THE ANXIETY EFFECT OF A GENERALIZED EPILEPSY RAT MODEL TREATED WITH ZOLPIDEM

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by

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LIST OF SYMBOLS AND ABBREVIATIONS

%	percentage
α	alpha
β	beta
γ	gamma
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	analysis of variance
ARASC	Animal Research and Service Centre
BAI	Beck anxiety inventory
BWG%	body weight gain percentage
℃	degree Celsius
CACNA1A	calcium voltage-gated channel subunit alpha-1 A
cm	centimetre
CO_2	carbon dioxide
CRL	central research laboratory
CT	computed tomography
DG	dentate gyrus
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
EEG	electroencephalography
ELISA	enzyme-linked immunosorbent assay
EPM	elevated plus maze
EPSP	excitatory post-synaptic potential
FER	feed efficiency ratio
fMRI	functional magnetic resonance imaging
g	gram
GABA	gamma-aminobutyric acid

$GABA_A$	gamma-aminobutyric acid type A
GAD	generalized anxiety disorder
Gal4	Galactose metabolism regulatory gene 4
GCD	granule cell dispersion
GFAP	glial fibrillary acidic protein
GluK	glutamate ionotropic receptor kainate type subunit
GWAS	genome-wide association study
H&E	Haematoxylin and eosin
HAM-A	Hamilton anxiety rating scale
hGAT-1	human gamma-aminobutyric acid transporter 1
HPA	hypothalamic-pituitary axis
i.p.	intraperitoneal
IACUC	Institutional Animal Care and Use Committee
ILAE	International League Against Epilepsy
iPSC	induced pluripotent stem cell
KA	kainic acid
KAR	kainate receptor
KCNQ2	potassium voltage-gated channel subfamily Q member 2
KCNQ3	potassium voltage-gated channel subfamily Q member 3
L	litre
M	molar
mL	millilitre
mM	millimolar
mol	mole
N	sample size
n	number of animals per group
NaCl	sodium chloride

NMDA	N-methyl-D-aspartate
OCD	obsessive-compulsive disorder
OFT	open field test
OHSC	organotypic hippocampal slice culture
OSC	organotypic slice cultures
p	p-value
PET	positron emission tomography
pH	potential of hydrogen
PTSD	post-traumatic stress disorder
PTZ	pentylenetetrazol
RNAi	RNA interference
RT-qPCR	reverse transcription quantitative polymerase chain reaction
SCN1A	sodium voltage-gated channel alpha subunit 1
SCN2A	sodium voltage-gated channel alpha subunit 2
SD	Sprague Dawley
SE	status epilepticus
SEM	standard error mean
SPECT	single photon emission computed tomography
STAI	state-trait anxiety inventory
SWS	slow-wave sleep
UAS	upstream activation sequence
USM	Universiti Sains Malaysia
w/v	weight per volume
ZOL	zolpidem

LIST OF APPENDICES

KESAN GANGGUAN KEBIMBANGAN DALAM MODEL TIKUS EPILEPSI MENYELURUH YANG DIBERIKAN RAWATAN ZOLPIDEM

ABSTRAK

Epilepsi menyeluruh (*generalized epilepsy*) dicirikan oleh sawan berulang yang melibatkan kedua-dua hemisfera otak, sering dikaitkan dengan gangguan kebimbangan (*anxiety*) yang menjejaskan kualiti hidup pesakit. Kajian ini menilai kesan anxiolitik zolpidem, modulator reseptor $GABA_A$, dalam model tikus epilepsi menyeluruh yang diaruhkan oleh asid kainik (KA). Zolpidem dihipotesiskan dapat mengurangkan kebimbangan dengan meningkatkan fungsi GABAergik melalui subunit α_2 pada reseptor $GABA_A$. Sebanyak 24 ekor tikus jantan Sprague-Dawley (SD) dibahagikan kepada enam kumpulan: Sham, KA sahaja, KA + 1mg/kg zolpidem, KA + 3mg/kg zolpidem, 1mg/kg zolpidem sahaja, dan 3mg/kg zolpidem sahaja. Epilepsi menyeluruh diaruhkan melalui suntikan intraperitoneal (i.p.) KA pada dos 7.5mg/kg hingga 15mg/kg mengikut keperluan. Zolpidem diberikan secara oral sekali sehari selama 14 hari berturut-turut. Tingkah laku kebimbangan dinilai menggunakan ujian lapangan terbuka (*open field test*, OFT) sebelum dan selepas rawatan zolpidem, manakala parameter fisiologi seperti berat badan, pengambilan makanan dan air dipantau setiap hari. Analisis histopatologi hipokampus dilakukan dengan pewarnaan haematoxylin dan eosin (H&E) bagi menilai perubahan morfologi, termasuk kehilangan neuron dan gangguan struktur tisu yang berkait dengan patologi kebimbangan akibat epilepsi meyeluruh, serta menilai potensi kesan neuroprotektif zolpidem. Hasil menunjukkan suntikan KA berjaya menginduksi epilepsi menyeluruh, dengan semua tikus mencapai skor tahap 4 berdasarkan skala *Modified Racine*. Dalam OFT, kumpulan KA + 1mg/kg zolpidem menunjukkan peningkatan signifikan dalam kekerapan memasuki zon tengah selepas intervensi berbanding sebelum intervensi ($p < 0.01$), mencadangkan potensi

kesan anxiolitik pada dos ini. Sebaliknya, kumpulan KA + 3mg/kg zolpidem turut menunjukkan peningkatan signifikan ($p < 0.05$), namun kesannya adalah kurang ketara berbanding kumpulan dos rendah, menunjukkan keberkesanan yang lebih rendah pada dos yang lebih tinggi dalam konteks epilepsi. Manakala, kumpulan zolpidem 3mg/kg sahaja menunjukkan penurunan dalam kekerapan memasuki zon tengah dan tempoh berada di zon tengah selepas intervensi (kedua-duanya $p < 0.01$), mencadangkan kemungkinan kesan anxiogenik pada dos tinggi dalam konteks bukan epilepsi. Walau bagaimanapun, jumlah jarak pergerakan tidak menunjukkan sebarang perbezaan signifikan antara kumpulan, menunjukkan bahawa kesan zolpidem lebih tertumpu kepada tingkah laku eksplorasi yang berkaitan dengan kebimbangan, dan bukannya aktiviti motor keseluruhan. Histologi menunjukkan degenerasi neuron dalam hipokampus tikus yang menerima KA, termasuk hipereosinofilia sitoplasma, nukleus piknotik, dan penyebaran sel granula (*granule cell dispersion*). Kumpulan KA + 1mg/kg zolpidem menunjukkan tahap kemerosotan neuron lebih rendah berbanding KA sahaja dan KA + 3mg/kg zolpidem. Namun, kumpulan zolpidem sahaja juga mengalami neurodegenerasi, menunjukkan kemungkinan kesan negatif akibat pendedahan berpanjangan tanpa epilepsi. Secara keseluruhan, zolpidem 1mg/kg berpotensi mengurangkan gangguan kebimbangan dalam epilepsi menyeluruh, tetapi dos lebih tinggi (3mg/kg) mungkin memburukkan status kebimbangan dan meningkatkan neurodegenerasi. Penentuan dos yang tepat adalah penting untuk mengoptimumkan manfaat terapeutik sambil mengurangkan kesan sampingan zolpidem.

THE ANXIETY EFFECT OF A GENERALIZED EPILEPSY RAT MODEL TREATED WITH ZOLPIDEM

ABSTRACT

Generalized epilepsy, characterized by recurrent seizures originating from both cerebral hemispheres, is often associated with anxiety, significantly impacting patients' quality of life. This study investigated the anxiolytic effects of zolpidem, a $GABA_A$ receptor modulator, in a kainic acid (KA)-induced generalized epilepsy rat model. A total of 24 male Sprague-Dawley (SD) rats were assigned to six groups: Sham, KA only, KA + 1mg/kg zolpidem, KA + 3mg/kg zolpidem, 1mg/kg zolpidem only, and 3mg/kg zolpidem only. Generalized epilepsy induced through intraperitoneal (i.p.) administration of KA (7.5mg/kg to 15mg/kg, as needed). Zolpidem was administered orally at a daily dose for 14 consecutive days. Anxiety-related behaviours were assessed using the open-field test (OFT) before and after zolpidem treatment, while body weight, feed intake, and water consumption were monitored daily. Histopathological analysis of the hippocampus was performed using haematoxylin and eosin (H&E) staining to assess morphological alterations, including neuronal loss and disruption of tissue architecture, associated with anxiety-related pathology in kainic acid-induced generalized epilepsy, and to evaluate the potential neuroprotective effects of zolpidem treatment. KA injection successfully induced generalised epilepsy, with all KA-injected rats reaching a seizure severity score of 4 on the Modified Racine Scale. In the OFT, central zone entries significantly increased ($p < 0.01$) following intervention in the KA + 1mg/kg ZOL group, indicating a potential anxiolytic effect. The KA + 3mg/kg zolpidem group also showed a significant increase ($p < 0.05$), but the effect was less pronounced than at the lower dose. In contrast, the 3mg/kg zolpidem-only group exhibited reduced entries and time spent in the central zone post-intervention (both

showing $p < 0.01$), suggesting a potential anxiogenic effect at higher doses in non-epileptic animals. However, total distance travelled showed no significant differences across groups, indicating zolpidem's effects were more specific to anxiety-related exploratory behaviour than general locomotion. These behavioural findings align with cage-side observations, which associated KA-induced generalized epilepsy with anxiety-like behaviour. No significant differences in body weight, feed efficiency, or water consumption were observed across groups. Histological analysis revealed characteristic neuronal damage in the hippocampus of KA-treated rats, including hypereosinophilia of the cytoplasm, pyknotic nuclei, and granule cell dispersion (GCD). Notably, the KA + 1mg/kg zolpidem group exhibited reduced neuronal damage compared to the KA only and KA + 3mg/kg zolpidem groups. Paradoxically, both zolpidem-only groups also displayed neurodegenerative changes, suggesting potential adverse effects of prolonged zolpidem exposure in the absence of epilepsy. These findings suggest that zolpidem at 1mg/kg reduces anxiety in generalized epilepsy, potentially mitigating the epilepsy-associated psychiatric comorbidity. However, a higher dose of 3mg/kg may exacerbate anxiety and neuronal damage, highlighting the importance of precise dosing to optimize therapeutic outcomes while minimizing adverse effects.

CHAPTER 1

INTRODUCTION

1.1 Study Background

Epilepsy, a complex neurological condition marked by recurrent seizures, represents sudden and uncontrolled electrical disturbances within the brain (Akyüz *et al.*, 2021). Presenting in various forms, seizures can be focal, affecting specific regions which are limited to part of one cerebral hemisphere (Lu & Triesch, 2019), or generalized, involving the entire brain (Li *et al.*, 2020). Focal seizures induce subtle sensations like tingling or minor muscle twitches, while generalized seizures trigger dramatic full-body convulsions and loss of consciousness (Ighodaro *et al.*, 2023). Furthermore, the seizures are often accompanied by sensory disturbances including visual or auditory hallucinations and changes in the perception of time and space (Jaballah *et al.*, 2022). The impact of the mentioned physical risks, along with epilepsy's unpredictable nature can lead to a constant state of anxiety, a significant comorbidity of the condition (Sehlo *et al.*, 2022).

Anxiety is a mental health condition which significantly impairs the quality of life in individuals with epilepsy (Hingray *et al.*, 2019). It manifests as excessive worry and fear (Rauh *et al.*, 2022), leading to disruptions in daily life marked by restlessness, irritability, muscle tension, and difficulty concentrating (DeMartini *et al.*, 2019). The physical symptoms include rapid heartbeat (Li *et al.*, 2020), shortness of breath (Szuhany & Simon, 2022), and an elevated sense of panic (Salpekar *et al.*, 2020). Moreover, the increased levels of cortisol, the stress hormone associated with anxiety, have been linked to increased risk of cardiovascular diseases, hypertension and

dysregulation of immune functions (Vinkers *et al.*, 2021). Considering these outcomes, it is apparent that managing anxiety in individuals with generalized epilepsy is crucial to alleviate its wide-ranging impact on their quality of life.

Based on a clinical trial conducted by Chen *et al.* (2017), it has been found that zolpidem not only improves insomnia but also enhances seizure control in patients with epilepsy. This observation aligns with previous research conducted in rat models, which supports the concept that zolpidem has anticonvulsant properties, reducing both the frequency and severity of seizures (Sheikhi *et al.*, 2016; Vlainić & Peričić, 2010; Peričić *et al.*, 2008). Although the precise mechanism underlying this effect is not fully understood, it is found that zolpidem's action on gamma-aminobutyric acid type A ($GABA_A$) receptors contributes to its antiepileptic activity (Neumann *et al.*, 2019). By enhancing GABA-mediated inhibition, zolpidem reduces neuronal excitability, preventing the abnormal electrical activity that leads to seizures (Sheikhi *et al.*, 2016). Anxiety is often comorbid with epilepsy, and zolpidem's ability to enhance GABA-mediated inhibition may help alleviate symptoms of anxiety (Wisden *et al.*, 2017). However, the use of zolpidem in especially generalized epilepsy, and its effects on anxiety symptoms remain underexplored.

Rats are widely used in epilepsy research due to their susceptibility to experimentally induced seizures, which can be achieved through various methods such as chemical agents, electrical stimulation, or genetic manipulation (Reddy & Kuruba, 2013). One commonly used method involves inducing generalized epilepsy using kainic acid (KA), which reliably triggers seizures resembling those seen in human epilepsy (Lévesque & Avoli, 2013). Additionally, this particular model in rats allows researchers to study the progression of epilepsy and its associated comorbidities, offering a reliable

approach for testing potential antiepileptic drugs and therapeutic interventions (Rusina *et al.*, 2021).

The proposed study aims to investigate the anxiety effect of a generalized epilepsy rat model treated with zolpidem. By elucidating the impact of zolpidem on anxiety symptoms within the context of generalized epilepsy, this research could provide valuable insights into the potential of zolpidem as a treatment option not only for seizure control but also for the psychiatric comorbidities that significantly affect epilepsy patients' quality of life.

1.2 Problem Statement and Study Rationale

Anxiety is a prevalent comorbidity of epilepsy, impacting up to 20.2% of affected individuals (Scott *et al.*, 2017). This emotional distress significantly impairs mental well-being and can lead to disruptions in physical functionality, ultimately reducing the overall quality of life for those living with the condition (Wilmer *et al.*, 2021). Despite its prevalence and impact, the underlying mechanisms linking epilepsy and anxiety remain largely unknown. In contemporary practice, drugs commonly used to manage epilepsy and its associated anxiety along with other comorbidities include carbamazepine (Beydoun *et al.*, 2020), valproate (Li *et al.*, 2019), gabapentin (Ziganshina *et al.*, 2017), pregabalin (Panebianco *et al.*, 2022) and phenobarbital (Trinka, 2023). However, in terms of anxiety, these options are often ineffective or further exacerbate the problem (Miziak *et al.*, 2023; Sáenz-Farret *et al.*, 2022; Besag & Vasey, 2021). This is due to the fact that, for some individuals, the effectiveness of medication tends to wear off over time, either because of the particular way in which their bodies function or because of the nature of their epilepsy and its underlying causes (Zayed, 2023). Given the complex and often bidirectional relationship between epilepsy

and anxiety, there is a pressing need to comprehensively understand the association between these conditions to better guide treatment and improve outcomes for those affected.

The proposed research aims to fill this gap in understanding by investigating the efficacy of zolpidem, a $GABA_A$ receptor modulator, in alleviating anxiety in generalized epilepsy patients. The $GABA_A$ receptor, a primary target of zolpidem, is critically involved in regulating neuronal excitability and neurotransmission in the brain (Sheikhi *et al.*, 2016). This aligns with the known neuropathological basis of epilepsy, characterized by excessive neuronal excitability and aberrant neurotransmission (Barker-Haliski & White, 2015). Therefore, modulating $GABA_A$ receptor function with zolpidem may offer a novel approach to addressing anxiety in individuals with generalized epilepsy (Ghit *et al.*, 2021). Moreover, utilizing a rat model specifically designed to mimic generalized epilepsy in humans allows for a more detailed investigation into the neurobiological underpinnings of anxiety and epilepsy (Reddy & Kuruba, 2013). By replicating generalized epilepsy in a controlled and reproducible manner, this model enables researchers to uncover key insights into the mechanisms involved in epilepsy-related anxiety and explore potential treatment avenues. Ultimately, this study has the potential to revolutionize the management of epilepsy comorbidities and significantly enhance the overall quality of life for individuals living with epilepsy.

1.3 Research Questions

1. What is the severity of anxiety caused by generalized epilepsy?
2. How does generalized epilepsy-associated anxiety influence physiological and behavioural factors?
3. Is zolpidem an effective pharmacological intervention for treating anxiety acquired through generalized epilepsy?
4. How does zolpidem affect neurodegeneration associated with generalized epilepsy?

1.4 Research Hypotheses

1. The induction of KA-induced generalized epilepsy leads to the manifestation of anxiety in rats.
2. Anxiety associated with generalized epilepsy has an impact on physiological and behavioural factors as measured through daily observations.
3. Administration of zolpidem reduces the anxiety levels observed during testing with the OFT.
4. Zolpidem exhibits neuroprotective properties against seizure-induced hippocampal neurodegeneration in epilepsy through histological analysis.

1.5 Research Objectives

1.5.1 General Objective

To investigate the effect of zolpidem treatment on anxiety and neurodegeneration in a generalized epilepsy rat model.

1.5.2 Specific Objectives

1. To develop a generalized epilepsy rat model induced by kainic acid (KA).
2. To evaluate the impact of generalized epilepsy-associated anxiety on physiological and behavioural factors through daily measurements.
3. To assess the anxiety effect of the generalized epilepsy rat model via open field test (OFT).
4. To evaluate the neurodegenerative effect of the generalized epilepsy on the hippocampus in a rat model treated with zolpidem through histological analysis.

CHAPTER 2

LITERATURE REVIEW

2.1 Epilepsy

Epilepsy is a multifaceted neurological disorder characterized by recurrent seizures, resulting from abnormal electrical activity in the brain (Llanos *et al.*, 2023). It has become a global health concern, affecting around 50 million people worldwide (Trinka & Lee, 2019). Nearly half of these cases are concentrated in Asia (Lim *et al.*, 2020), and particularly in Malaysia, the active prevalence of epilepsy stands at 4.3 per 1000 population, with a higher occurrence observed among males, especially between the ages of 30 to 49 years old (Fong *et al.*, 2021). Moreover, despite rural areas having a population nearly three times lower than urban, the prevalence of epilepsy does not significantly differ between the two. This suggests that cultural and sociodemographic factors do limit access to advanced healthcare and epilepsy-related knowledge among residents of remote regions (Fong *et al.*, 2021). Thus, increasing awareness about epilepsy and its social impact can help reduce discrimination and stigma against individuals affected by the condition, while also advancing understanding and treatment options, thereby addressing the disparities in healthcare access highlighted by the regional prevalence data (Moilanen, 2023).

Defined by the International League Against Epilepsy (ILAE), epilepsy requires the occurrence of at least two unprovoked seizures separated by at least 24 hours or one unprovoked seizure with a high likelihood of recurrence, which arises from abnormal synchronous neuronal firing, eventually causing transient disruptions in normal brain function (Sazgar & Young, 2019). The resulting manifestations reflect the underlying

pathophysiology and the specific brain regions affected. Although the exact causes of epilepsy are still unknown, it is known to be a complex condition involving a variety of factors such as genetic predispositions, brain trauma, infections, tumors, cerebrovascular illnesses, developmental disorders, or abnormalities of metabolism (Vezzani *et al.*, 2015). Thus, a thorough assessment, involving detailed clinical history, neurological examination, electroencephalography (EEG) recording, and neuroimaging approaches like functional magnetic resonance imaging (fMRI) or computed tomography (CT) is essential, not only to diagnose epilepsy but also to differentiate it from other transient neurological events such as syncope, transient ischemic attacks, migraine auras, or psychogenic non-epileptic seizures (Harris & Angus-Leppan, 2020).

2.1.1 Classification of Epilepsy

Epilepsy encompasses a diverse range of seizure types and epileptic syndromes, each characterized by distinct clinical features, underlying mechanisms, and prognostic implications (Fisher, 2017). Understanding these variations is crucial for precise diagnosis, appropriate treatment selection, and prognostic assessment. Focal epilepsy, also referred to as partial epilepsy, originates from specific regions or focal areas within the brain (Ghulaxe *et al.*, 2023). Seizures associated with focal epilepsy may present with localized symptoms, depending on the affected brain region (Chowdhury *et al.*, 2021). They can be further classified into two primary subtypes: simple focal seizures, which do not impair consciousness and may manifest as focal motor, sensory, autonomic, or psychic symptoms; and complex focal seizures, which involve altered consciousness or awareness, often accompanied by abnormal behaviour or automatisms (Ghulaxe *et al.*, 2023).

In contrast, generalized epilepsy involves bilateral cortical involvement and includes seizures that affect both cerebral hemispheres simultaneously (Warren *et al.*, 2024). Unlike focal seizures, which arise from specific brain regions, generalized seizures originate from diffuse and synchronized neuronal activity (Luijtelaar *et al.*, 2014). Generalized epilepsy encompasses various seizure types, each with its own unique clinical manifestations and electroclinical features (Stefan & Trinka, 2022). Absence seizures, for instance, are characterized by brief episodes of impaired consciousness, often accompanied by cyclic blinking of eyelids, abruptly stopped speech and movement, mouth chewing, lip smacking, and rubbing fingers (Bilal, 2021). Tonic-clonic seizures, on the other hand, involve a sequence of tonic (stiffening) and clonic (rhythmic jerking) phases, affecting the entire body and often leading to loss of consciousness (Reddy, 2020). Myoclonic seizures manifest as sudden, brief lightning-like involuntary muscle jerks (Pena & Caviness, 2020), while atonic seizures result in sudden loss of muscle tone, causing the individual to collapse abruptly (Perkins, 2019).

Additionally, there are syndromic epilepsies representing a distinct subset of epileptic disorders characterized by specific age of onset, seizure types, electroclinical features, and comorbidities (Syrbe & Lemke, 2015). For instance, childhood absence epilepsy, juvenile myoclonic epilepsy, and Lennox-Gastaut syndrome typically align with generalized epilepsy, as they often manifest with bilateral involvement and may not have a clear focal onset (Albuja & Khan, 2022). On the other hand, Dravet syndrome, Pseudo-Lennox syndrome, and Landau-Kleffner syndrome exhibit features of focal epilepsy alongside potential generalized seizure activity (Mironov *et al.*, 2023). This means that while seizures may originate from specific areas within one hemisphere, they can also spread and affect both hemispheres.

2.1.2 Cellular Mechanism of Epilepsy

The biological manifestations of epilepsy include synchronized firing patterns and abnormal neuronal excitability, which are frequently caused by an imbalance in excitatory and inhibitory neurotransmission (Sumadewi *et al.*, 2023). Glutamate is the main neurotransmitter involved in excitatory neurotransmission (Chen *et al.*, 2022). It works by activating ionotropic receptors such as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors (Chen *et al.*, 2023). For instance, persistent depolarization and excitotoxicity might result from excessive glutamate release or deregulation of glutamate receptors, causing more sodium ions to enter the neurons, which triggers the onset and propagation of seizures (Essiz *et al.*, 2021). Gamma-aminobutyric acid (GABA), on the other hand, is necessary for inhibitory neurotransmission because it reduces neuronal excitability and inhibits excessive firing synchronization through the influx of chloride ions (Staley, 2015). This equilibrium can be disrupted (refer Figure 2.1), and the onset of seizures is facilitated by either reduced production of GABA or compromised receptor function (Feng *et al.*, 2022).

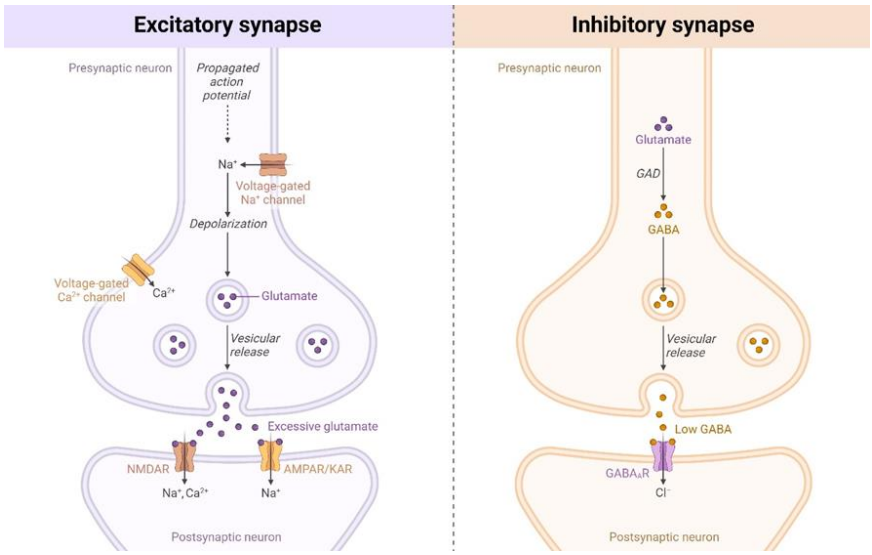

Figure 2.1 The Excitatory and Inhibitory Neurotransmission causing Epilepsy [Revised from "Mechanisms of Antiepileptic Drugs" by Nashed, BioRender.com]

Furthermore, changes in the function of voltage-gated sodium, potassium, and calcium channels also have a significant impact on the pathophysiology of epilepsy. For example, mutations in sodium channel genes like sodium voltage-gated channel alpha subunit 1 (*SCN1A*) and -subunit 2 (*SCN2A*), as seen in Dravet syndrome, increase neuronal excitability and seizure susceptibility (Yamakawa, 2016). Similarly, mutations in potassium channels, as in potassium voltage-gated channel subfamily Q member 2 (*KCNQ2*) and -member 3 (*KCNQ3*) genes, disrupt the balance of excitation and inhibition (Portale *et al.*, 2021), while dysregulation of calcium channels, is observed in calcium voltage-gated channel subunit alpha-1 A (*CACNA1A*) mutation, which exacerbates seizure activity by affecting neurotransmitter release and neuronal excitability (Li *et al.*, 2022). In addition to that, increased neuronal excitability and seizure susceptibility can also result from inflammatory processes in the brain as events like infection or damage, leading to an upregulation of inflammatory mediators (Rana & Musto, 2018). Oxidative stress can damage brain tissue and intensify epileptic activity because it is caused by an imbalance between reactive oxygen species and antioxidant defences (Olowe *et al.*, 2020). Also, any modifications to synaptic strength or connectivity, can facilitate recurrent seizures and sustain the dysfunction within the neural circuits (González *et al.*, 2019).

2.1.3 Models of Epilepsy

In epilepsy research, scientists use a variety of tools to understand the complexities of the disorder. One key approach involves using animals like rats and mice, which offer advantages such as genetic manipulability and well-established behavioural tests (Burrows & Hannan, 2016). The common strains used include Sprague-Dawley (SD) and Wistar for rats, and C57BL/6 or BALB/c for mice (Basso *et al.*, 2023). In the rodent

models, researchers induce seizures using chemical agents like KA, pilocarpine, or pentylenetetrazol (PTZ) which simulate conditions observed in human epilepsy, notably temporal lobe epilepsy (D'Amora *et al.*, 2023). These agents can trigger both generalized and focal seizures, with the specific type determined by factors such as dosage and route of administration (Peterson & Albertson, 2020). Focal epilepsy is established through targeted delivery of the convulsant into specific brain regions, achieved via methods like intracerebral injections (Welzel *et al.*, 2019). On the other hand, generalized epilepsy models involve systemic administration to produce seizures affecting both brain hemispheres simultaneously, typically via intraperitoneal or intravenous injections (Riffault *et al.*, 2016; Hosseini & Mirazi, 2014). The dose administered may vary depending on factors such as the animal species, strain and duration (Rusina *et al.*, 2021), but it is often calibrated to elicit focal seizure activity without causing widespread effects throughout the brain (Leclercq *et al.*, 2019).

Additionally, seizures can be triggered in rodents through precise electrical stimulation, allowing researchers to investigate neurophysiological mechanisms underlying seizure initiation and propagation (Marshall *et al.*, 2021). Electrodes, the instruments commonly used for electrical stimulation, can be inserted into specific brain regions to deliver precise electrical impulses (Ranjandish & Schmid, 2020). Depending on the research needs, electrodes can be either invasive or non-invasive. In invasive methods, electrodes are surgically implanted into the brain tissue, allowing for direct and precise stimulation of targeted brain areas in the case of focal epilepsies (Caldwell *et al.*, 2019). This approach is often used for studies requiring high spatial resolution and specific targeting of neuronal circuits involved in seizure generation (Caldwell *et al.*, 2019). On the other hand, non-invasive methods may involve surface electrodes placed on the scalp or other parts of the body to deliver electrical stimulation (Missey

et al., 2021). Parameters such as stimulation intensity, frequency, and duration are adjusted to fine-tune the type of seizure induced, providing knowledge into the underlying neurophysiological mechanisms of epilepsy (Fisher & Velasco, 2014).

As for zebrafish models, generalized seizures can be provoked using pro-convulsants or through genetic manipulations that affect widespread neuronal activity (Gawel *et al.*, 2020). Focal seizures are triggered by targeting specific brain regions using techniques like optogenetics or microinjections of chemicals. Optogenetic stimulation selectively activates or inhibits neurons expressing light-sensitive proteins (Ledri *et al.*, 2023), thereby facilitating the investigation of specific cell types and their roles in seizure initiation and propagation (Berglind *et al.*, 2018; Krook-Magnuson *et al.*, 2015). There is also the technique of microinjecting specific oligonucleotides and cDNA of interest into zebrafish embryos to induce epilepsy-like phenotypes by disrupting the expression and function of the genes involved in seizure regulation (de Calbiac *et al.*, 2018).

In fruit fly (Drosophila) models, generalized seizures are induced through genetic manipulation targeting genes involved in neuronal excitability or synaptic transmission throughout the nervous system, such as γ-aminobutyric acid transporter 1 (hGAT-1) (Kasture *et al.*, 2023), while focal seizures are elicited by the galactose metabolism regulatory gene 4/ upstream activating sequence (Gal4/UAS) system (Liu *et al.*, 2023). Gal4 is used to selectively activate or inhibit the expression of specific genes in targeted neuronal populations (Dolan *et al.*, 2017). By crossing Gal4 driver lines with upstream activating sequence-RNA interference (UAS-RNAi) lines or introducing mutations in genes encoding ion channels or neurotransmitter receptors, researchers can modulate the activity of specific neurons, thus mimicking the alterations seen in focal epilepsy (Liu *et al.*, 2023). Moving onto the *Caenorhabditis elegans* (*C.*

elegans) model, while it may not precisely replicate focal or generalized seizures seen in higher organisms, seizure-like behaviours can be induced through RNAi or transgenic overexpression (Caldwell *et al.*, 2020), providing information into epilepsy mechanisms, with calcium imaging techniques revealing associated neuronal activity patterns (Sun *et al.*, 2014).

There are *in vitro* models like organotypic slice cultures (OSCs) and induced pluripotent stem cell (iPSC) models, in which researchers can induce both generalized and focal seizures, offering insights into epilepsy mechanisms (Grainger *et al.*, 2018). The induction of seizures *in vitro* models closely mirror the methods used in rodents (Dulla *et al.*, 2018). Furthermore, this approach enables researchers to investigate epilepsy mechanisms and test therapeutic interventions in a controlled setting (Dulla *et al.*, 2018). Additionally, iPSC models offer the advantage of personalized medicine approaches due to their ability to replicate patient-specific characteristics in epilepsy research and drug discovery (Simkin & Kiskinis, 2018).

Computational models, such as mathematical simulations and network modelling, are essential for understanding epilepsy dynamics and treatment responses (Montgomery, 2024). These models adjust parameters related to neuronal excitability, synaptic transmission, and network connectivity to simulate seizure dynamics, allowing for the specific exhibition of generalized or focal seizures (Kapustnikov *et al.*, 2022; Simula *et al.*, 2022). High-performance computing systems and specialized software, such as MATLAB/Simulink, NEURON, and NEST, enable the execution of complex simulations, involving the definition of mathematical equations and parameters governing neuronal behaviour and network connectivity, followed by simulation runs to observe seizure dynamics (Karimi *et al.*, 2022; Najafi *et al.*, 2021). It requires a researcher to possess access to computational resources, programming skills,

knowledge of neurobiology, and familiarity with modelling software to perform this technique (Rokem *et al.*, 2021).

2.1.4 Assessments of Epilepsy

Various assessment methods have been developed and utilized in epilepsy research to measure seizure severity, monitor treatment responses, and track disease progression (Sharma *et al.*, 2018). The Racine scale, established in 1972 (Van Erum *et al.*, 2019), categorizes seizures in rodent models into five stages based on observed behaviours classified in terms of mobility, head nodding, facial movements, forelimb clonus, tonic-clonic seizures and postural control (Jabir, 2018). Observers monitor and score these behaviours to measure seizure severity during experiments (Phelan *et al.*, 2015). This scale is used in preclinical research to assess seizure activity, monitor disease progression, and evaluate potential treatments in animal models of epilepsy (Phelan *et al.*, 2015). The Modified Racine Scale, an adaptation of the original Racine scale, incorporates additional parameters such as salivation (Chiprés-Tinajero *et al.*, 2020), vocalization (Copping *et al.*, 2019), rhythmic head or body movements (Arshad & Naegele, 2020), and urination or defecation (Rojas *et al.*, 2020). Scoring these behaviours alongside the original scale, provides a more comprehensive evaluation of seizure activity. This modified version of the scale, in particular, is utilized in experiments aiming to investigate subtle or atypical seizure behaviours or assess the efficacy of treatments targeting specific seizure characteristics (Tse *et al.*, 2014).

EEG analysis is an important method for assessing the severity and characteristics of seizure activity in both preclinical and clinical settings (Wei *et al.*, 2021). In order to record the electrical signals generated, electrodes are placed on the scalp or directly on the surface of the brain (Feyissa *et al.*, 2021). These recordings enable researchers to identify seizure onset, track propagation patterns, and detect

interictal spikes and epileptiform discharges (Gschwind *et al.*, 2023). It is also a way to quantify changes in brain waves observed during seizures, providing a comprehensive understanding of epileptiform activity. Kindling is the process of repeatedly generating seizures using either electrical or chemical stimulations so that the seizure gradually intensifies (Jiruska *et al.*, 2023). The kindling score is a standardized measure to determine epileptogenesis and seizure susceptibility through the quantification of the severity and course of seizures (Singh *et al.*, 2021). In studies using this approach, animals are repeatedly stimulated, and the frequency, intensity, and duration of seizures are recorded using the Racine scale (Lu *et al.*, 2022).

Seizure frequency counts involve attentively recording and assessing the number of seizures experienced by animals over a specific period of time, typically monitored via continuous video-EEG recordings (Gschwind *et al.*, 2023). By manually analyzing the recordings, seizures experienced are categorized based on behavioural and electrographic aspects, providing information on the frequency, duration, and intensity of it, which will be measured with Racine scale (Gschwind *et al.*, 2023). Moreover, continuous EEG recordings are essential because they aid in detecting electrographic signs of seizures mentioned earlier. This method improves the precision of data, minimizes observer bias, and facilitates high-throughput screening in preclinical investigations (Bassett *et al.*, 2014). The process of video monitoring animal subjects with recording devices is a technique frequently utilized in behavioural studies (Pegoraro *et al.*, 2020). Researchers are able to observe and analyze seizure-related behaviours in real-time through these recordings (Orciani *et al.*, 2023). In the context of epilepsy, it allows various behavioural manifestations to be identified, including convulsions, automatisms, repetitive movements, and movement patterns (Orciani *et al.*, 2023). The reason for incorporating this approach together with other methods is

because it improves the accuracy of seizure parameters to be measured by allowing close and detailed capturing of even subtle changes in animal behaviour that may not be evident through other assessments alone (Grieco *et al.*, 2021).

Apart from that, there is histological analysis, which involves examination of brain tissue to understand structural changes associated with epilepsy (Göbel-Guéniot *et al.*, 2020). In this procedure, samples of brain tissue are obtained and prepared for microscopic inspection using methods including immunohistochemistry and histological staining. Histopathological staining techniques include dyes that provide contrast to neuron structures according to their acidic or alkaline properties, permitting the visualization of cellular architecture and changes in tissue morphology (Krassner *et al.*, 2023). This provides information about neuronal loss, gliosis, and abnormal synaptic connections (Rossini *et al.*, 2020). On the other hand, immunohistochemistry facilitates the identification of particular proteins or markers linked to epileptic activity (Bosque *et al.*, 2021). It works based on the principle of antigen-antibody interactions. The antigen found on the surface of a protein of interest is visualized through the binding of the corresponding antibody, which will be labelled with a chromogen or fluorophore (Kuczkiewicz-Siemion *et al.*, 2022).

2.2 Anxiety

Anxiety is a complex emotional state characterized by feelings of apprehension, worry, and unease, encompassing cognitive, emotional, and physiological reactions to perceived threats or stressors (Tabangcora, 2023). It varies in intensity, duration, and triggers, serving as an adaptive mechanism aimed at fostering survival and coping strategies (Telloian, 2021). Central to anxiety are cognitive processes influencing perceptions of threat, uncertainty, and susceptibility, often leading to heightened

vigilance and anticipation of potential hazards (Günther *et al.*, 2022). Emotional responses include fear, nervousness, and distress, with individuals experiencing tension and emotional overwhelm due to perceived threats or stressors (Kwon & Park, 2014). Anxiety manifests across different life stages, exhibiting age-related variations in symptomatology, from separation anxiety in children to generalized anxiety disorder in adults (Copeland *et al.*, 2013) and anxiety co-occurring with medical conditions in older adults (Wuthrich *et al.*, 2021). They may be triggered by specific events, traumas, or life transitions, shaped by cultural norms and personal coping mechanisms (Chentsova-Dutton & Maercker, 2019).

Physiologically, anxiety elicits a cascade of reactions preparing the body to confront threats, such as activation of the autonomic nervous system and the release of stress hormones like cortisol and adrenaline (Cacha *et al.*, 2019). These responses to anxiety are contextually contingent, influenced by individual variances, environmental factors, and situational cues (Robinson *et al.*, 2013). An increase in cortisol levels can influence food intake by triggering cravings for high-calorie comfort foods, causing changes in body weight (Chao *et al.*, 2017). These changes have been studied in relation to food intake behaviours such as uncontrolled eating, emotional eating, and cognitive restraint eating (Aoun *et al.*, 2019; Janjetic *et al.*, 2019). Interestingly, these behaviours are often utilized as coping mechanisms to alleviate negative emotions arising from anxiety, ultimately leading to overeating (Hussenoeder *et al.*, 2021).

2.2.1 Classification of Anxiety

Anxiety disorders include a range of psychiatric conditions, all distinguished by elevated levels of fear, worry, and apprehension (Star, 2020). These disorders, outlined in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), present distinct

features, triggers, and symptom presentations (Buser & Cruz, 2022). Generalized Anxiety Disorder (GAD) entails persistent and disproportionate worry across various life domains, lasting at least six months and accompanied by symptoms like restlessness and irritability (Cohen *et al.*, 2016). Moreover, Panic Disorder involving repeated, unexpected panic attacks characterized by sudden fear and physical symptoms like palpitations, chest pain, sweating, and trembling, with chronic stages potentially involving depersonalization, fear of losing control, and thoughts of death (Cackovic *et al.*, 2023). Social Anxiety Disorder, or Social Phobia, involves intense fear of scrutiny in social situations, leading to avoidance behaviours and impairment in occupational or academic spheres (Ambusaidi *et al.*, 2022). Furthermore, Specific Phobias entail irrational fear of specific objects or situations, prompting avoidance and significant distress (Eaton *et al.*, 2018). Obsessive-Compulsive Disorder (OCD) is characterized by intrusive thoughts and repetitive behaviours, impairing daily functioning and relationships (Benzina *et al.*, 2016). Lastly, Post-Traumatic Stress Disorder (PTSD), which arises following exposure to traumatic events, resulting in symptoms like intrusive memories, avoidance, and hyperarousal, which can persist for extended periods and impair various aspects of life (Al Jowf *et al.*, 2023).

2.2.2 Assessments of Anxiety

In clinical and research settings, the evaluation of anxiety severity relies on standardized scales and questionnaires validated to quantify symptom frequency, intensity, and impact (Muris *et al.*, 2016). Some of the widely used assessments include the Hamilton Anxiety Rating Scale (HAM-A), a clinician-administered scale assessing anxiety severity across psychological and somatic domains (Marks *et al.*, 2022), the Beck Anxiety Inventory (BAI), a self-report questionnaire measuring anxiety severity via

cognitive, affective, and physiological aspects (Sisay *et al.*, 2024), and the State-Trait Anxiety Inventory (STAI), which evaluates state and trait anxiety providing insight into situational fluctuations and enduring predispositions (Pretorius & Padmanabhanunni, 2023). Additionally, the Generalized Anxiety Disorder 7-item (GAD-7) Scale screens for generalized anxiety disorder and assess symptom severity based on seven items (Niwenahisemo *et al.*, 2024). Physical assessments include vital signs such as heart rate, blood pressure, and respiratory rate, reflecting physiological arousal accompanying anxiety (Vinkers *et al.*, 2021), along with observational cues like restlessness, sweating, and facial expressions (Giannakakis *et al.*, 2017).

The OFT is a commonly used technique for evaluating anxiety-like behaviour in rodents (Acikgoz *et al.*, 2022). It provides insights into rodents' inherent fear and anxiety reactions, by monitoring their exploratory behaviour and preference for closed versus open areas within the field setup (Chen *et al.*, 2024). An extensive characterization of anxiety-related behaviours is provided by the parameters including freezing, rearing, grooming, and locomotion (Võikar & Stanford, 2022). Rodents with heightened anxiety levels may exhibit increased vigilance (Suárez-Pereira *et al.*, 2022), avoidance of open spaces (Chowdhury *et al.*, 2020), and reluctance to explore unfamiliar territories (Ungurianu *et al.*, 2020) within the arena. The OFT's realistic design replicates the experiences of rodents in potentially threatening environments in the wild, which in turn increases the translational relevance of findings to human anxiety disorders (Võikar & Stanford, 2022). Similar to the function of the OFT, there is elevated plus maze (EPM) test, in which rodents are placed in a plus-shaped maze with two open arms and two enclosed arms (Chen *et al.*, 2024). By monitoring the time spent in each type of arm and the number of entries into each, researchers can gauge the rodents' anxiety levels (Tucker & McCabe, 2021). Increased time spent in enclosed