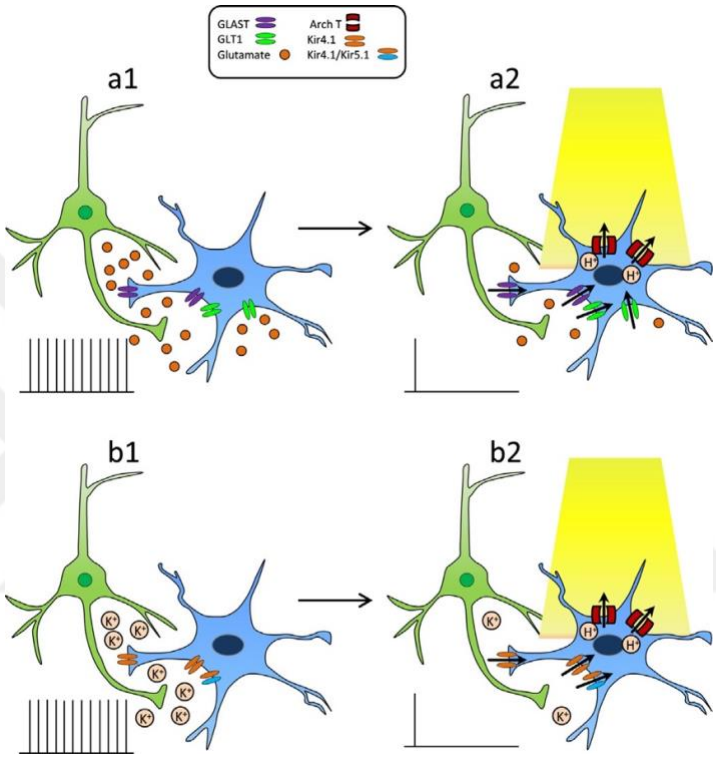


Figure 14. Optogenetic manipulation in astrocytes. a1 Excess glutamate in the extracellular environment can cause hyperexcitation in neurons. a2 Optogeneticaly activated Arch T-expression in astrocyte membranes cause hyperpolarization and alkalization in astrocytes (Beppu et al., 2014). Both effects lead to an increase in the uptake of glutamate via GLAST and GLT1 transporters, therefore terminating the hyperexcitation of the neuron. b1 Excess K^+ in the extracellular environment of a neuron can cause hyperexcitation. b2 Photoactivation of an Arch T-expressing astrocyte results hyperpolarization and alkalization of astrocytes, which could enhance the K^+ uptake via Kir4.1 and/or Kir4.1/Kir5.1 channel in the astrocyte membrane, thus reducing the hyperexcitation of the neuron. Retrieved from Zhi, J. et al. 2014 [20].

2.6. Glial Fibrillary Protein Structure and Applications

Glial fibrillary acidic protein (GFAP) is an intermediate filament protein expressed almost exclusively in astrocytes (34). This specificity gives an opportunity to use GFAP promoter for targeting transgene expression to astrocytes (248,249). To identify the effect of optogenetic stimulation of astrocytes on any physiological action, the promoter for the astrocyte-specific cytoskeletal protein, GFAP, can be used to direct the expression of channelrhodopsin-2 (ChR2) and the linked reporter gene, enhanced yellow fluorescent protein (EYFP), in astrocytes (24). rAAV-GFAP-ChR2 (H134R)-EYFP plasmid construction (Figure 15) and expression (Figure 16) can be seen below.

Applications can be varied from in vivo functional tests of various gene products, use of astrocytes in bioactive molecules and developing disease models (34).

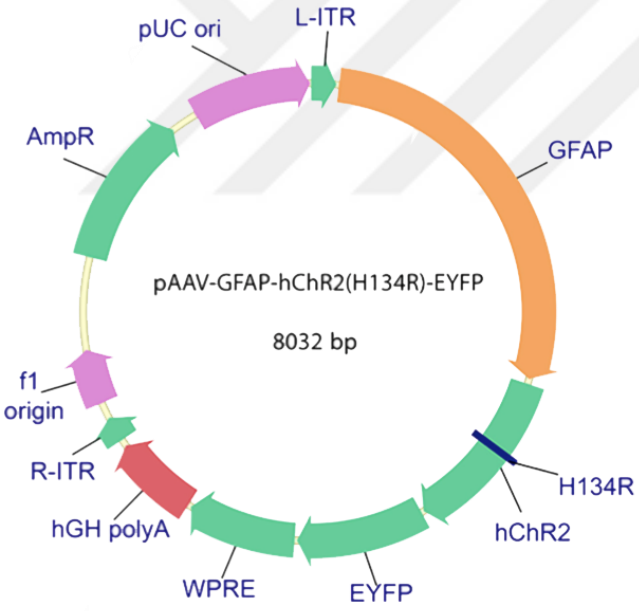


Figure 15. A map of channelrhodopsin-2 (ChR2) linked glial fibrillary acid protein (GFAP) reporter plasmid containing enhanced yellow fluorescent protein (EYFP) that is packed into recombinant adenoassociated virus (rAAV). Retrieved from Pelluru, D. et al. 2016 [24].

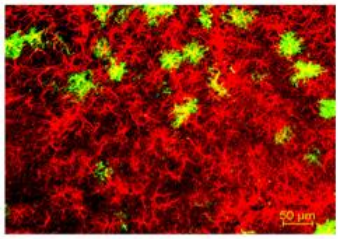


Figure 16. The photograph illustrate the astrocytes containing GFAP (red) and EYFP (green) in a mouse. Retrieved from Pelluru, D. et al. 2016 [24].

Adeno-associated virus (AAV) is a nonenveloped parvovirus with a single-stranded DNA genome of 4.7 kb (250) that is widely used as a backbone for recombinant viral vectors (43). The virus shows no pathogenicity and low immunogenicity, that can also be produced in high quantities with high purity (251).

Several evolutionarily distinct AAV serotypes have been found (252) with diverse cell tropism, transduction strength and gene expression distribution (253,254,255). The transduction occurs by an axonal terminus, retrograde transport to the cell body (256,257) or via uptake by a projection neuron, anterograde transport along the axonal projection, secretion from the axon terminus to a second-order neuron (258). The transduction difference among AAV serotypes relies on the differential uptake at the plasma membrane rather than the principal differences in transport mechanisms (43). AAV vectors undergo long-distance axonal transport when locally injected into the specific brain structures. AAVs employ several receptors and attachment factors, such as the laminin for AAV8 (259), galactose for AAV9 (260), and sialic acid for AAV1 (261).

AAV serotypes 1,8, and 9 can be actively transported along axons in both anterograde and retrograde directions in which serotype 8 can rarely spreads beyond the injection site (43). The vectors utilize the late endosome/lysosome for retrograde axonal transport (43,262) and a shared intracellular compartment for anterograde axonal transport (43). There is a substantial difference in regard to time course of retrograde and anterograde AAV transport. Retrograde transport can easily be peaked and remain constant, whereas anterograde transport increases gradually over time suggesting that AAV rapidly enters the retrograde-directed late endosome/lysosome carrier, while entry into the anterograde carrier occurs preferentially at later time points once AAV has accumulated inside the cell body (43).

Among the novel AAV serotypes, AAV8 has been proven to have a tremendous potential for in vivo gene delivery. It shows nearly complete transduction of many tissues in rodents (45). The efficiency of AAV8 is due to a presumably ubiquitous receptors that confers its wide-ranging tropism, and the rapid release of vector genomes into the transduced cell nucleus (263). These findings suggest that AAV8 can be very efficient for gene delivery to the CNS.

Chapter 3

3. MATERIALS AND METHODS

3.1. Materials

Chemicals, antibodies, and devices that were used in this study are presented in Table 8, 9, and 10 respectively.

Table 8. Chemicals

Name	**Brand name**	**Catalog No**
Ketamine (Ketasol 10%)	Richer pharma Ag	-
Xylazine (Rompun 2%)	Bayer	-
Saline (NaCl)	Sigma Aldrich	31434
Ethanol	Merck Millipore	1009862500
Paraformaldehyde	Sigma Aldrich	158127
Phosphate Buffer Tablet	Sigma Aldrich	P4417
DPBS no Ca no Mg	Gibco	14190-169
Triton™ X-100	Fisher Bioreagent	BP151-100
Tween-20	Sigma Aldrich	P9416
Normal Goat Serum	Sigma Aldrich	G9023
Hoechst 33342	Thermo Fisher	62249
Glycerol	Sigma Aldrich	49782
Optic fiber glue	Epoxy Technology	301 UGM/ BSEAL
Selfcure Acrylic Repair Material, (Powder)	IMICRYL	-
Selfcure Acrylic Repair Material, (Liquid)	IMICRYL	-
Hamilton Cleaning Solution	Hamilton	Part #18310
Tears Naturale II	Alcon	-
Antibiotic	Terramycin	-

Table 9. Antibodies

Name	**Brand name**	**Catalog**
Anti-GFAP antibody	Abcam	ab7260
Recombinant Anti-NeuN antibody [EPR12763]	Abcam	ab177487
Goat anti-Rabbit IgG (H+L) Alexa Fluor 594	Jackson Immunoresearch	-
Albumin from Bovine Serum (BSA)	Thermo Fisher	-

Table 10. Devices and Apparatus

Name	**Brand name**
Microinjection syringe pump	World Precision Instruments
Stereotaxic apparatus	Stoelting
Hamilton syringe 26 ga	Hamilton
Confocal Microscopy	Leica Dmi8/Cs/Sted
Cryostat	Leica Cm 1950
EEG recording machine	Biopac, MP36
EEG cable connectors	Tripolar Cable with 50 cm spring covering
EEG-recording electrodes	Plastics1, MS333/1-A/SPC
EEG-cable	Plastics1, 335-340/3
Optogenetic Stimulator	Thorlabs Starter Kit 2
Optogenetic ferrul	Thorlabs
Optogenetic cannula (fiber)	Thorlabs
Stereotaxic cannula holder	Thorlabs XCF #8-32-LH
Optogenetic cable	Prizmatix, 500SMA-2.5mm NA 0.63
Micromotor control HP4-917	Foredom Electric Company
DIY Cannula Kit for Optogenetics 2.5mm/200 μm	Prixmatix
Polishing Kit Lapping Film	Prizmatix
Poly-lysine coated glass slides	Sigma Aldrich P0425
Coverslip	GT Vision, Product no.: 5915-5922

3.2. Animal Experiments

In the study, 250-300 gr, 4-6 months old male GAERS, WAG-Rij and Wistar animals used. All animal experiments were held at Koç University Research Center for Translational Medicine ‘’KUTTAM’’. The study was approved by the Local Animal Ethics Committee of Koç University under the approval number 2018.HADYEK.017. Experimental design of the study is shown in Figure 17. The total animal number for the study is n=16.

***Virus for experimental group:** GFAP-ChR2: AAV8-GFAP-hChR2(H134R)-EYFP

***Virus for control group:** AAV5-CaMKlla-EYFP

Experimental Groups:
1. GAERS (Σn=4) GFAP group
2. GAERS (Σn=3) Control group
3. WAG-Rij (Σn=4) GFAP group
4. WAG-Rij (Σn=4) Control group

Sub-group:
1. Sham group: Wistar (Σn=1)
   (For virus injection and target location study)
2. Immunofluorescence
   (For virus expression verification)

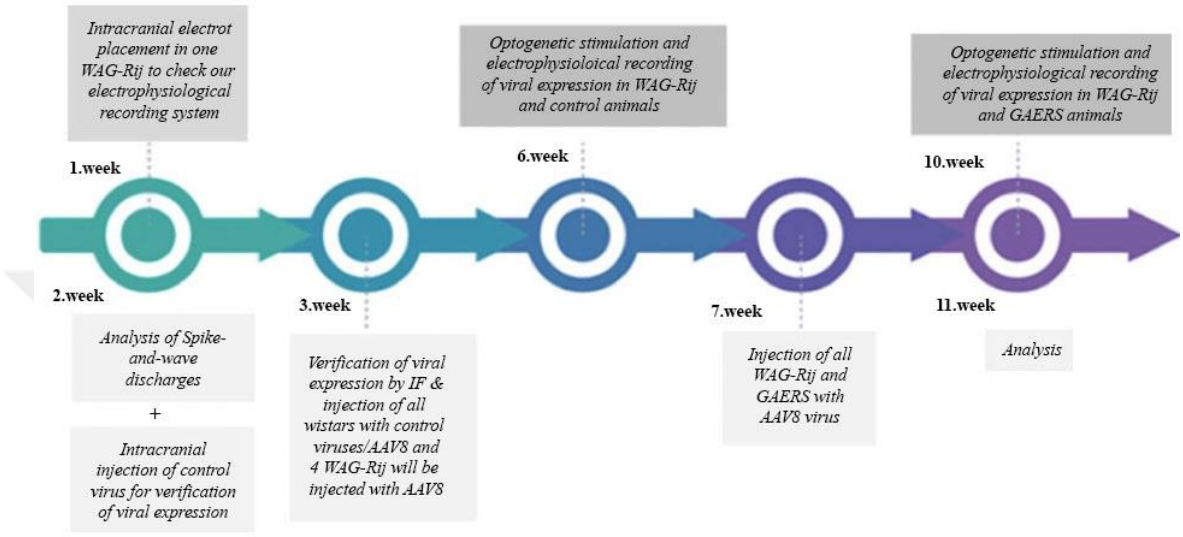


Figure 17. Experimental design of the study.

3.2.1. Stereotaxic injections

3.2.1.1. Implementation of electrodes for electroencephalogram measurements

We first checked whether our system was suitable for recording from rat cortex with our electrodes. For this purpose, 1 WAG-Rij rat, due to readily available SWDs, was stereotaxically implemented only with monopolar electrophysiological electrodes (tip-active silver fine-wire recording electrodes with Teflon (75 μm in core diameter; Medwire, USA) and from the same wires with an open tip as a reference electrode (the active part of 1 cm)) under 3% isoflurane anesthesia. Rat was injected with analgesic as a single peri-operative carprofen subcutaneous administration half an hour before the surgery.

1. Rat was immobilized in a stereotaxic frame by fixing the head from external ear canal and nose.
2. The hair over the skull was shaved with an electric razor and the skin was disinfected by Batticon.

3. Eye cream was applied, Terramycin, on both corneas to avoid dehydration and infection.

4. Tail and toe-pinch reflexes were checked to ensure adequate anesthesia.

5. An anterior-posterior incision was made of about 2.5 cm on the midline of the scalp. Bulldog clamps were used to pinch off the skin and to keep the incision open. The conjunctive tissue around was removed with a spatula and cotton swabs to expose the skull surface.

6. To get a rigorous attachment of the cement, several scratches were done over the skull with a tip of the surgical blade.

7. The level of head was checked by way of comparing the dorsoventral depth of bregma and lambda. First, guide cannula was exactly located over Bregma and dorso-ventral coordinate was recorded. Next, guide cannula was slightly touched the skull over lambda, and the dorsoventral coordinate was noted. The head was adjusted by nose bar until the two coordinates clashed.

8. Two small burr holes were in the skull for fixing the skull screws by using hand drill (one approximately 5 mm lateral to the location of frontal electrode in the contralateral hemisphere of the intended side for virus and electrode placement and the other was 5 mm behind the frontal electrode in the ipsilateral side). Two clean screws then were placed into these holes until they are tightly anchored, without being completely inserted.

9. Guide cannula was exactly placed over bregma to measure the anterior-posterior (AP) and lateral (ML) coordinates as reference points. Then the coordinates for three channel electrodes were calculated by adding/subtracting from bregma according to the stereotaxic atlas (429).

10. Exact locations of the target areas (Figure 19) were marked with pen, and after the burr holes were made with the drill, the size and the correct locations were checked.

11. The three stipes of electrode were located at their coordinates and externalized through the pedestal and the installation was mounted to the skull using acrylic cement. (Figure 18).

12. The dental cement was prepared and generously applied around optrode, electrode as well as the screws. And any surplus was removed from the skin before the cement was allowed to dry completely.

13. The whole surface of the skull was then covered with dental acrylic to stabilize it.
14. After the cement has completely dried, wound area was cleaned with sterile saline and one suture was made at the front and the back sides of it.
15. Animal was removed from the stereotaxic apparatus, replacing the gas tubing in front of its nose. The body temperature and oxygen saturation were checked for 1 hour in a recovery cage.
16. Animal was then returned to the vivarium room.

Figure 18. Stereotaxic implementation of EEG electrode on the skull of a WAG-Rij rat.

Recovery of rat was checked every two days by keeping the records of weight and other physiological observations in laboratory logbooks. Animals that showed overt sign of sickness, infection of wound, loss of body weight or other sings of well-being received extensive care, or they were dismissed from the study. The main drawback seen was the dislocation of implemented acrylic that contained electrodes and optrode. These animals were discarded from study.

3.2.1.2. Intracranial injections of virus for target location and virus expression

For this purpose, Wistar rat was injected with virus carrying the rhodopsin. At this point, rat was kept under anesthesia with 3% isoflurane and placed onto the stereotaxic frame as explained above. Apart from the controlling the target location, we examined the optogenetic stimulation approach in the animal, so electrodes and optogenetic ferrul were implemented in the same animal as detailed below.

1. Electrode placement was done as explained above.
2. Additionally, the coordinates for ventrobasal nucleus was calculated after electrodes were placed and Hamilton syringe was moved to target area.
3. For the viral injection, Hamilton was placed carefully until the dura, after recording the dorsoventral level of dura, calculation of the ventral point of ventrobasal nucleus

was done. The coordinates for thalamus ventrobasal nuclei are for AP: -3.6 mm, for ML: -3 mm and for DV: -6.4 mm. The titer of concentrated virus for in vivo injection was $3x10_{12}$ virus molecules/ml.

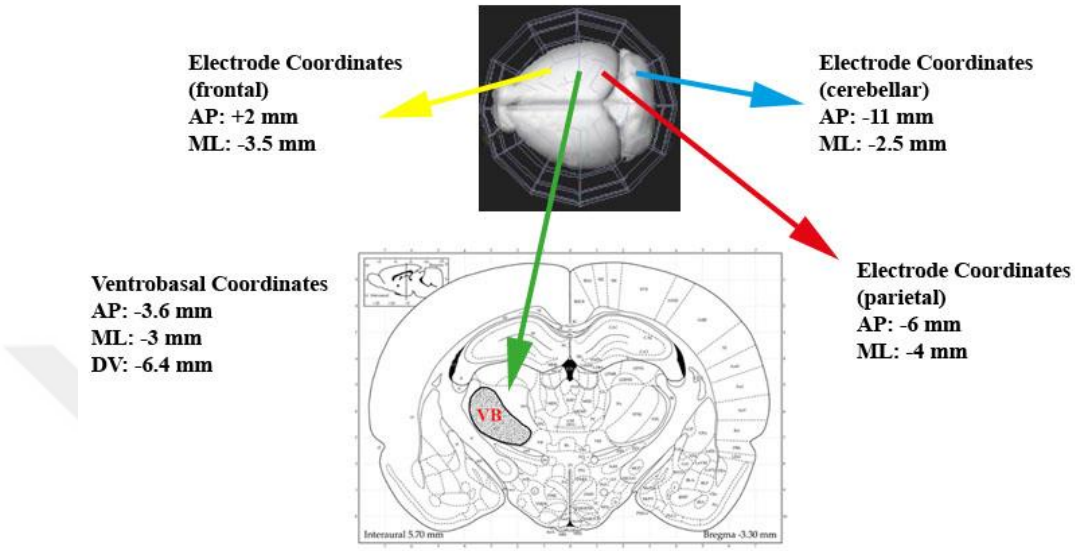


Figure 19. Representation of brain section from injection site and electrode placements (Adapted from the web site: http://labs.gaidi.ca/rat-brain-atlas/?ml=-2.8&ap=-3.3&dv=-6.5)

4. Hamilton syringe (26 ga) was lowered until it reached the final ventral coordinate and the total volume of 0.5 µl virus was injected at a rate of 0.1 µl/min with an injection pump.
5. Hamilton syringe was left in place for 10 min to avoid any virus retraction, and then gradually withdrawn.
6. Finally, optrode was inserted just 200 µm above the area of virus injection by stereotaxic cannula holder (preparation can be seen in methods section 3.2.2.2)
7. Afterwards, the cannula support was carefully removed by turning the dorsoventral bar upwards.
8. The dental cement was prepared, and the surface of skull was covered with it as explained above.
9. The cement was allowed to dry completely, and wound area was sutured at the front and back edges (Figure 20).

10. Animal was removed from the stereotaxic apparatus and followed for health conditions for at least one hour. Then returned to the vivarium room.

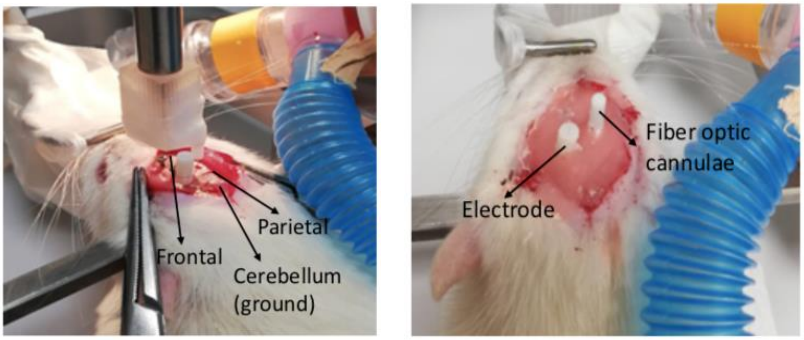


Figure 20. Schematics of Stereotaxic injections.

3.2.1.3. Intracranial injections of virus to GAERS and WAG-Rij animals

Animals for experimental groups were injected with optogeneticaly modified virus after the preliminary results were obtained. The injections were made in the order of WAG-Rij group being the first that followed the GAERS injections.

3.2.2. Optical Stimulations

All animals that have been injected with viruses were allowed to recover for three weeks as requested for virus expression. After 3 weeks, recording and stimulation cables were inserted to bipolar electrode and fiber optic cannula, respectively under isoflurane anesthesia of 2% (v/v). These animals were then stimulated using 473 nm blue light in a plexiglass cage. The optical stimuli (with 9.68 mW intensity calculated from fiber optic cannula tip) were delivered as 200 multiple square pulses (each pulse's duration 50 ms) at 10 Hz to the VB nuclei where the virus have been injected in both GAERS and WAG-Rij rats (Figure 21).

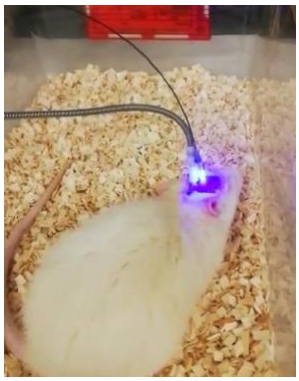


Figure 21. Optogenetic stimulation was delivered via an optical fiber with the configuration of 10 Hz. for 50 msec (200 pulses).

3.2.2.1. Setting up the optogenetic hardware

Components of the optogenetics hardware was obtained from ThorLabs (Starter Kit 2 with 590 nm additional light source) and set up manually according to the instructions and literature.

3.2.2.2. Preparation of the optrode

The optical fiber and ferrul were prepared with the help of DIY kit which contains the Polishing Kit Lapping Fims, Polishing Puck and Hex key, scribe and uncleaved fibers.

3.2.2.2.1. Construction of the cannula

To construct the cannula, first the length of optical fibers was adjusted for ventral coordinate of the ventrobasal thalamus. Epoxy glue was prepared according to manufacturer's instructions. A bead of epoxy glue was placed on flat side of cannula, after a few seconds uncleaved end of fiber was inserted into flat side of ferrul. Fiber was pushed through to the desired length, as measured with caliper to get exact length and awaited to dry for 24 hours.

3.2.2.2.2. Polishing of the cannula

Scribe was gently scratched around the fiber on the convex side until the excess fiber falls off. Cannula was then inserted into the puck with a very small part of it sticking out. The puck was tightened with the hex key and placed on the polishing paper. Polishing was done with small circles and after a few rounds moved on to increasingly finer polishing papers which followed the 5- μm black, 3- μm pink, 1- μm green, 0.3- μm white papers respectively. For the finer papers, a few drops of water added onto it.

3.2.2.2.3. Inspection

The cannula was hold to the light with round end towards us and ensured a full circle of light was visible through it.

3.2.2.3. Optimization of the light delivery

The light stimulation was manually controlled by a digital TTL pulser, delivered by a LED light source (Prizmatix, Dual LED, Holon, Israel) and the average light intensity targeted at thalamus was chosen from several articles in which same/similar optrode used and it was at the highest intensity available in our device, 9.68 mW.

3.2.2.4. Light stimulation protocol

Light stimulation method was adjusted with an animal attached to the optical cable (ferrul) as well as with the EEG cable.

1. On the day of stimulation, animal was connected to plexiglass and handled carefully while connecting to light stimulating cable and to EEG electrodes.
2. Excitatory stimulation was applied with 473 nm blue light wavelength.
3. Electrophysiological recording was continuously recorded during optical stimulation which allowed us to manipulate the stimulation duration and intensity according to the online result we obtained on the EEG screen.

3.2.3. Electroencephalogram recordings

EEG was continuously recorded via a four-channel electrophysiology system (Biopac MP36, CA, USA). The active electrodes with bipolar configuration were placed on the frontal and parietal cortex, ground electrode was placed on the cerebellar cortex. The recording was performed with a sampling rate of 500 Hz and 0.5-100 Hz online bandpass filter.

3.2.3.1. EEG Recordings from preliminary study

1. We recorded the characteristics of SWDs in a WAG-Rij rat.
2. After one week of the implementation of electrodes, on the recording day WAG-Rij rat was put under light anesthesia and the three-channel connector cable (Plastics 1, 335-340/3) was connected to the surgically placed electrode. Awake rat afterwards was placed in a plexiglass cage and attached the EEG recording system to detect the SWDs.
3. After a short adaptation period, around half an hour, basal EEG signals were recorded which also includes the spontaneous SWDs.

3.2.3.2. EEG Recordings from experimental groups

EEG recordings were performed as explained above. Each session was started with a 1-hour baseline recording (first 30 minutes for adaptation and second 30 minutes for background EEG activity), then after the optogenetic stimulation protocol, the changes were recorded for half an hour. Three recording sessions for each animal were repeated in different days and every session was adjusted to be in the different hour of a day to cover the whole spectrum of SWD occurrence in the animal.

3.2.4. Statistical Analysis:

EEG recordings: after performing the Shapiro-Wilk test to find out the distribution, parametric paired t-test was used for normally distributed pairs of pre- post-stimulation protocol and nonparametric Mann-Whitney test was used for non-normal distributed firing rates of the SWDs in GAERS and WAG-Rij rats. The level of significance was selected as $p<0.05$.

3.3. Perfusion

After the completion of third set of EEG recordings, animals were injected with high dose of ketamine (200 mg/kg) and xylazine (30 mg/kg) anesthesia prior to cardiac perfusion. The chest of the animal was then excised with scissors, a small cut to the right atrium was performed and bolus infusion of approximately 200 mL saline was administered to the left ventricle of rat. Then the fixation process is continued by introducing a 300-400 mL fixative solution (4% PFA). At the end of perfusion process, the brains were removed and transferred to the same fixative for overnight. Afterwards, the brains were displaced into 10% sucrose solution for one day, followed by 20% and 30 % every other day until the brains dehydrated. The brains were cut in cryostat (Leica CM 1950, Leica Biosys., Germany) at 20 μm and free-floating sections were placed in six well plate in PBS-NaAzide at 4^0C. The sections from injection area were specially collected in a single well and whole sections were held at the dark room until for further staining and confocal imaging.

3.4. Stainings

3.4.1. Protocols

Blocking solution:

1. 0.1% triton-X / Dulbecco's phosphate buffered saline (DPBS) was prepared.

2. 1 mg of Bovine serum albumin (BSA) was weighted and dissolved in triton-X / DPBS solution.

3. 10% Normal goat serum (NGS) was dissolved in 0.1% triton-X /DPBS.

4. 1 mg in 100 μL was added to finalize the solution.

Primary antibodies:

1. 2% NGS, 2 µL

2. Single staining: GFAP- (1:100), primary antibody

3. Single staining: NeuN- (1:100), primary antibody

- 0.1% triton-X/DPBS, 99 µL in total 100 µL solution.

Secondary antibodies:

1. 1% NGS

2. Single staining: 1:100 secondary antibody, (Alexa 594)

- 0.1% triton-X/DPBS, 99 µL in total 100 µL solution.

Nucleus dye:

1. 1 µL of 1x DPBS was mixed with 1 µL glycerol.

2. 10 µL Hoechst was added into the solution and covered with aluminium foil in order to protect from light.

3.4.2. Procedure:

1. Tissue sections were protected in PB buffer with 0.1%NaAzide and stored at 4^{0}C until staining.

2. Two sections from each injection area were stained per animal. Free floating sections were placed in 6 well-plate.

3. Sections were washed with 0.1%triton-X-DPBS solution for 3x5 minutes.

4. Blocking solution was then added to sections and incubated for 1 hour at room temperature on a shaker.

5. Primary antibody was prepared as explained above.

6. Free floating sections without prior washing were then transferred into small PCR tubes which contain 100 µL antibody solution for each sections (two) from each animal.

7. The sections were then allowed to be incubated with primary antibody overnight at 4^{0}C.

8. In the following day, sections were washed with 0.1% triton-X/DPBS solution for 3x5 minutes in 6-well plate.

9. Secondary antibody for both GFAP and NeUN was Alexa 594 (1:100).

10. After washing step, sections were again transferred to small PCR tubes for secondary antibody incubation for 1 hour.

11. Finally, sections were washed with 0.1% triton-X/DPBS solution for 3x5 minutes.

12. Sections were then mounted on poly-lysine coated glass histology slides and allowed to dry for a short time.

13. Nucleus dye was prepared as described and applied over the sections on slides.

14. Sections were then covered with a coverslip and edges of the coverslips were further glued with transparent nail polish.

Confocal microscopy was used to analyze the stainings and the analysis of images were done by ImageJ software.

The analysis of GFAP-Alexa Fluor 594 and NeuN-Alexa Fluor 594 stainings were performed by Leica Application Suite X (Las X) software.

* Precise target locations were checked for correct injections and the location for each animal can be seen in figure 26 and figure 27 below.

* To check the virus expression in experimental groups, GFAP and NeuN stainings were performed in consecutive sections of two animals from each group. The images can be seen below.

Chapter 4

4. RESULTS AND DISCUSSION

4.1. Preliminary Results

4.1.1. EEG recordings from sham animal

We first characterized the course of seizure development in a sub-group of sham control animal which was not transduced with virus. The occurrence of spontaneous SWDs were recorded with our system. The typical SWDs pattern contain 7-11 Hz as can be seen in figure 22.

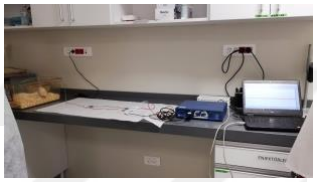
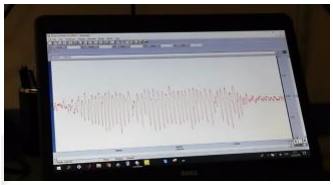
a  b  c  d 

Figure 22. (a) Cortical EEG recording was achieved with three channel electrodes; (b) the electrodes were connected to EEG system; (c) Biopac MP36 system was recruited; (d) Half an hour baseline activity revealed the characteristic SWD oscillations in WAG-Rij.

Our system is approved to be applicable for animal research, and the efficacy of minimum signal recording has been tested with ‘’wave generator’’. Also, the high sampling frequency with its various filtering options and the capacity of signal amplification makes the system flexible for signal conditioning.

4.1.2. Results from optogenetic experiments showed stimulation paradigm

Optogenetic stimulation was checked in virus injected WAG-Rij and Wistar animals.

4.1.2.1. The injection of optogenetic virus

Correct injections of virus carrying the light-sensitive rhodopsin to our target area, ventrobasal thalamus was achieved as can be seen in the figure 23.

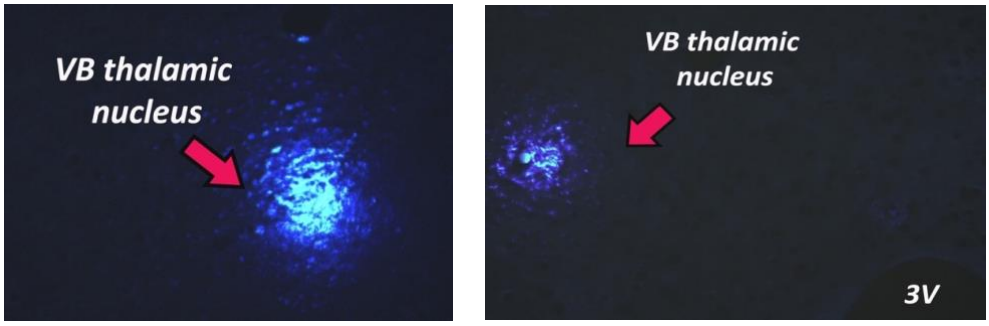


Figure 23. The arrows show the virus injection area in (a) Wistar and in (b) WAG-Rij rat.

4.1.2.2. Optogenetic stimulation

Optogenetic stimulation parameters were adjusted and modified in one WAG-Rij rat. The results can be seen in the figure 24. Spike-and-wave discharges from fronto-parietal cortex showed decrease duration after optogenetic stimulation in one WAG-Rij rat (Figure 25).

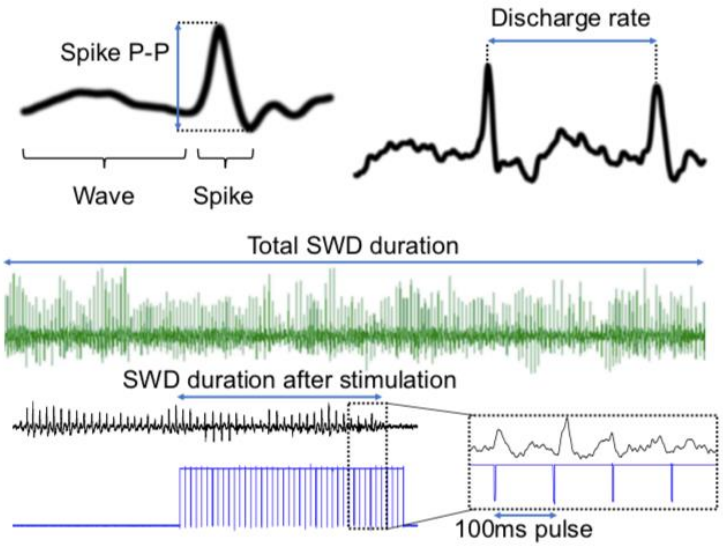


Figure 24. Optogenetic stimulation was achieved with optical fiber with pulses at 10 Hz. for 50 msec 200 times.

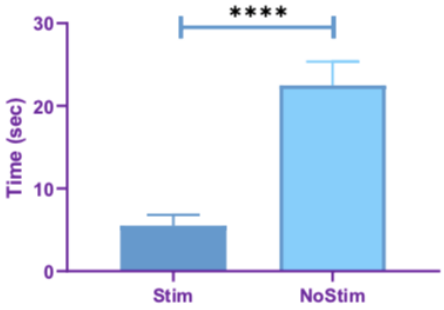


Figure 25. The optogenetic stimulation lead to a decreased SWD duration in WAG-Rij rat.

4.2. Results

Once we gathered the initial electrographic and optogenetic recordings, we performed experiments with our experimental groups. For each optogenetic session, after a successive 30-min baseline period, the effect of optogenetic stimulation was analyzed by cumulative duration of SWD expressed as a percentage of total period.

Data sets were analyzed (with trigger of optogenetic stimulation) using frequency-based method peristimulus frequency gram (PSF) and the effect of excitatory postsynaptic potentials (EPSP) was evaluated in terms of frequency range. Addition to PSF, probability-based analysis, peristimulus time histogram (PSTH) were used to evaluate the firing probability of the discharges of the astrocytes. Also, their cumulative summation (CUSUM) graphs were created to show the properties of the EPSP in detail.

The seizure start was characterized by a simultaneous appearance of 7-11 Hz frequency, high amplitude epileptiform spike activity on cortical electrodes. Behavioral manifestations including facial movements such as chewing, jaw-opening, head nodding, automatisms of the extremities, followed by myoclonic jerks and clonic seizures with rearing, falling and loss of postural control. Mild behavioral manifestations such as mouth movements, chewing preceded the appearance of spike activity on the EEG by 1-3 min.

*During our experimental set-up, due to the possible effect of intense light coming to the eyes of rat from optogenetic illumination, we tried to cover the surrounding of ferrul to prevent any misevaluation. The results below show the opposite of what we observed in preliminary results.

4.2.1. Virus expression in VB thalamus

4.2.1.1. VB injections

Immunofluorescent images for VB target location was done by using LEICA DMI8 SP8. Images of GAERS and WAG-Rij animals can be seen below.

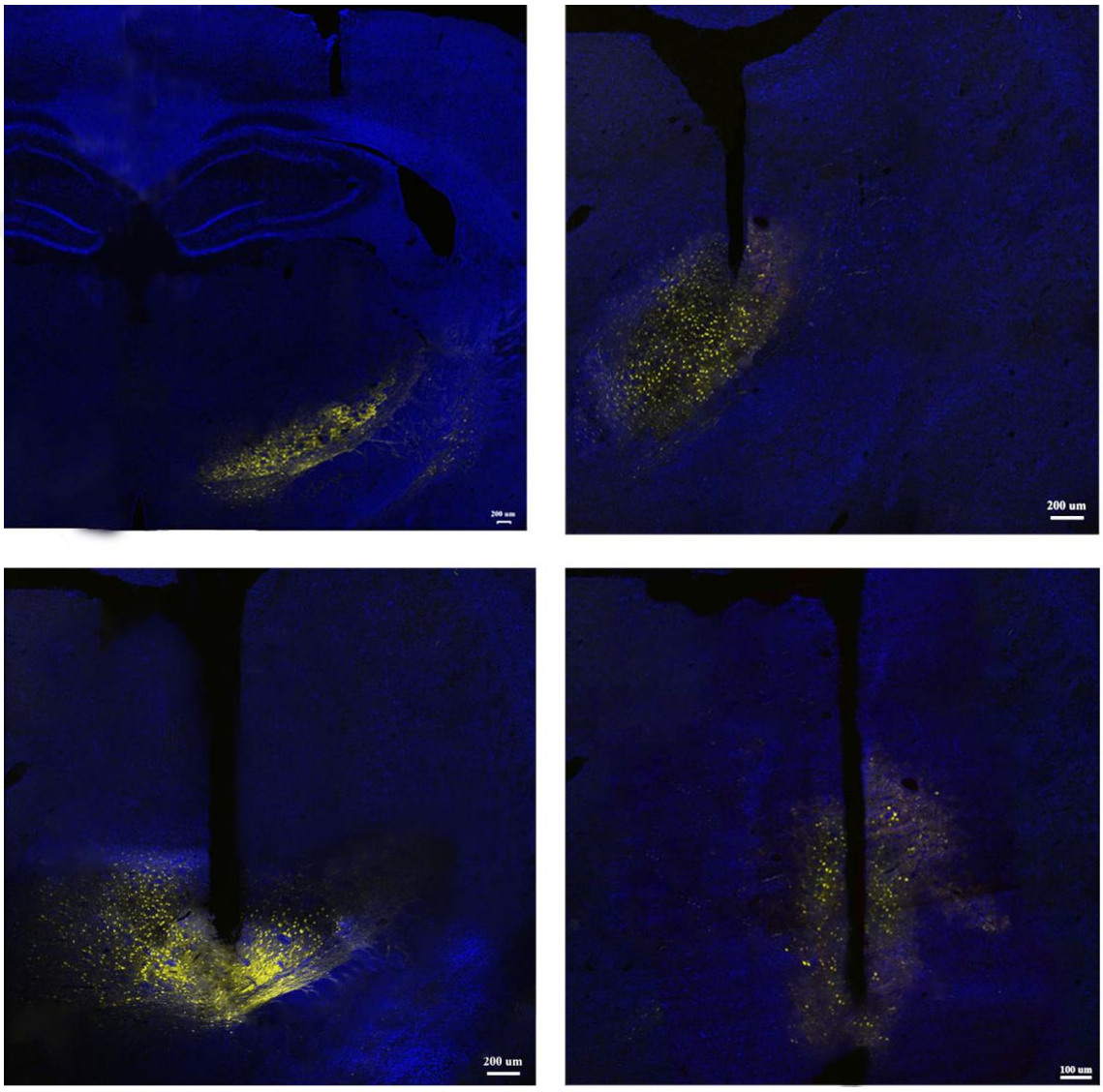


Figure 26. Immunofluorescent images of virus injection sites of VB thalamus in GAERS.

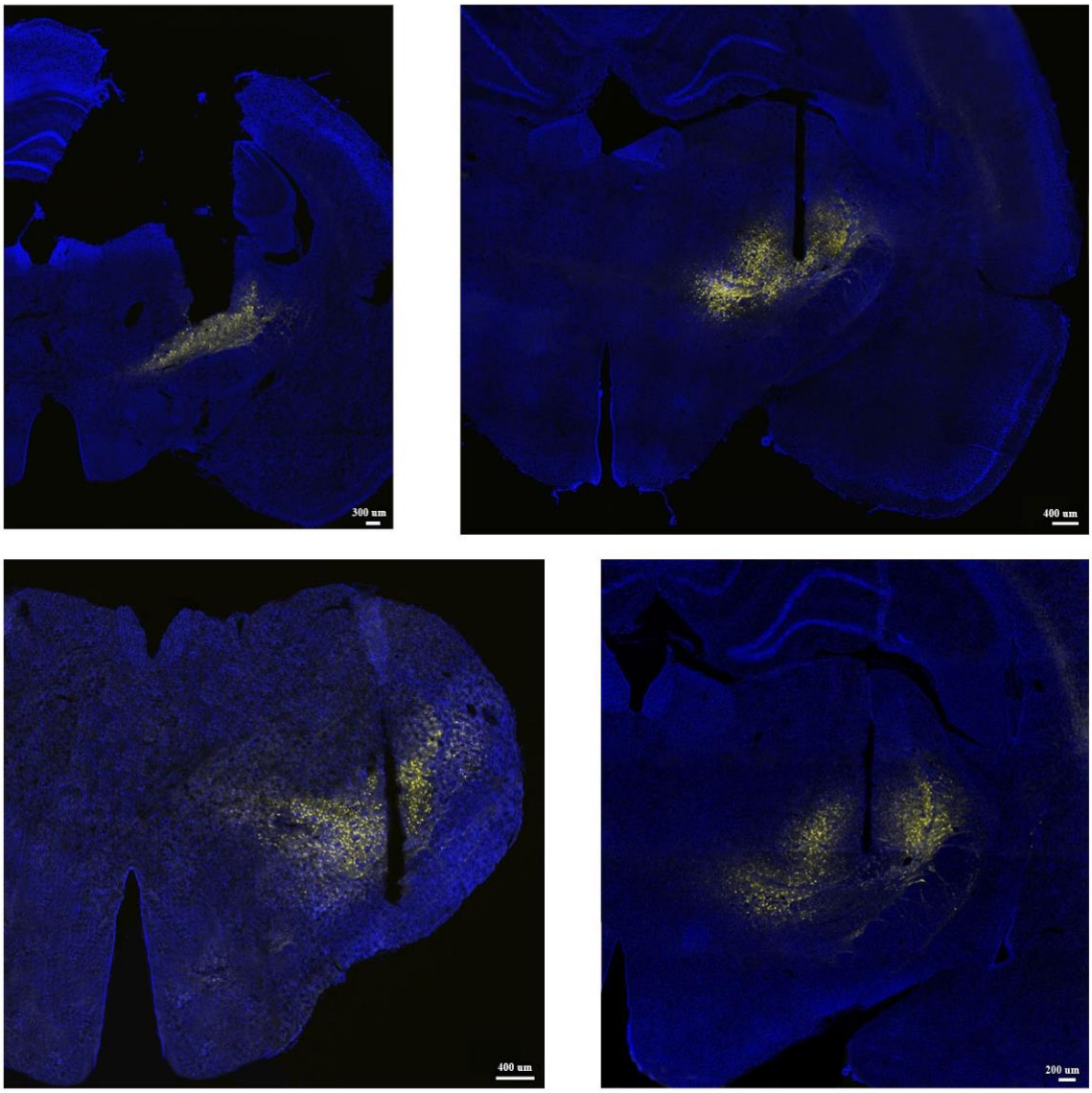


Figure 27. Immunofluorescent images of virus injection sites of VB thalamus in WAG-Rij.

4.2.1.2. Virus expression in astrocytes

Targeted expression of virus in astrocytes can be seen at images below (Figure 28,29,30,31).

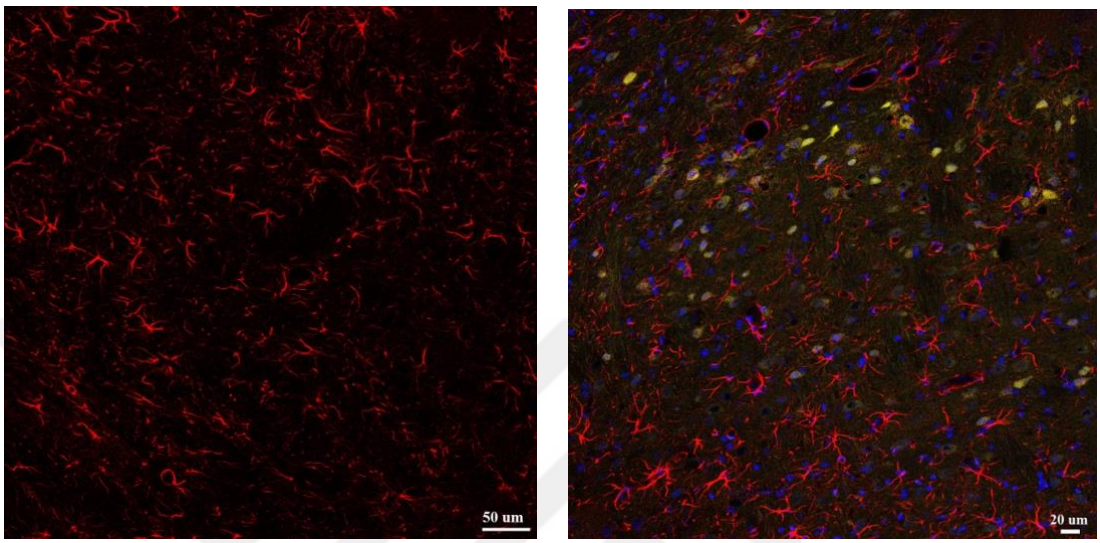


Figure 28. Immunofluorescent images of astrocytes infected by viruses.

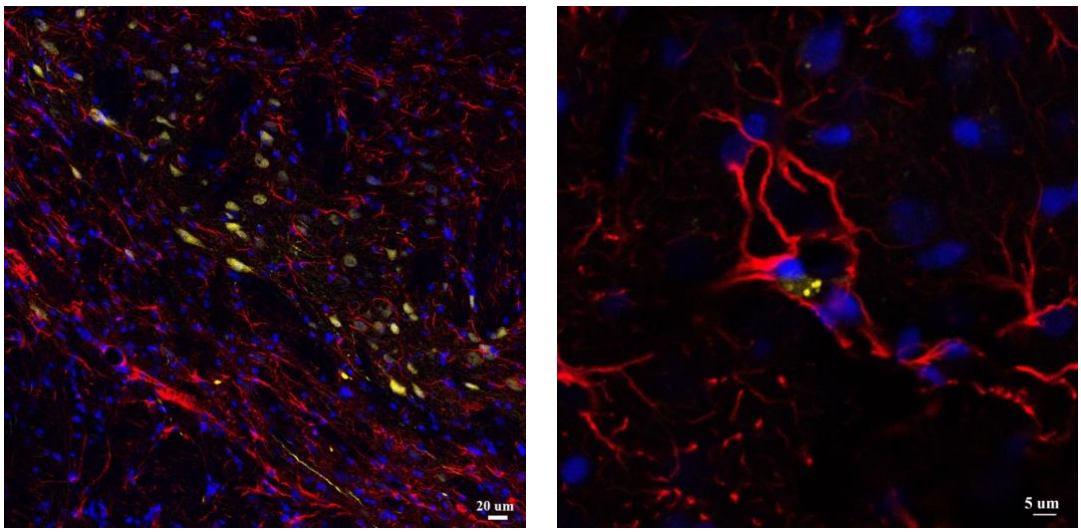


Figure 29. Immunofluorescent images of astrocytes infected by virus at a 40x magnification.

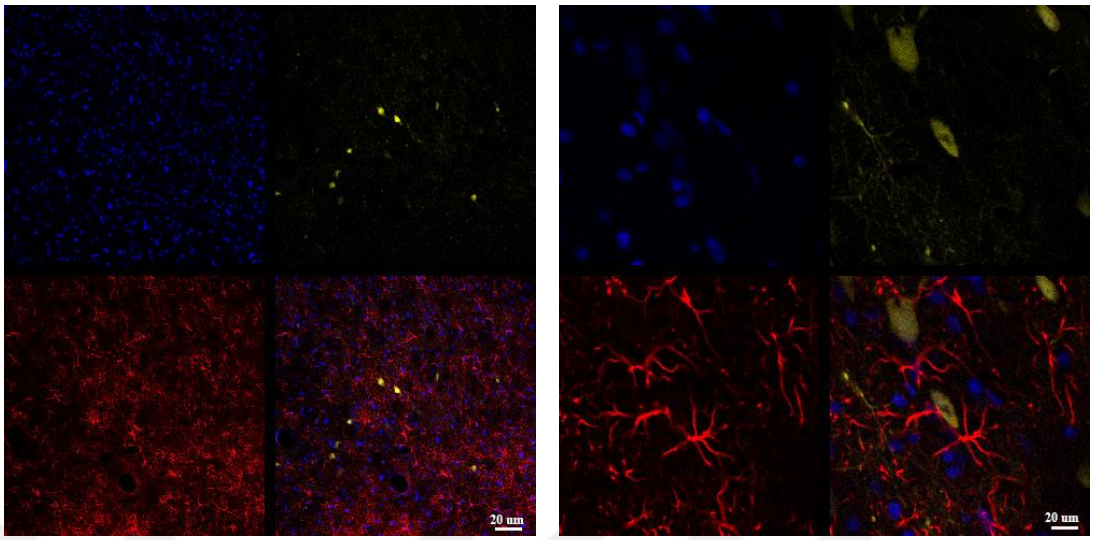


Figure 30. Immunofluorescent images of astrocytes infected by virus in GAERS.

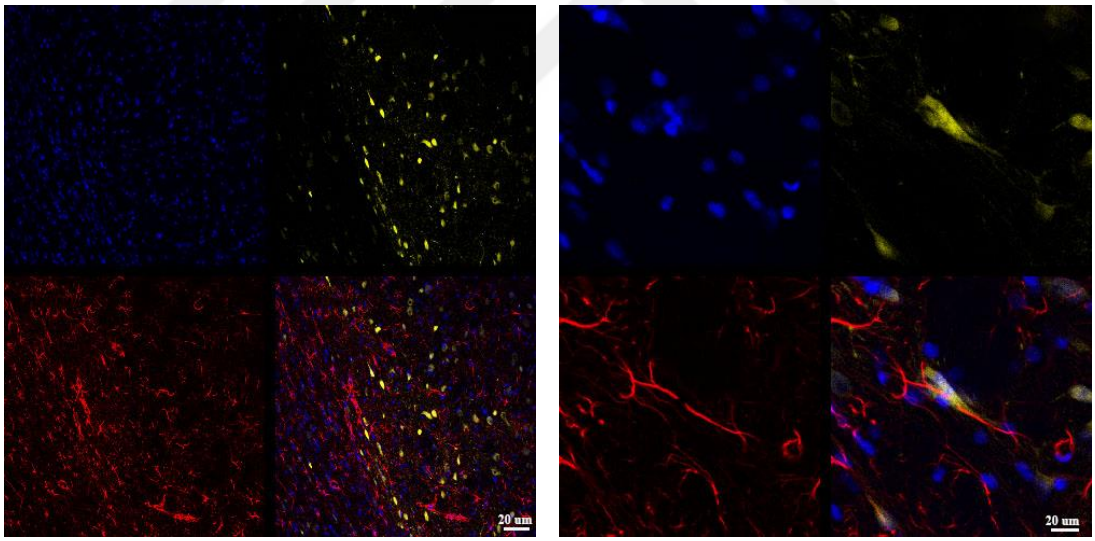


Figure 31. Immunofluorescent images of astrocytes infected by virus in WAG-Rij.

Virus expression was also observed in some neurons as can be seen in figure 32.

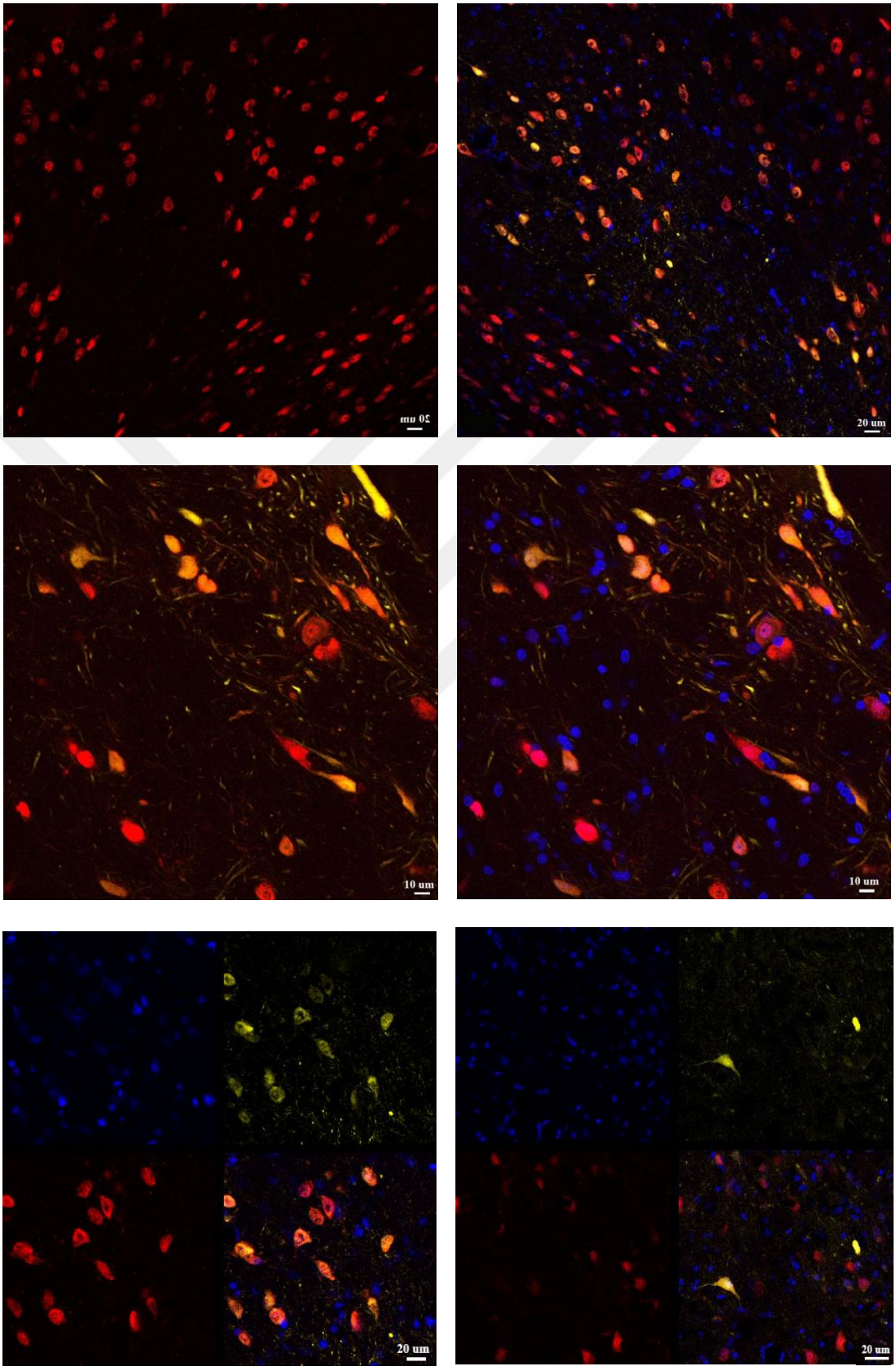


Figure 32. Immunofluorescent images of neurons infected by virus.

4.2.2. Optogenetic seizure control in vivo

We used a protocol for light-mediated excitation of astrocytes. Light was turned on for 50 msec 200 times at 10 Hz (Figure 33). The illumination mode leads to change of seizure dynamic in terms of an increase in the duration of SWD activity in both GAERS and WAG-Rij animals.

To quantify the dynamics of seizure development, the cumulative line-length for cortical channels as a measure of amplitude and frequency dynamics of each seizure (403).

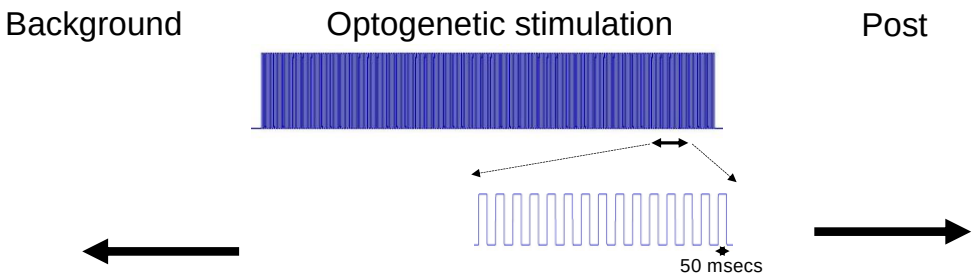


Figure 33. Representation of the optogenetic stimulation pulses.

4.2.3. EEG from GAERS

The properties of the SWDs and their response to the optogenetic stimulation of the ventrobasal thalamic nucleus were evaluated in the GAERS. The characteristics of SWDs in the GAERS were found as long and continuous (Figure 34) before and after the delivery of stimuli (Figure 35).

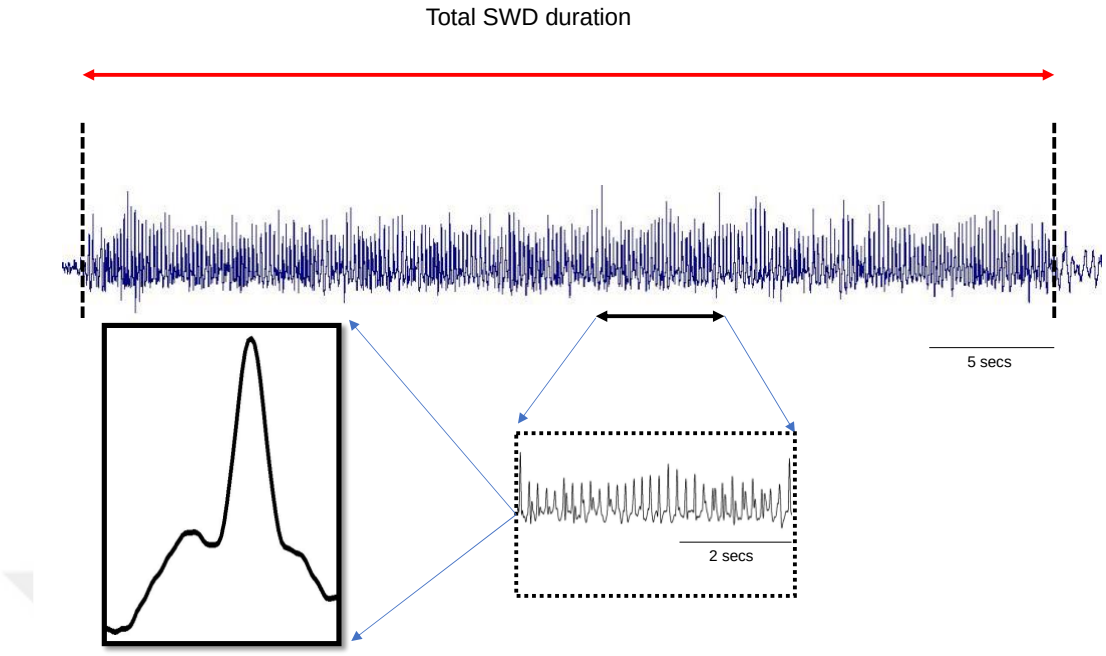


Figure 34. Sample recording of a continuous SWD.

We found significantly increased duration of the SWDs in GFAP-AAV injected GAERS compared to their own prestimulus background level (paired t-test: 12.50 ± 4.41 secs vs 17.44 ± 6.07 secs (mean ± SD), p=0.0043) (Figure 36a).

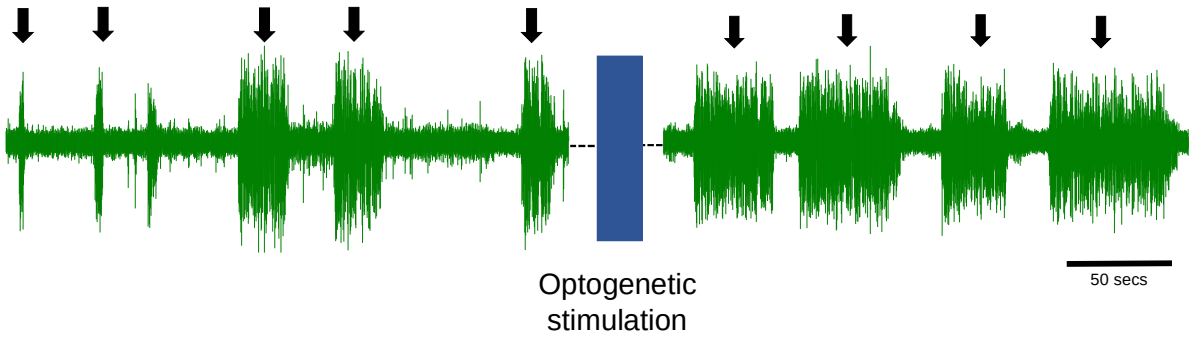


Figure 35. A sample recording before and after optogenetic stimulation. Black arrows show the SWDs.

On the other hand, optogenetic stimulation did not change the average SWD duration in control virus injected GAERS (paired t-test; 18.62 ± 4.94 secs vs 20.17 ± 4.67 secs (mean ± SD), p=0.3906) (Figure 36b).

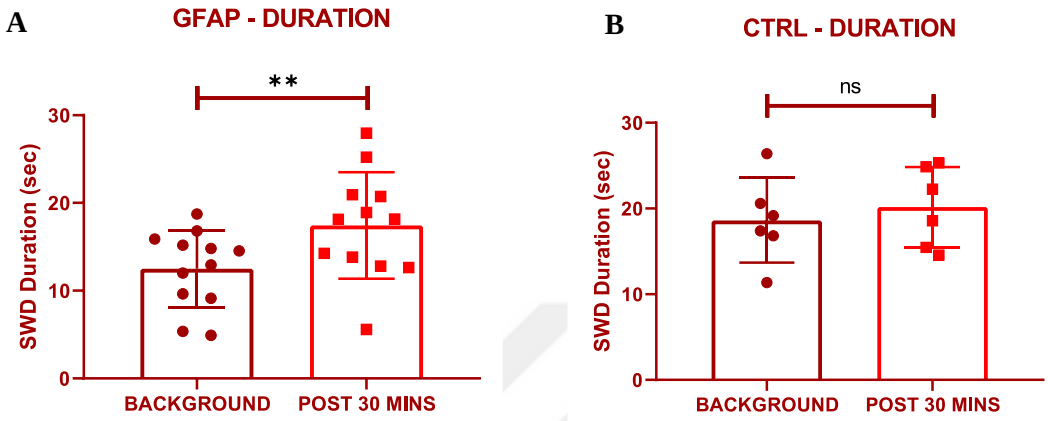


Figure 36. Average duration of SWDs in AAV-GFAP injected GAERS (a) and control rats (b).

While, the number of SWDs in GFAP (28 ± 12 vs 24 ± 9, p=0.1325) and control (21 ± 9 vs 22 ± 8, p=0.9204) virus injected GAERS were not significantly affected compared to their own background level (Figure 37a,37b).

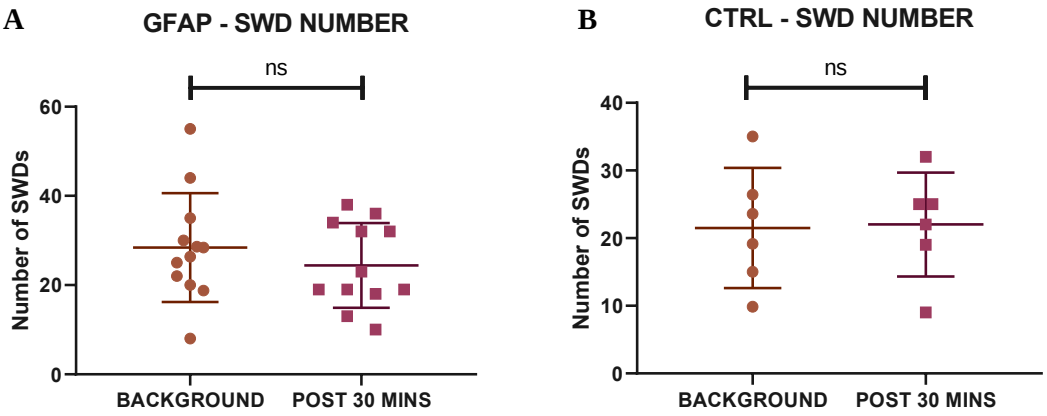


Figure 37. Average number of SWDs in AAV-GFAP injected GAERS (a) and control rats (b).

The distribution of the SWDs regarding the individual duration can be seen by histograms. For GFAP injected GAERS, although both background and post stimulus SWDs were about 5 to 10 secs, majority of the SWD duration was between 5 and 35 secs after optogenetic stimulation while it was between 0 and 25 secs in the background (Figure 38).

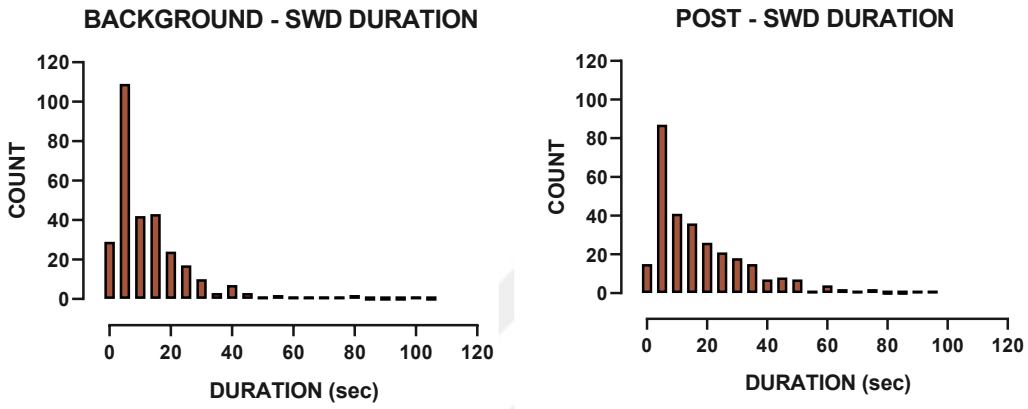


Figure 38. Histograms showing the distribution of the SWDs by their duration in background and post stimulus region.

All of the responses were compared with the prestimulus background SWDs. ** p< 0.01. Error bars are standard deviation. The background region and post stimulus region were 30 minutes long.

4.2.4. EEG from WAG/Rij

SWDs represent 7-11 Hz discharges in GAERS different than human absences which shows 3 Hz slow frequencies. Additionally, WAG-Rij rats present two types of SWDs, SWD type I and II. SWD type I is more generalized and dissipate into entire cortex meanwhile SWD type II is more localized to the occipital area (420). Due to the resemblance of SWD type I to human SWD profile, SWD type I is the type of consideration in our study.

The effect of optogenetic stimulation of the ventrobasal thalamic nucleus on SWD characteristics were investigated similarly in the WAG-Rij rats (Figure 39).

The characteristics of the SWDs in WAG-Rij rats represented below. The shapes of SWDs were more variable within each burst compared to GAERS (Figure 40).

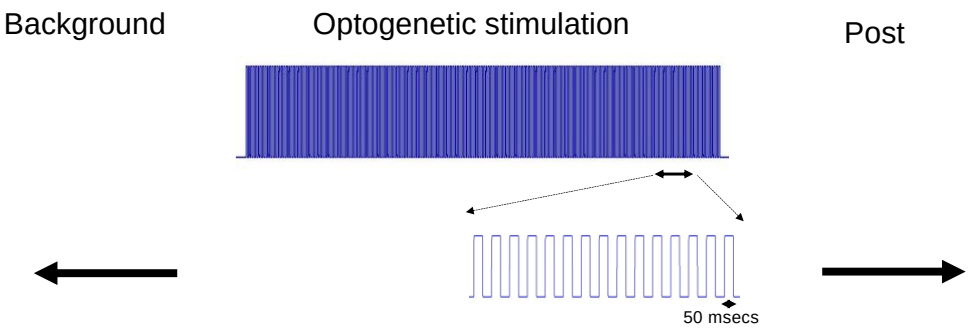


Figure 39. Representation of the optogenetic stimulation pulses.

Optogenetic stimulation did not significantly change the duration of the SWDs in both GFAP injected (paired t-test; 3.59 ± 1.94 secs vs 3.47 ± 2.07 secs (mean ± SD), p=0.6202) WAG-Rij rats (Figure 41a), and control rats (paired t-test; 3.81 ± 1.37 secs vs 3.46 ± 1.21 secs (mean ± SD), p=0.4461) (Figure 41b), in contrast to GAERS.

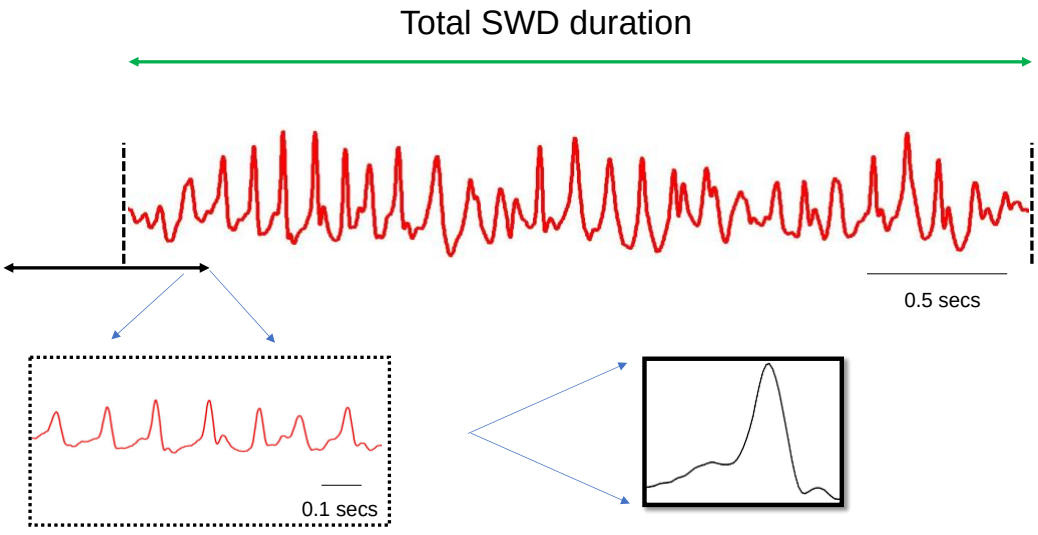


Figure 40. A sample recording of a continuous SWD.

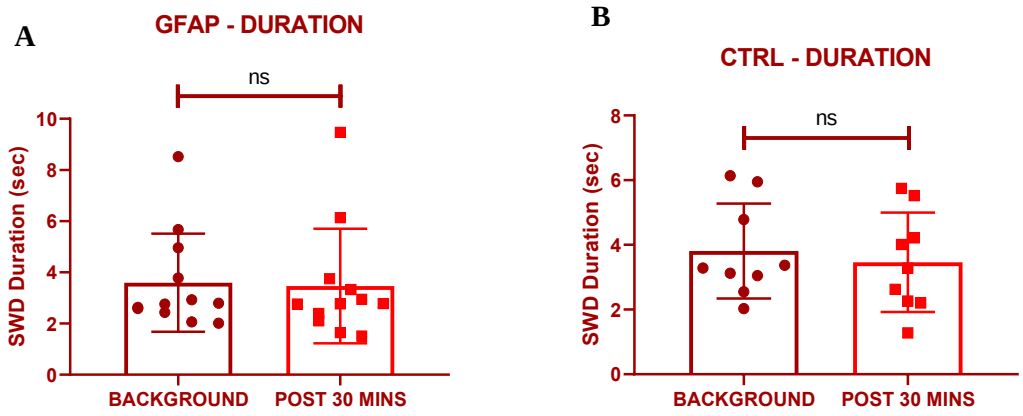


Figure 41. Average duration of SWDs in AAV-GFAP injected WAG-Rij rats (a) and control rats (b).

However, ventrobasal nucleus stimulation significantly increased the number of SWD bursts in GFAP injected WAG-Rij rats compared to background (paired t-test; 18.52 ± 11.46 bursts/30 mins vs 30.17 ± 18.43 bursts/30 mins (mean ± SD), p=0.0015), SWD number were not changed in the control virus injected WAG-Rij group (paired t-test; 16.17 ± 10.77 bursts/30 mins vs 13.67 ± 7.86 bursts/30 mins (mean ± SD), p=0.2813) (Figure 42a,42b).

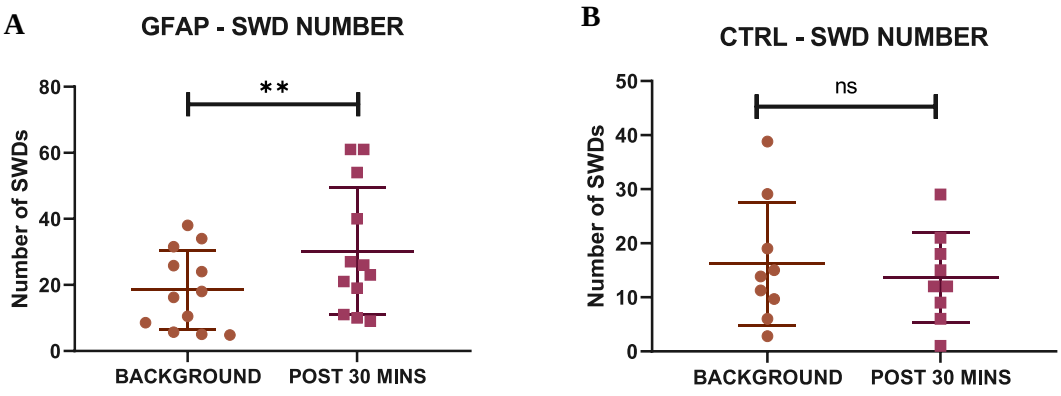


Figure 42. Average number of SWDs in AAV-GFAP injected WAG-Rij rats (a) and control rats (b)

These SWD bursts (regarding the individual duration) had similar duration in background and post stimulus region with the latter had slightly but insignificantly longer duration. The majority of SWDs were ranging between 1-3 secs in background while it was between 1-4 secs in post stimulus region (Figure 43).

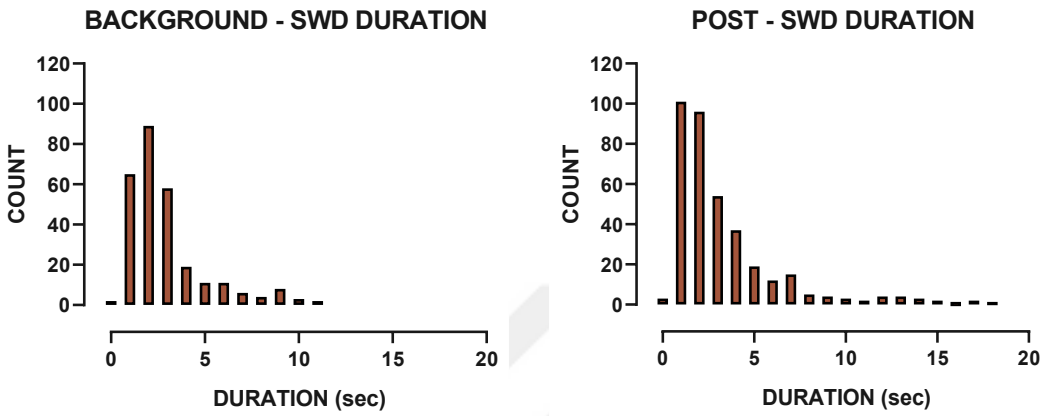


Figure 43. Histograms (with 1 sec bin width) showing the distribution of SWDs by their duration in background and post stimulus region. ** $p<0.01$. Error bars are standard deviation. The background and post stimulus duration were 30 minutes

4.2.5. Comparison of GAERS vs WAG-Rij

In addition to various characteristics of SWDs in both rat stains, the discharge rate of the SWDs was calculated and compared.

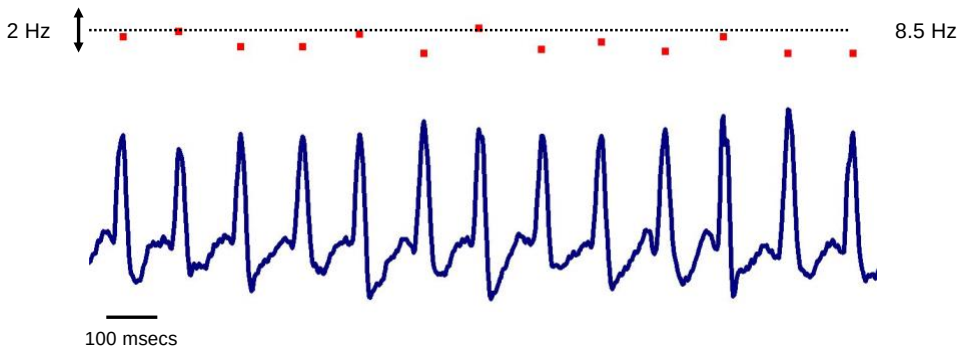


Figure 44. A sample figure showing the instantaneous discharge rate of the SWDs in GAERS (red dots in peer trace) and SWD recording.

After detection of the instantaneous discharge rate in randomly selected 3 SWDs from each animals (3 pre- and 3 post stimulus SWDs) (Figure 44), we did not observe any change in discharge rate before and after the optogenetic stimulation not only in GAERS (background: 6.77 ± 0.42 Hz (mean ± SD), post stimulus: 6.80 ± 0.43 Hz (mean ± SD), paired t-test; p=0.8179) (Figure 45) but also in WAG-Rij rats (background: 7.80 ± 0.60 Hz (mean ± SD), post stimulus: 7.90 ± 0.72 Hz (mean ± SD), paired t-test; p=0.6614) (Figure 46).

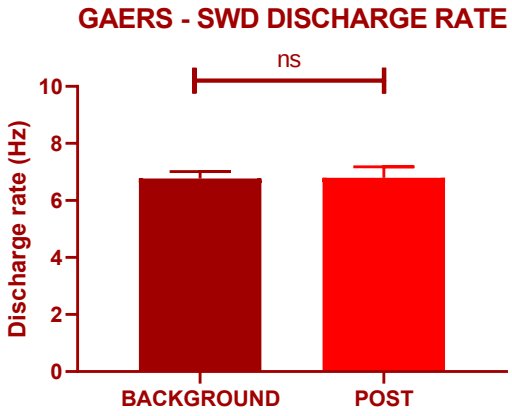


Figure 45. The difference between the background and post stimulus SWD discharge rate in GAERS is illustrated.

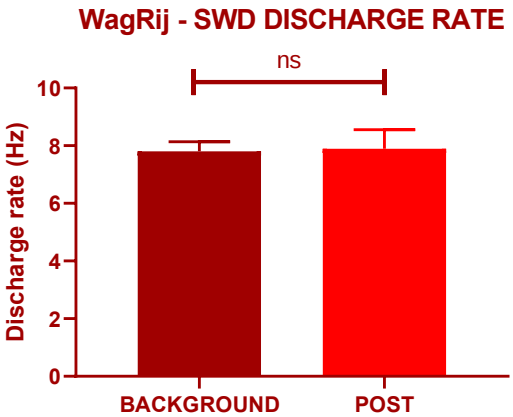


Figure 46. The difference between the background and post stimulus SWD discharge rate in WAG-Rij is illustrated.

However, both background (Mann-Whitney test; p<0.0001) (Figure 47) and post stimulus (Mann-Whitney test; p<0.0001) (Figure 48) average discharge rate of individual spike and wave-discharges in WAG-Rij rats were significantly longer than the discharge rate recorded in GAERS.

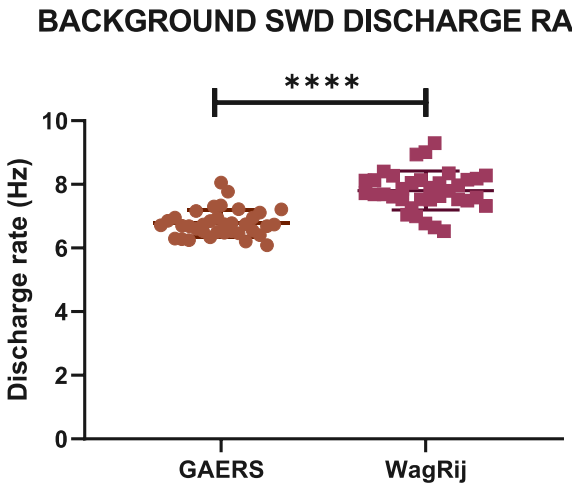


Figure 47. Comparison of the background discharge rate of SWDs between GAERS and WAG-Rij.

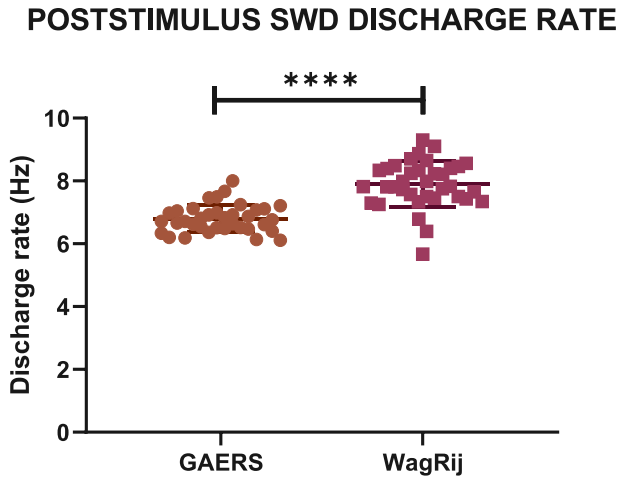


Figure 48. Comparison of the post stimulus discharge rate of SWDs between GAERS and WAG-Rij. **** p< 0.0001. Error bars are standard deviation.

4.2.6. Discussion

The excitation of astrocytes in VB thalamus was studied to understand the roles of glial cells in the occurrence of SWD in typical absence epilepsy. We aimed to manipulate these cells by optogenetic technique with the help of specific astrocytic marker and simultaneously measure the pathological cortical activity represented as spike-and-wave discharges.

We successfully introduced optogenetic rhodopsin targeting astrocytes into ventrobasal thalamic nuclei and subsequently recorded from and directed light into this subcortical structure in a freely behaving animal. Exciting activity in VB exacerbated the occurrence of SWD in two genetic epileptic animal models.

In our study, when thalamic astrocytes were excited optogenetically, SWD duration was significantly increased without causing any effecting on the SWD number in GAERS. The distribution of the SWD in terms of duration changed from 5-10 sec to 5-35 sec after optogenetics stimulation. On the other hand, optogenetic manipulation of astrocytes did not cause a significant change in the duration of SWD while resulting a profound increase in the number of SWD burst in WAG-Rij rats (from 18.52 ± 11.46 burst/30 mins at prestimulation to 30.17 ±18.43 bursts/30 mins at poststimulation).

This result suggests that seizure activity is correlated with excitatory transmission within VB and subsequent propagation of the cerebral cortex. Our results provided that manipulation of astrocytes with light which drive the excitation has a profound influence on SWD propagation/dynamics with a different effect SWD characteristics in GAERS and WAG-Rij rats. The EEG reveals that astrocytes excitation cause a significant pattern difference between two above mentioned strains.

In epilepsy, the loss of organization of astrocytic domains (290), cellular neuropathology of astrocytes (404) has been shown in absence epilepsy studies using genetic rat models. The functional consequences of astrocyte manipulation might be related with the formation, function and expression of gap junctions, ion channels, receptors, and transporters. In fact, in WAG-Rij model of absence epilepsy, cortical pyramidal neurons show several abnormalities in respect to dendritic arborization, branching and orientation (408) for which astrocyte plays a pivotal role both in

development and in the mature state modified via plastic changes. Since astrocytes, rather than neurons, are considered to be the responsible elements for homeostatic balance, epilepsy could be related with the improper functioning of astrocytes (19). The present study correlates with this notion and provides that astrocyte manipulation has a functional effect on the occurrence of epileptic discharges.

Glia would not only have a role in information processing in the brain or in the generation of animal behavior. Evidence for neurons communicating with glia is solid, however signaling pathway leading back from glial-to-neuronal activity was often difficult to study. Selective stimulation of astrocytes in vivo triggered neuronal activation. Glial photo stimulation leads to release of glutamate which was sufficient to activate receptors in thalamo-cortical neurons which then eventually lead to increase the seizure provocation (425).

Although it has long been believed that neurotransmitters released from neurons is the main signaling pathway between neurons and glia, if was first shown by Parpura et al., that astrocyte provides an additional pathway via the release of glutamate from their vesicles which then causes an NMDA receptor-mediated increase in neuronal activity in the neighboring neurons (406). In the literature, several studies have confirmed that the astrocyte-mediated neuronal activity (293,295,348,410). The result of the present study is in correlation with the notion of astrocytic modulation of the neuronal activation, as a result of this activation in their soma thalamo-cortical neurons might increase their glutamate release in their cortical target leading to excessive spike-wave discharges observed in EEG.

The direct cooperation of astrocyte and neurons in supporting ictal and interictal epileptiform events has been studied in an in-vitro model of focal seizure induced by NMDA application (405). It was found that Ca^{2+} elevation in astrocytes is highly correlated with initiation and maintenance of a focal, seizure-like discharges. Additional support came from the study of Tian (407), it was shown that paroxysmal depolarization shifts that function as the driving mechanisms of firing of group of neurons into synchronous bursting, can be initiated by the glutamate release originating from extrasynaptic sources or by the photolysis of stored Ca^{2+} in astrocytes.

In fact, this Ca^{2+}-dependent astrocytic glutamate release takes place through volume-sensitive channels expressed in the astrocyte's membranes.

However, in a transgenic animal expressing the Gq-coupled metabotropic receptor only in astrocytes, researchers showed that wide-spread Ca^{2+} elevations in astrocytes do not either increase neuronal Ca^{2+} elevations or produce slow inward currents (409). However, authors speculated that the results of their study might be related with the specific technique they used to stimulate the astrocytes. The astrocytic release of glutamate could be influenced by the speed of the synchronized intracellular Ca^{2+} wave or the fast and repetitive pattern of Ca^{2+} elevations. In fact, Chen et al., (411) provided the concept of ''kiss-and-run'' style of glutamate release as a result of Ca^{2+} mobilization lead by agonist administration following mechanical stimulation. This type of vesicle release is fast but with an incomplete demise of the vesicular content. Therefore, the choice of technique for astrocyte stimulation might not yield a necessary stimulation of astrocytes needed for efficient Ca^{2+} elevations in their study.

The discrepancy was untied with the discovery of new mechanisms described below (figure 49). It is revealed that the most significant calcium transients occur in the fine processes of astrocytes that was not discovered earlier as well as new mechanisms by which astrocyte $[Ca^{2+}]i$ is increased and manifests its effects (421). Indeed, different astrocyte processes produce $[Ca^{2+}]i$ transients at different times (422), and spatially localized $[Ca^{2+}]i$ transients occur more frequently in the astrocyte processes than their soma (423). The release of GABA and glutamate can be achieved from astrocytes via Ca^{2+}-activated bestrophin-1 anion channels and K^{+}-selective two-pore domain channels (424). However, it has been proposed that $[Ca^{2+}]$ changes in astrocytes affects neurons not by releasing neurotransmitters rather via changes the activity of transporters located at the astrocyte membrane (421). As a consequence of increase $[Ca^{2+}]$ in astrocytes, G_q-coupled mGluRs increase their glutamate uptake (426) and the insertion of GLAST glutamate transporters onto their membranes (427), leading to a decrease effect of glutamate on nearby neurons (427). In the case of TRPA1 channel-mediated $[Ca^{2+}]$ rise in astrocytes, the insertion of GABA transporters into the membrane of astrocyte is facilitated therefore leading to a regulation of GABAergic effect on nearby neurons without any astrocytic release of GABA (428).

Additionally, increases in astrocytic [Ca^{2+}]i can raise enough [Na^{+}]i to increase sodium pump activity, which then results a decrease in the extracellular K^{+} concentration and local hyperpolarization of neurons (425). This lower spontaneous excitatory synaptic activity but also reduces any loss of action potential triggered excitatory synaptic transmission.

A gliotransmission is therefore influenced by mainly modifications in the activity or expression of membrane transporters and also from the changes in the amplitude of synaptic currents which is correlated with Ca^{2+}-driven changes of neurotransmitter transporter level in the astrocyte membrane (426). The mechanism of neurotransmission is way complicated, therefore the mechanism of how optogenetic manipulation affected the SWDs is needed to be explored at the molecular level.

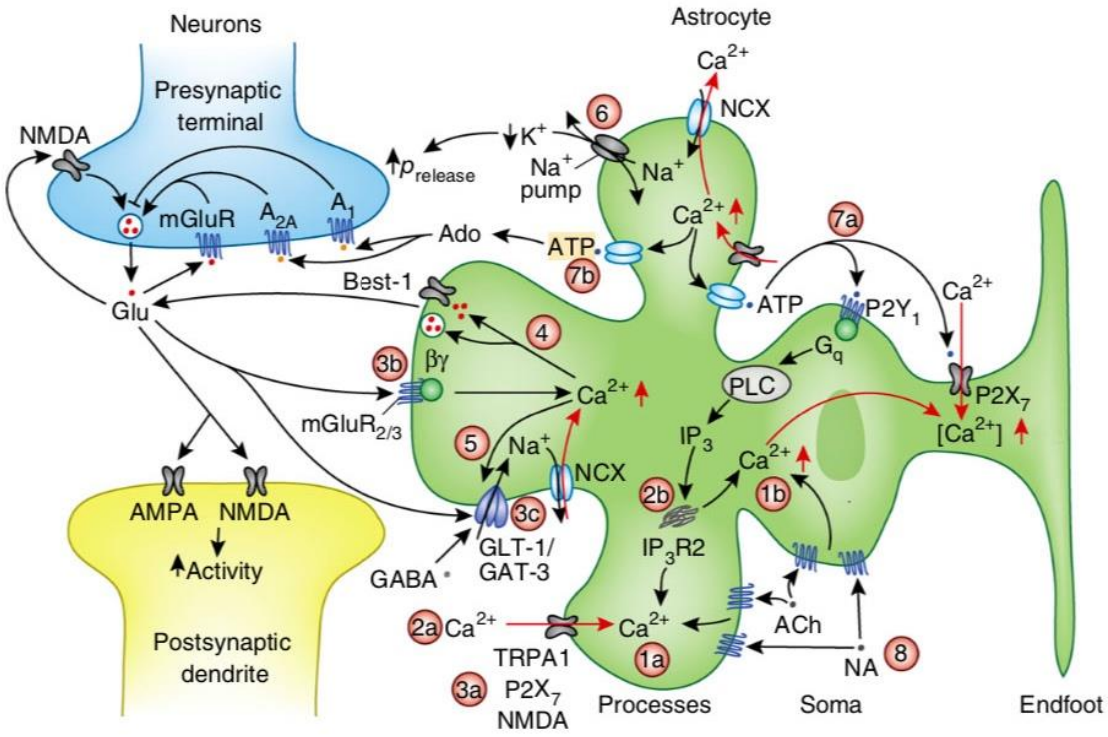


Figure 49. The third wave astrocyte role in Ca^{2+} signaling. 1. The frequency, kinetics and spatial spread of [Ca^{2+}]i transients in astrocytes processes (1a) differ from those in the soma (1b). 2. [Ca^{2+}]i transients in the processes depend on the Ca^{2+} entry from extracellular space (2a) by way of ion channels (40%) and on Ca^{2+} release from intracellular stores (60%), on the other hand [Ca^{2+}]i transients in the soma (2b) primarily depend on Ca^{2+} release from intracellular stores. 3. [Ca^{2+}]i transients can be

produced by [Ca^{2+}] entry via spontaneously opening TRPA1 channels or neurotransmitter-gated channels (3a), by mGluR2 or mGluR3 (3b), and by neurotransmitter uptake raising [Na^{+}]i and reversing Na^{+}/Ca^{2+} exchange (3c). 4. [Ca^{2+}]i rises may release transmitters through ion channels like Best-1, or via exocytosis. 5. [Ca^{2+}]i rises alter the surface expression of neurotransmitter transporters. 6. Activation of Na^{+}/Ca^{2+} exchange by a [Ca^{2+}]i rise can raise [Na^{+}]i and activate the sodium pump, lowering [K^{+}]o and hyperpolarizing nearby neurons. This increases the release probability ($p_{release}$) for action potential–driven vesicle release and thus decreases synaptic failure rate. 7. ATP released by a [Ca^{2+}]i rise may act on P2X or P2Y receptors to raise [Ca^{2+}]i farther along the cell, propagating a Ca^{2+} wave along the cell (7a), or be converted to adenosine, which acts on presynaptic receptors to increase (A2A) or decrease (A1) transmitter release (7b). 8. Noradrenaline (NA) released from locus coeruleus neurons and acetylcholine (ACh) released from nucleus basalis neurons produce large [Ca^{2+}]i rises in astrocytes.

Although original thought that astrocytes could well be segregated by molecular markers, primarily by the expression of intermediate filament protein glial fibrillary acidic protein - GFAP, the heterogeneous expression of GFAP among astrocyte types dissuaded this notion. For instance, GFAP expression is more pronounced in fibrous astrocytes than protoplasmic astrocytes with a great heterogeneity between protoplasmic astrocytes in different brain regions.

Identification of classical astrocytes is suggested to involve the criteria including absence of electrical excitability; very negative membrane potential (-80 to -90 mV); expression of neurotransmitter transporters (GABA, glutamate); expression of intermediate filaments (particularly GFAP); glycogen granules; processes contacting blood vessels; perisynaptic processes; connection to other astrocytes by gap junctions (391,392). There are exceptions to these criteria as well such as the existence of : (1) GFAP-negative protoplasmic astrocytes; (2) GFAP-positive cells that do not form gap junctions; (3) ‘’GluR’’ cells that express neurotransmitter receptors but not transporters (393,394) and (4) cells that may/may not send projections to distinct areas/boundaries like blood vessels, pia etc. There are several reports showing the high level of astrocytes targeting for GFAP transgenes using adenovirus (395), showing the

high level of astrocyte targeting for GFAP transgenes using adenovirus (395), herpes simplex virus (396) and lentivirus (397). Therefore, it become so important to specify the astrocyte types and be able to manipulate them exclusively.

With the advent of molecular biology, several researchers have achieved the specific manipulation of the cells of their interest including astrocytes. Several studies have achieved to target astrocytes by optogenetic manipulations (243,405,412,414,415,416,417,418). In our study, virus was mostly expressed in astrocytes in thalamus, in addition to neuronal expression of GFAP-AAV in some injections as parallel with previous studies of the same transgene used where the astrocyte transduction efficiency was calculated as 88% for astrocytes (413,414). The transduction profile is dependent on the titer and brain region injected and the neuronal expression could be due to other promoters present in the viral vector (figure 15), this promoter might well override the GFAP promoter specificity. Even under this conditions, the effects of astrocyte stimulation cannot be undermined, in a study of Tian et al., when neurons were stimulated as the same method with astrocytes, there was no production of field potential while astrocyte stimulation could trigger Ca^{2+}-dependent glutamate release that resulted in paroxysmal discharge shifts in neighboring neurons (407).

The different characteristics of naturally occurred SWD in two genetic models of absence epilepsy, GAERS and WAG-Rij rats, have been examined by Akman et al. (419). In general, the WAG-Rij rats generate faster SWDs than GAERS, our study is highly correlated with this knowledge, and reveals that WAG-Rij rats display SWD mostly in between 1-3 sec while SWDs in GAERS were hang between 5-10 sec. Besides, the number, cumulative total duration and mean duration of SWDs were significantly higher in GAERS compared to WAG-Rij, on the other hand WAG-Rij rats show more elevated discharge frequency (419). The results of the present study were also revealed different response characteristics of SWDs following optogenetic manipulation in these two genetic models. As a result of manipulation, SWD duration was increased in GAERS, while the number of SWDs was the affected parameter in WAG-Rij. The difference in SWDs features in these two strains might be correlated with relative activation of different GABA receptor in the thalamo-cortical cells (419). Since it was earlier shown that $GABA_A$ and $GABA_B$ receptors are employed

differentially as a consequence of corticothalamic input (420), the role of these receptors become important in the generation of oscillation frequencies when their role in different types of oscillations are considered as longer lasting IPSPs are mediated by $GABA_B$ receptors and short lasting IPSPs are set by $GABA_A$ receptors. These differences might be related with the underlying pathological mechanism of these two different strains and suggest that it should be taken into consideration when two strains are studied. It remains to be seen what kind of SWD responses can be observed with the manipulation of specific astrocytes and the resulting molecular changes in terms neurotransmitter turnover and transmission need to be investigated.

In summary, the work described above has highlighted the astrocytes importance in contributing to the pathophysiology of typical absence epilepsy. In particular, novel substances related with astrocytes that can either decrease gap-junction communications negatively, increase GAT-1 function or reduce the activity of extrasynaptic $GABA_A$ receptors may prove to be useful in the pharmacological treatment of absence seizures. All the current drugs are developed to affect neurons. However, the connection between astrocytes and neurons has been ignored. Increase interest and intense study on astrocytes in absence epilepsy may well be pave the way for the development of astrocyte-related therapeutic interventions. Then therapeutic intervention should be aimed at restoring homeostasis in astrocytes, putatively more astrocyte specific. The advantage of these therapeutic interventions would be the sparing of any side-effect of the inhibition of neuronal components and synaptic transmission, as in the case of antiepileptic drug.

CHAPTER 5

5. CONCLUSION AND FUTURE DIRECTIONS

It is of unique opportunity to use the optogenetic stimulation of thalamus in conjunction with electrophysiological recordings in thalamus because it does not introduce the same electrical artifact caused by thalamic microstimulation. Using depolarizing opsins, we achieved to control the relative depolarization of the thalamus to directly investigate the effect of thalamic state on neural activity propagation in the thalamocortical circuit.

Across nearly all sensory modalities, the thalamus is a common stage of processing that links the peripheral sensory information to the cortex. Before reaching the thalamus, sensory information is transduced at the receptor and processed by a diverse set of prethalamic circuits. Therefore, the observations of thalamic and cortical activity always confounded by prethalamic dynamics, making it difficult to established what happens where. This obstacle can be readily overcome by specifically targeting the area and cell type of interest with optogenetic techniques.

It is suggested for years that the distinct firing modes of thalamus, known as burst and tonic firing modes, present a mechanism to differentially transmit information to cortex. Before the development of optogenetic tools, the primary method to control thalamocortical state was through the activation of the natural neuromodulation arousal mechanisms in the brain. However, with the advent of optogenetics, it has become possible to quickly and easily shift the thalamocortical state locally and bidirectionally. Here, we tested the role of astrocytes of VB thalamus in the propagation of neural activity as SW discharged in frontal cortex in response to thalamic stimulation during the tonic and burst firing modes of the thalamus.

Since optogenetics is technically demanding, there is a lot of important steps to consider before starting the experiments. These practical hurdles include: determining the optimal methods to achieve opsin expression, the wavelength, intensity and duration of incident light, and even recent illumination history (If fewer channelrhodopsin molecules begin in or have returned to the dark-adapted state, the initial transient response to a light pulse will be smaller, through the steady-state photocurrent may remain the same. Nonspecific effects of opsin expression and light

delivery is a possible outcome. These parameters should carefully be evaluated before starting experiments.

Through the use of optogenetics, recent work has demonstrated that the artificial hyperpolarization or depolarization of the thalamus is sufficient to drive cortex into the synchronized (399) or desynchronized (400) state, and yet the effect of astrocytes in this process was shown by our work.

Optogenetics offer a promising strategy to control activity od several cell types. The manipulation of astrocytes offers a unique therapeutic avenue with a real time control. However, much remains to be developed before any clinical application. There is a huge need to identify specific promoter for each subpopulation of astrocytes.

Astrocytes could be subdivided into at least two types: passive astrocytes and complex astrocytes. The passive astrocytes express glutamate transporters and display glutamate transporter currents; while complex astrocytes contain ionotropic glutamate receptors (Glu R) and exhibit AMPA receptor activity. Furthermore, even passive astrocytes could be divided into GLAST and GLT-1 subpopulations that do not overlap (401). It should be true that more subpopulations will be identified with the efforts of molecular scientists in the near future. The main advantage of optogenetics is that channels can be targeted to specific cell types and manipulate the activity of individual cells under the control of specific promoters. However, the specific promoters for subpopulation astrocytes remain undeveloped. The different subpopulations of astrocytes could play different roles in the neural network. Thus, identifying specific promoters for subtypes of astrocyte deserves the efforts of further studying the mechanisms and treatment of astrocyte- related epilepsy.

Second, it is important to improve the properties of optogenetic actuators. One possible risk in optogenetic treatments is heating damage of brain tissue by the light illuminating the optogenetic actuator. There are two ways to reduce this risk. The first way is to improve the membrane expression of optogenetic actuators or the photocurrents of individual optogenetic actuator. Indeed, efforts have already been made on this. For example, Yawo's group engineered a mutation of ChR2, ChR2-WR, which significantly increased the membrane expression and photocurrents of individual ChR2 (238). Thus, the photocurrents could be evoked by lower light power

density. The other way is to develop new variants or identify new optogenetic actuators that are red shifted. Red light more easily penetrates the brain tissue, due to lower absorption by hemoglobin, myoglobin and lipids and scatters less than blue light (402). Therefore, one can get the same effects with lower light power. There is ongoing progress on this, as well. For instance, Lin et al. developed ReaChR (red-activatable ChR), a variant of ChR which could be activated by light with wavelengths up to 655 nm (401). More recently, jaws derived from *H. salinarum* strain of shark, was demonstrated to be activated by the most red-shifted spectrum of any hyperpolarizing rhodopsin so far (402).

Thirdly, studies with non-human primates are necessary. There are at least two significant differences between rodents and humans: the brain size and life span. To date, most studies are performed by using rodents. Due to the difference in brain size between the rodents and humans, one has to consider the different volume of virus for targeting optogenetic actuator and the different light power density. Optogenetics is safety for rodents; however, the side effects for the much longer life span of humans remain untested. Although there is a long way until clinical application, optogenetics offers a new method to investigate the communication between astrocytes and neurons and an alternative strategy for the treatment of epilepsy.

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