**ESCOLA UNIVERSITÁRIA VASCO DA GAMA**

Ciclo de Estudos Integrado Conducente ao Grau de Mestre em Medicina Veterinária

Dissertação de Mestrado

# THE ROLE OF NEUROINFLAMMATION IN IDIOPATHIC EPILEPSY IN DOGS: A SYSTEMATIC REVIEW

Diogo Manuel Francisco Manso

**Coimbra, julho de 2024**

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Dissertação do Estágio Curricular do Ciclo de Estudos Conducente ao

Grau de Mestre em Medicina Veterinária na EUVG

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*AA* - Arachidonic acid

*AED* - Antiepileptic drug

*AMPA* - Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

*ANOVA* - One-way analysis of variance

*ASC* - Apoptosis-associated speck-like protein with a caspase recruitment domain

*BBB* - Blood-brain barrier

*CA3* - Cornu Ammonis 3

*CARD* - Caspase activation and recruitment domain

*CCL2* - C-C motif chemokine ligands 2

*CCR2* - C-C chemokine receptor type 2

*CD44* - Cluster of differentiation 44

*CNS* - Central nervous system

*COX* - Cyclooxygenase

*CSF* - Cerebrospinal fluid

*CTRExp* - Experimental control group

*CTRPat* - Patient control group

*CXCL13* - C-X-C motif ligand 13

*CXCR5* - C-X-C chemokine receptor type 5

*DAMPs* - Damage-associated molecular patterns

*EEG* - Electroencephalogram

*EP1R* - EP1 receptor

*GABA* - Gamma-aminobutyric acid

*GABAaR* - GABAA receptor

*GFAP* - Glial fibrillary acidic protein

*GPCRs* - G protein-coupled receptors

*HSP70* - Heat shock protein 70

HMGB1 - High mobility group box 1

*IFN-β* - Interferon-beta

*IFN-γ* - Interferon-gamma

*IL-1β* - Interleukin-1 beta

*IL-1Rα* - Interleukin-1 receptor antagonist

*IL-1R1* - Interleukin-1 receptor type 1

*IL-6* - Interleukin-6

*IL-17* - Interleukin-17

*ILAE* - International League Against Epilepsy

*IVETF* - International Veterinary Epilepsy Task Force

*Kir* - inward-rectifier potassium

*KA* - Kainic Acid

*LTP* - Long-term potentiation

*MAPK* - Mitogen-activated protein kinase

*MFS* - Mossy fiber sprouting

*MMP-9* - Matrix metalloproteinase-9

*MRI* - Magnetic resonance imaging

*NLR* - Nucleotide-binding oligomerization domain-like receptors

*NMDA* - N-methyl-D-aspartate

*NF-κB* - Nuclear factor kappa-light-chain-enhancer of activated B cells

*NLRP3* - Nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3

*OD* - Optical density

*PAMPs* - Pathogen-associated molecular patterns

*PGs* - Prostaglandins

*Pgp* - P-glycoprotein

*PYD* - Pyrin domain

*PRRs* - Pattern recognition receptors

*RAGE* - Receptor for advanced glycation end-products

*ROS* - Reactive oxygen species

*SE* - Status epilepticus

*SMAD* - Suppressor of mothers against decapentaplegic

*TBI* - Trauma brain injury

*TGF-β* - Transforming growth factor beta

*TGF-βR* - Transforming growth factor beta receptor

*TLR* - Toll-like receptors

*TNF-α* - Tumour necrosis factor-alpha

*VE* - Vascular endothelial

*ZO-1* - Zonula occludens-1

**THE ROLE OF NEUROINFLAMMATION IN IDIOPATHIC EPILEPSY IN DOGS: A SYSTEMATIC REVIEW**

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**RESUMO**

Este estudo explora o impacto da neuroinflamação na epilepsia idiopática em cães através de uma revisão sistemática abrangente que engloba estudos em animais de laboratório, cães e seres humanos. Os objetivos incluem a investigação da correlação entre a neuroinflamação e a epilepsia idiopática, a compreensão das causas da inflamação do sistema nervoso central em cães e a avaliação da eficácia das modalidades de tratamento atuais.

As descobertas revelam que a neuroinflamação é um fator comum tanto em cães como em humanos, potencialmente levando a epilepsia idiopática. Os fatores que conduzem à neuroinflamação incluem a proliferação glial, o aumento da produção de mediadores inflamatórios, a rutura da barreira hematoencefálica e o aumento das vias de sinalização no sistema nervoso central. Vários mediadores inflamatórios, incluindo citocinas, quimiocinas e componentes do inflamassoma, estão elevados em cães com epilepsia idiopática.

O estudo conclui que os tratamentos mais eficazes para a epilepsia idiopática canina dependem frequentemente de uma análise caso a caso, envolvendo normalmente a administração de fenobarbital, brometo de potássio ou imepitoína. Embora alguma evidência sugira que os fármacos anti-inflamatórios podem ajudar a aliviar os sintomas da epilepsia ao atuarem sobre a neuroinflamação subjacente, é necessária mais investigação para estabelecer a sua eficácia.

As limitações deste estudo incluem fontes de dados variáveis, potenciais enviesamentos e desafios inerentes à investigação de condições raras. É imperativo efetuar mais investigação, tanto em medicina humana como veterinária, para obter informações mais aprofundadas sobre estes aspectos.

**Palavras-chave:** Etiologia, sistema nervoso central, epilepsia idiopática, mediadores, neuroinflamação, convulsão, tratamento

**ABSTRACT**

This research explores the impact of neuroinflammation on idiopathic epilepsy in dogs through a comprehensive systematic review encompassing studies on laboratory animals, dogs, and humans. Objectives include investigating the correlation between neuroinflammation and idiopathic epilepsy, understanding the causes of central nervous system inflammation in dogs, and evaluating the effectiveness of current treatment modalities.

The findings reveal neuroinflammation as a common factor in both dogs and humans, potentially leading to idiopathic epilepsy. Factors driving neuroinflammation encompass glial proliferation, increased production of inflammatory mediators, disruption of the blood-brain barrier, and heightened signaling pathways within the central nervous system. Various inflammatory mediators, including cytokines, chemokines, and inflammasome components, are elevated in dogs with idiopathic epilepsy.

The study concludes that the most effective treatments for canine idiopathic epilepsy often depend on a case-by-case basis, typically involving the administration of phenobarbital, potassium bromide, or imepitoin. While some evidence suggests that anti-inflammatory drugs may help alleviate epilepsy symptoms by addressing the underlying neuroinflammation, further research is needed to establish their efficacy.

Limitations of this study include variable data sources, potential biases, and inherent challenges in investigating rare conditions. Further research is imperative in both human and veterinary medicine to gain deeper insights into these aspects.

**Keywords:** Aetiology, central nervous system, idiopathic epilepsy, mediators, neuroinflammation, seizure, treatment

## CHAPTER 1: INTRODUCTION

### 1.1 Background of the Research

Epilepsy is a complex neurological disorder characterised by a persistent disposition to generate epileptic seizures (Fisher et al., 2014). It is prevalent in both humans and domestic dogs, rendering dogs an ideal translational model for studying epilepsy, as they can exhibit striking similarities in etiology, clinical manifestation, and disease course when compared to human patients (Potschka et al., 2013). As valuable large-animal models, dogs bridge the translational gap between rodent models and human clinical applications. Their size allows for the use of intracranial electroencephalogram (EEG) and responsive neurostimulation devices designed for humans, facilitating direct studies of epilepsy. Consequently, research on dogs benefits both veterinary and human medicine, as they function as both preclinical models and clinical patients (Löscher, 2022).

In the general dog population, epilepsy ranks among one of the most common neurological diseases (Nettifee et al., 2017). While its exact prevalence remains undetermined, it is estimated to be around 0.6% to 0.75% (Hamers et al., 2023; Kearsley-Fleet et al., 2013). This research aims to investigate the role of neuroinflammation in idiopathic epilepsy in dogs by conducting a detailed systematic review of various experimental studies on laboratory animals, dogs and humans.

Witnessing a domestic dog endure extreme symptoms of epilepsy, characterised by severe seizures and convulsions, is not only distressing from a veterinary medicine standpoint but also traumatic for pet owners (Wessmann et al., 2016). Dogs experiencing epilepsy, similar to humans, may exhibit clinical signs, including motor manifestations such as running, clonus, tonus, and a combination of these two; disturbances of consciousness; and autonomic symptoms such as urination, defecation, and excessive salivation. Additionally, they may display abnormal or unusual behaviors such as circling, restlessness, apparent blindness, aggression, jaw chomping, and inappropriate barking, among others (Berendt et al., 2004; Holliday et al., 1970).

Research by Stanslaski et al. (2018) and Packer & Volk (2015) suggest that epilepsy in dogs result from excessive and potentially uncontrollable electrical activity within the central nervous system (CNS), particularly the cerebral cortex. While classic clinical signs such as loss of consciousness, collapsing, body stiffness, and loss of balance have been covered, Cerda-Gonzalez et al. (2021) emphasise that seizures in dogs may manifest in various ways, collectively referred to as any form of abnormal involuntary behavior.

The prevalence of seizures and epileptic clinical signs in domestic dogs in Europe and the USA emphasises the significance of this study. For instance, Erlen et al. (2020) suggest that in the UK, from 2834 incident seizure cases, at least 20% of the dogs suffer from epilepsy. Among these cases, according to the International Veterinary Epilepsy Task Force (IVETF) classification system, at least 17% of the reported cases of epileptic dogs are diagnosed with idiopathic epilepsy. While various researchers and veterinarians have explored the underlying causes of seizures in dogs and proposed potential treatments, there has been limited research into how neuroinflammation may trigger, influence, or impact idiopathic epilepsy in dogs. These gaps present crucial areas with significant implications that warrant further comprehensive and analytical investigation.

Recent advancements in animal models have elucidated the significant role of inflammatory processes in the pathogenesis of epilepsy, highlighting the complexity of idiopathic epilepsy which involves various inflammatory cytokines and mediators in the neuroinflammation process (Barker-Haliski et al., 2017). Potential triggers for neuroinflammation include factors like trauma, viral infections, severe allergies, hormonal disorders, brain tumours, as well as environmental factors such as contaminants (Amanollahi et al., 2022). Despite these aetiological factors, high blood levels of cytokines such as interleukin-1 beta (IL-1β) have experimentally been confirmed to play key roles in the neuroinflammation processes in dogs (Kostic et al., 2019). From a veterinary science standpoint, this implies that neuroinflammation is associated with idiopathic epilepsy in dogs. Therefore, it is crucial to delve into and comprehend the aetiologies of neuroinflammation and its corresponding neurogenic mediators and molecules.

### 1.2 Research Problem

As research suggests, epilepsy in dogs, much like in humans, is influenced by a variety of factors. Mutated genes, such as the LGI2 mutation observed in the Lagotto Romagnolo breed, have been associated with focal, juvenile remitting epilepsy (Ekenstedt et al., 2011). Moreover, neurotropic viruses like Theiler's murine encephalomyelitis virus, have been linked to viral encephalitis and subsequent epilepsy development (Libbey & Fujinami, 2011), along with other factors such as inheritance (Hesselink, 2020). Determining the precise cause of the pets' seizures and understanding the underlying conditions has proven to be practically complex. This complexity of epilepsy's aetiologies has posed challenges for veterinary medical practitioners seeking permanent therapeutic solutions (Balestrini et al., 2021).

Our research contributes a detailed analytical systematic review focusing on the role of neuroinflammation in dogs and its specific impact on idiopathic epilepsy. We emphasise understanding

the inflammatory processes in the CNS of dogs and how these mechanisms may lead to extreme neurological responses, including unprovoked seizures and convulsions, with a particular focus on idiopathic epilepsy.

### 1.3 Relevance of the Study

This systematic review seeks to investigate the influence of neuroinflammation on idiopathic epilepsy in dogs. It aims to uncover the underlying causes often overlooked in this condition. Ultimately, a better understanding of the disorder from a neuroinflammation perspective holds paramount importance for the veterinary field.

### 1.4 Aim

This analytical systematic review explores the link between neuroinflammation and idiopathic epilepsy, along with the underlying causes of neuroinflammation in dogs. By delving into the origins of CNS inflammation in dogs, the study endeavors to clarify the aetiologies and understand how these processes trigger, progress the disease, and impact its treatment and prognosis.

### 1.5 Objectives

To address the research questions at hand, this study established four primary objectives:

1. Analyse the overlooked causes of neuroinflammation in dogs;
2. Research the causes of idiopathic epilepsy in dogs;
3. Examine the intricate relationship between neuroinflammation and idiopathic epilepsy in dogs;
4. Critically assess the findings from experimental studies on the treatment mechanisms for the development of idiopathic epilepsy and evaluate their effectiveness.

### 1.6 Research Questions

1. What are the subtle aetiologies of neuroinflammation that lead to idiopathic epilepsy in dogs?
2. How do the main neuroinflammatory mediators/molecules correlate with each other to increase the recurrence of idiopathic epilepsy in dogs?
3. What is the most effective treatment mechanism for neuroinflammation-initiated idiopathic epilepsy in dogs?

## CHAPTER 2: LITERATURE REVIEW

### 2.1 Overview

In the context of human health, epilepsy ranks as one of the most prevalent neurological disorders globally (World Health Organization, 2023). This condition affects individuals of all ages and exhibits a wide range of causes and triggers, which vary significantly across regions (Beghi, 2020). In less developed regions, epilepsy diagnosis can be particularly challenging due to financial constraints and cultural factors (Ba-Diop et al., 2014). The global variation in the course, frequency, and outcomes of epilepsy can be attributed to these limitations and the uneven distribution of environmental risk factors (Beghi, 2020).

Research conducted in humans by Cano et al. (2021), Marchi et al. (2014), and Sirven (2015) highlights a diverse range of factors that may lead to recurrent epilepsy, including infections, inflammatory conditions, neurodegenerative diseases, brain tumours, traumatic brain injuries (TBI), strokes, and congenital anomalies.

Over the past decade, an accumulating body of evidence has emphasised the significant role of active inflammatory pathways in the development of human epilepsy (Aronica et al., 2017). To understand the implications of neuroinflammation in idiopathic epilepsy in both humans and dogs, it is crucial to study the adverse effects of brain inflammation.

Epileptogenesis, the process leading to symptomatic epilepsy, is intricately connected to various complex factors, posing challenges for medical researchers and veterinary practitioners seeking a comprehensive understanding (Pitkänen et al., 2015). According to the majority of studies using animal models, it is typically initiated by a sudden, unforeseen brain insult, significant disruption of the CNS, or a genetic alteration. Ultimately, it culminates in ictogenesis, marking the occurrence of the first spontaneous seizure (Devinsky et al., 2018).

Prior to the onset of the initial spontaneous seizure, epileptogenic events gradually alter neuronal excitability, establish critical connections, and potentially prompt extensive morphological changes, as observed in both human and animal studies (Webster et al., 2017). These changes can be categorised into three distinct phases: acute, sub-acute, and chronic (Tunthanathip, 2022). The acute transformation phase, initiating within minutes and persisting for days, is characterised by immediate early gene activation, receptor control changes, ion channel kinetics adjustments, post-translational modifications to functional proteins, and the release of various cytokines and other inflammatory mediators. On the other hand, the sub-acute phase, spanning from hours to weeks, involves neuronal

death, sustained activation of the inflammatory cascade, and neurotrophic factor production. The chronic phase, lasting weeks to months or even years, includes anatomical alterations such as neurogenesis, gliosis, mossy fiber sprouting (MFS), and network reorganization. As shown (Figure 1), early spontaneous seizures tend to occur during the acute and sub-acute stages, while late spontaneous seizures are more likely during the chronic phase (Mukhtar, 2020; Webster et al., 2017). In humans, the chronic phase poses an increased risk of developing mental comorbidities (Mula et al., 2020). Additionally, in humans, late seizures may contribute to epilepsy progression, often showing resistance to medication (Mukhtar, 2020). Although there is not much known directly about epileptogenesis in companion animals, data from rodents and human studies are likely to also be informative for all mammals including dogs (Patterson, 2013).

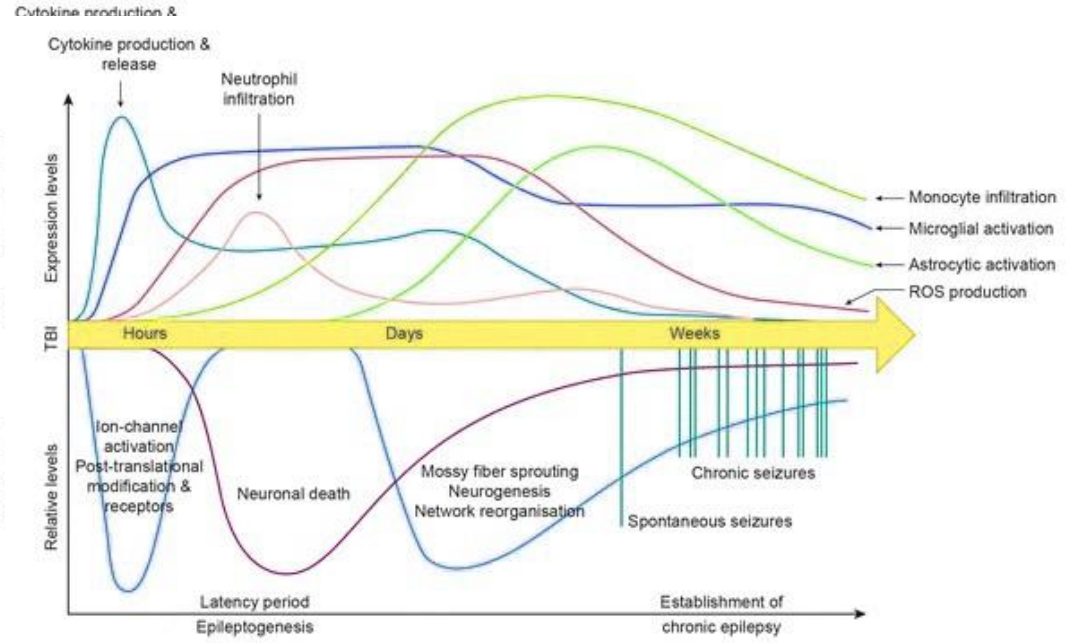


*Figure 1. Synopsis of the development of inflammatory factors and the onset of epileptogenesis* (image adapted from Webster et al., 2017).

### 2.2 Epidemiology of Idiopathic Epilepsy in Comparison to Human Beings

The IVETF categorises epilepsy into two primary classifications: structural and idiopathic epilepsy. Unlike idiopathic epilepsy, which is characterised by a lack of evident structural brain alterations, structural epilepsy is defined by acquired or inherited changes in the brain's structural framework (Berendt et al., 2015). Idiopathic epilepsy in dogs constitutes a complex neurological condition on its own, often involving a genetic or hereditary component, and it is absent of structural cerebral abnormalities (Hülsmeyer et al., 2015). The nomenclature adopted by the IVETF is distinct from that

used by the International League Against Epilepsy (ILAE) for human epilepsy. The ILAE has replaced the term "idiopathic" with "genetic" and "unspecified causes" or "unknown aetiology" (Löscher, 2022).

According to the American Association of Neurological Surgeons (2019), a human-focused organisation, seizures exhibit considerable variation, leading epilepsy specialists to frequently reclassify seizure types. Generally, seizures fall into two main categories: generalised seizures and focal seizures, differing in their initiation. Generalised seizures start with a widespread electrical discharge involving both sides of the brain simultaneously, while focal seizures originate from an electrical discharge in a specific area of the brain. A more detailed categorization is made by the Centers for Disease Control and Prevention (2019), Sarmast et al. (2020), and Wirrell et al. (2022) classifying human epilepsy into four distinct groups: focal, generalised, generalised and focal epilepsy, and an undetermined category. Similar seizure types are observed in dogs with epilepsy (Chandler, 2006).

The ILAE has established a system to classify various epilepsy syndromes based on specific clinical patterns and EEG characteristics, as noted by Wirrell et al. (2022) and Sarmast et al. (2020). However, achieving such classification remains challenging in canine epilepsy due to inherent limitations in conducting EEG investigations in dogs. According to Löscher (2022), the classification of canine epilepsy primarily relies on presumed causes due to the limited availability of EEG-video, genetic, and brain imaging data in sufficient quantities.

Hall et al. (2020) elaborate on the clinical diagnosis of idiopathic epilepsy in dogs, which follows a three-tier system, with each tier increasing the confidence of the diagnosis. Tier I includes elements such as a history consistent with idiopathic epilepsy, a normal neurological examination, and unremarkable results from basic blood tests and urinalysis. Tier II involves additional diagnostic measures, such as bile acid stimulation tests, cerebrospinal fluid (CSF) analysis, and brain magnetic resonance imaging (MRI). The highest level of confidence for diagnosing idiopathic epilepsy, Tier III, is achieved through a combination of Tier I and Tier II investigations, along with the identification of electroencephalographic abnormalities characteristic of seizure disorders.

Historically, generalised tonic-clonic seizures were considered the most prevalent type in canine epilepsy. However, an increasing body of evidence suggests that focal onset seizures are the predominant form of seizure onset in dogs with epilepsy (Berendt & Gram, 1999).

According to an extensive retrospective study conducted by Steinmetz et al. (2013), spanning a duration of 11.5 years and involving 1000 dogs admitted to the University of Hannover's Department of Small Animal Medicine and Surgery, it can be extrapolated that idiopathic epilepsy stands as the predominant form in comparison to other distinct epileptic conditions (see Figure 2). Out of the studied

cases, 63% (n=630) of the dogs were categorised as having idiopathic epilepsy or unknow aetiology, and the remaining 37% (n=370) had a structural aetiology.

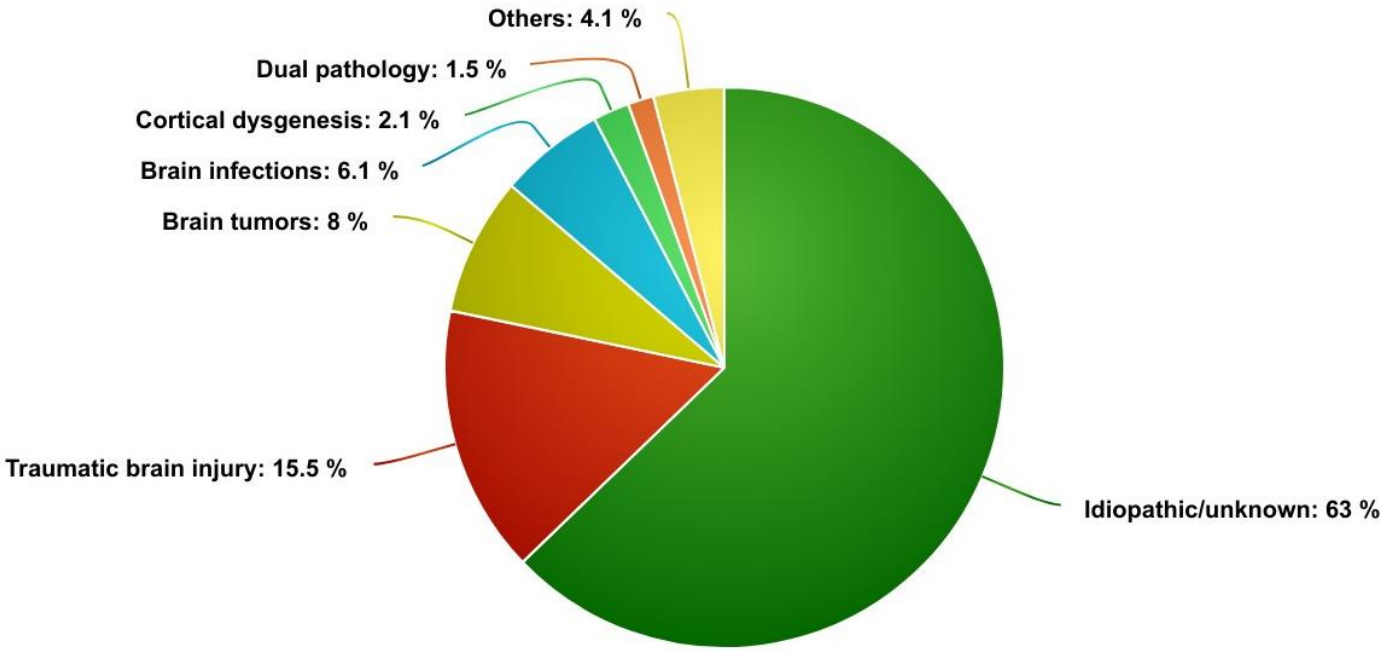


*Figure 2. Aetiologies by proportion in dogs with epilepsy* (image adapted from Steinmetz et al., 2013).

Most recently, empirical data with similar trends have been reported. For example, a study conducted by Hall et al. (2020) involving 900 dogs who underwent MRI for epileptic seizures revealed that structural lesions were the cause of seizures in 45.1% (n=406) of cases, while no structural lesions were identified in the remaining 54.9% (n=494) of cases. Presumed idiopathic epilepsy was diagnosed in 53.8% (n=484).

These findings are similar to research on human epilepsy, as represented by Hauser et al. (1993). Drawing upon data from an extensive population-based study spanning more than five decades in Rochester, Minnesota, USA, it is worth highlighting that 65% of patients were designated as idiopathic/cryptogenic, with the remaining 35% falling into the category of symptomatic.

However, it is important to note that the proportion of cryptogenic epilepsies, defined as an epilepsy of presumed symptomatic nature in which the cause has not been identified (Shorvon, 2011), has steadily decreased in human medicine over the course of the past few years. This has been made possible by the growing usage of high-resolution MRI and the development of innovative techniques that can identify the neuroinflammatory factors responsible for epilepsy, such as next-generation

sequencing. It is reasonable to anticipate that similar advancements will be made in the diagnosis of epilepsy in dogs in a few years (Löscher, 2022).

It is also crucial to emphasise that despite the fact that a multitude of pedigree analyses have convincingly demonstrated genetic influence on most epilepsy patients across various dog breeds, identifying the affected genes within the CNS as a result of either mutation or inflammation has been extremely challenging (Ekenstedt & Oberbauer, 2013).

**2.3 Neuroinflammation as a Defensive and/or a Pathological Systemic Process**

Highlighting neuroinflammation as a key player in epilepsy onset, research conducted by Parsons et al. (2022) and Villa et al. (2019) underscores its intricate and interconnected nature. The role of neuroinflammation as a substantial contributor to epilepsy onset has garnered increased recognition in recent times. A widely accepted consensus suggests that the activation of specific cell types, particularly microglial cells and astrocytes, acting as intrinsic innate immunity cells of the brain, coupled with the heightened release of neuroinflammation-associated cytokines such as IL-1β, interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF-α), collectively plays a significant role in the development of brain-related diseases (Dionisio-Santos et al., 2019; Liu et al., 2019; Wang et al., 2018).

These studies also emphasise that neuroinflammation can exhibit a dual nature, serving both as a protective and detrimental force to the brain. Microglia, extending beyond their immune surveillance function, actively contribute to maintaining the CNS stability, including the release of neurotrophic factors that enhance neuronal well-being (Dionisio-Santos et al., 2019; DiSabato et al., 2016). The inflammatory response represents a complex biochemical process characterised by cascading events, which are indispensable for preserving neuronal homeostasis (DiSabato et al., 2016; Woodburn et al., 2021).

Studies by Mukhtar (2020) and Woodburn et al. (2021) propose that immunogenic stimuli, such as pathogens or tissue injury, triggers neuroinflammatory processes. This activation prompts gliosis, a process that entails the activation and proliferation of microglia and astrocytes, leading to increased production of pro-inflammatory molecules like cytokines and chemokines. Additionally, it results in the generation of reactive oxygen species (ROS) and the disruption of the blood-brain barrier (BBB), allowing peripheral immune cell infiltration and serum albumin extravasation. These events collectively heighten and sustain neuroinflammation, contributing to the development of seizures during the epileptogenesis phase (Chen et al., 2018).

## 2.4 Connection Between Neuroinflammation, Central Nervous System's Immune Processes, and Idiopathic Epilepsy

Chemical inflammatory mediators and their intricate interaction with neurotransmitters are very important to analyse. Glutamate, the principal excitatory neurotransmitter, and gamma-aminobutyric acid (GABA), the primary inhibitory one, are central to this complex interplay, offering insights into CNS pathophysiology (Petroff, 2002). These mediators play a pivotal role in driving pathological conditions, contributing to impaired BBB, neuronal loss, and sustained hyper-excitability of nerve cells (Mukhtar, 2020). The array of these agents comprises cytokines such as IL-1β, IL-6, TNF-α, chemokines, prostaglandins, toll-like receptors (TLRs) and more (Chen et al., 2023; Mukhtar, 2020; Rana & Musto, 2018).

As previously mentioned, the activation of microglia in response to brain inflammation resulting from either a primary or secondary insult is characterised by distinct changes. These changes include a shift in their phenotype from a ramified to an amoeboid morphology, alterations in relevant protein markers, and a significant upsurge in cytokine production (Woodburn et al., 2021). It is noteworthy that various members of the glial cell family, such as astrocytes, as well as non-glial cells like neurons and endothelial cells, may also produce pro-inflammatory mediators (Erta et al., 2012).

Pro-inflammatory cytokines, including IL-1β, IL-6 and TNF-α are usually found in low concentrations in both CSF and blood serum. However, they demonstrate a gradual rise following epileptic seizures (Alyu & Dikmen, 2016; Han et al., 2016). Additionally, a histopathological analysis of brain tissue from individuals with epilepsy has revealed the presence of chronic inflammation marked by neuronal loss, reactive gliosis, and cortical morphological changes (Steinborn et al., 2014).

**Interleukin-1β (IL-1β)**

Implicated in various CNS-related pathophysiological conditions, IL-1β primarily interacts with the interleukin-1 receptor type 1 (IL-1R1), activating glutamatergic N-methyl-D-aspartate (NMDA) receptors, which are glutamate-gated ion channels (Balosso et al., 2008). Vezzani et al. (2008) underscored the pivotal role of IL-1β-IL-1R1 signaling in inducing hyperexcitability in neuronal networks, alongside the crucial involvement of tyrosine kinases and NMDA receptor phosphorylation. The binding of IL-1β to its receptor, IL-1R1, triggers the activation of Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathways. These pathways collectively participate to cytokine production, upregulation of inflammatory genes, and the generation of ROS. ROS, encompassing various derivatives of molecular oxygen, are normal

byproducts of aerobic life. Excessive ROS production results in molecular damage, referred to as oxidative stress (Sies & Jones, 2020).

Furthermore, these pathways also involve the activation of the Src family of kinases, enzymes responsible for phosphorylating tyrosine residues on proteins. Elevated levels of IL-1β induce Src kinase-mediated tyrosine phosphorylation of NMDA receptor subunits NR2A and NR2B. This phosphorylation is believed to enhance NMDA receptor functionality, potentially modifying their activity and sensitivity, leading to increased calcium influx, which subsequently reinforces glutamatergic neurotransmission. This, in turn, is associated with heightened neuronal cell death as a consequence of excitotoxicity (Vezzani et al., 2008; Viviani et al., 2003). Excitotoxicity occurs when excessive glutamate or related excitatory amino acids lead to the death of central neurons, particularly after prolonged or intense exposure (Fricker et al., 2018).

Additionally, Chen et al. 2023 suggest that IL-1β has the capacity to enhance the release of glutamate and downregulate GABAA receptor (GABAAR) within the hippocampus. As a result, an overstimulation of IL-1β could potentially damage the equilibrium between excitatory and inhibitory neurotransmission, consequently raising the susceptibility to hyperexcitability and cell damage. Moreover, Alyu & Dikmen (2016) and Rana & Musto (2018) emphasise that IL-1β not only facilitates the secretion of glutamate by neural cells but also reduces its reuptake, thereby increasing glutamate availability in neuronal synapses and promoting neuronal hyperexcitability.

**Interleukin-6 (IL-6)**

Regarding IL-6, this cytokine is typically produced in moderate quantities. However, upon stimulation, as seen in an inflammatory reaction, large amounts are produced (Erta et al., 2012). The increasing of other cytokines, like IL-1β, TNF-α, interferon-gamma (IFN-γ), and interleukin-17 (IL-17) also results in the up-regulation of IL-6. Evidence suggests that IL-6 overexpression reduces long-term potentiation (LTP) - a persistent increase in synaptic strength following high-frequency stimulation of a chemical synapse, which results in stronger neuronal connections (Cooke, 2006) - and neurogenesis in the hippocampus, promoting gliosis and contributing to epileptogenesis (Mukhtar, 2020).

Notably, IL-6 assumes a critical role in regulating the transition from innate to acquired immunity. It not only inhibits neutrophils but also recruits monocytes and T-cells for a late inflammatory response, a vital aspect in effectively addressing injuries. Moreover, IL-6 levels escalate in tandem with increasing levels of other cytokines, thereby creating conditions that may contribute to epileptogenesis (Erta et al., 2012).

**Tumour necrosis factor-α (TNF-α)**

TNF-α, an inflammatory cytokine within the TNF ligands, plays a pivotal role in regulating N-cadherin, a vital molecule responsible for cellular adhesion and synaptic structure, affecting both excitatory and inhibitory synapses. This control of N-cadherin by TNF-α leads to an increase of the number of neuronal synapses, particularly through the modulation of this intricate synapse-forming molecule (Kubota et al., 2009).

In conditions marked by low extracellular glutamate levels, TNF-α is released by various glial cells, including microglia and astrocytes. TNF-α has the capability to enhance microglial gap junctions and upregulate microglial glutaminase, ultimately resulting in elevated glutamate release. As previously mentioned, glutamate functions as a central excitatory neurotransmitter, inciting neuronal excitation at synapses. Additionally, TNF-α contributes to the increase of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptors at the cell surface of hippocampal neurons, further intensifying glutamatergic transmission and, consequently, enhancing neuronal excitability (Galic et al., 2012).

**Transforming growth factor- β (TGF-β)**

Transforming growth factor beta (TGF-β) is a pleiotropic cytokine involved in both suppressive and inflammatory immune responses. TGF-β1, TGF-β2, and TGF-β3 are all part of the same family of molecules, which signal via its receptors TGF-βR1 and TGF-βR2. TGF-β regulates the differentiation and function of various leukocyte types, controls the nature and intensity of immune responses, and maintains tolerance to self-antigens and innocuous antigens, such as food and commensal bacteria. It also regulates a diverse array of cellular processes, including cell proliferation, recognition, differentiation, and apoptosis (Sanjabi et al., 2017; Shi & Massagué, 2003).

TGF-β ligand binds to TGF-βR1 and TGF-βR2, and induce the phosphorylation of SMAD proteins. Upon phosphorylation, SMAD2 and SMAD3 form a heterodimeric complex with the common signaling mediator SMAD4, translocating together into the nucleus to regulate gene expression (Derynck & Zhang, 2003).

While TGF-β can act as a potential anti-inflammatory mediator and down-regulator of pro-inflammatory cytokines (Battista et al., 2006; Rustenhoven et al., 2016), there is substantial evidence indicating its significant participation in various epileptogenic conditions. This is particularly evident in its crucial role in blood-brain barrier (BBB) functions (Lu et al., 2009; Yu et al., 2014).

**Blood-brain barrier (BBB) breakdown**

BBB disruption appears be a common pathological hallmark during seizures, characterised by alterations in the tight junction that allow the infiltration of peripheral immune cells and specific soluble serum proteins. This occurs not only during acute seizure episodes but persists into the chronic epileptic phase, suggesting that compromised BBB integrity may contribute to the development and progression of epilepsy (van Vliet et al., 2007).

After BBB disruption, albumin infiltrates the brain parenchyma, traditionally utilised as an indicator of BBB dysfunction given that albumin typically does not breach the BBB under physiological conditions (Salar et al., 2014). Upon direct exposure of the brain to albumin, its uptake into astrocytes is mediated by TGF-β receptors. This activation of TGF-β signaling through SMAD2/3 induces an inflammatory response and astrocytic dysfunction. Inflammatory molecules produced by astrocytes, such as IL-1β and high mobility group box 1 (HMGB1), contribute to seizure onset (Kim et al., 2012).

Under normal physiological conditions, astrocytes efficiently take up extracellular potassium residues during the repolarization phase. Given that the levels of extracellular potassium critically influence the resting membrane potential, this reuptake serves as a vital mechanism to maintain homeostasis and prevent neural hyperexcitability. This regulatory process is called spatial potassium buffering (Kinboshi et al., 2020). Moreover, the uptake of albumin by astrocytes diminishes the buffering of extracellular potassium due to the downregulation of inward-rectifier potassium (Kir) 4.1 channels in astrocytes. This cascade of events leads to increased accumulation of extracellular potassium, resulting in the promotion of NMDA receptor-mediated neuronal hyperexcitability and seizure-like activity (Ivens et al., 2007). In addition, spatial potassium buffering is associated with glutamate reuptake via glutamate transporters. This disruption results in the downregulation of glutamate transporters in astrocytes, leading to the accumulation of extracellular glutamate, ultimately contributing to increasing the neuronal excitability (Cacheaux et al., 2009; Kinboshi et al., 2020).

**Cyclooxygenase (COX) cascade and Prostaglandins**

Prostaglandins (PGs) play a significant role in inflammation processes alongside inflammatory cytokines. The cascade begins with phospholipase A2, which releases arachidonic acid (AA) from cell membrane phospholipids. Cyclooxygenase (COX) then converts AA into PGH2, which is further transformed by specific PG synthases into various PGs, including thromboxane A2, PGE2, PGI2, PGD2 and PGF2 (Wang et al., 2021).

Early clinical observations have highlighted the increase of COX-2 and PG production in the brain following seizures. These events are linked to epileptogenesis and a lowered seizure threshold (Rantala et al., 2001). The role of COX-2 and PGs in epilepsy seems to exhibit a dual nature, with early neuroprotective and late neurotoxic effects following seizures. These contrasting outcomes can be attributed to the diverse functions of specific PGs. For instance, PGD2 has anticonvulsive properties, while PGE2 primarily acts as a promoter of epileptogenesis (Serrano et al., 2011; Shimada et al., 2014).

PGE2 is a pivotal player in epileptic neuronal cell death and the decrease in seizure threshold. It acts on G protein-coupled receptors (GPCRs) named EP1, EP2, EP3, and EP4, which are localised in brain cells. This interaction with neurons increases neuronal excitability, partly by inhibiting potassium currents, leading to elevated calcium influx. Furthermore, PGE2 can stimulate glial cells to release glutamate, contributing to the hyperexcitability seen in epilepsy. (Shimada et al., 2014). Additionally, inflammatory cytokines, like IL-1β, have been found to promote COX-2 and PGE2 synthesis in the brain following epileptic seizures. (O'Banion et al., 1996; Walters et al., 2012).

**Chemokines**

Chemokines, a broad family of small proteins known as chemotactic cytokines, signal through cell surface GPCRs. Renowned for their role in stimulating, especially leukocytes, chemokines are crucial for immune system development and homeostasis. They play a central role in various immune and inflammatory responses, functioning not only as inducers of directed chemotactic migration but also participating in protective or destructive processes (Hughes & Nibbs, 2018).

Within the brain, chemokines, primarily secreted by glial cells, have been involved in the development of epilepsy. In animal models of epilepsy, they influence leukocyte movement, contributing to BBB disruption and, consequently, the onset of seizures (Fabene et al., 2008). Furthermore, chemokines regulate neuronal activity by modulating voltage-dependent channels and enhancing the release of neurotransmitters, including GABA, glutamate, and dopamine, often through calcium-dependent mechanisms (Guyon & Nahon, 2007).

Early investigations revealed an upregulation of C-C motif chemokine ligands 2 (CCL2) and its receptor, C-C chemokine receptor type 2 (CCR2) in resected brain tissues from human epileptic patients (He et al., 2013). Furthermore, a study by Cerri et al. (2016) using mice models with chronic epilepsy demonstrated that systemic inflammation exacerbated seizures, concomitant with elevated levels of CCL2/CCR2 in the brain. Furthermore, the signaling pathway involving C-X-C motif ligand 13/C-X-C chemokine receptor type 5 (CXCL13/CXCR5) chemokines has also demonstrated significant

upregulation in brain tissues of both patients with epilepsy and in experimental epilepsy models using rats (Li et al., 2016).

**Toll-like receptors**

Toll-like receptors, like TLR1, TLR2, TLR3, TLR4 are part of a protein class expressed in all major glial cell types and have a more limited presence in neurons. As pattern recognition receptors (PRRs), the toll-like receptors play a crucial role in the innate immune system's proper functioning. These receptors are designed to identify two classes of molecules: pathogen-associated molecular patterns (PAMPs) linked to microbial pathogens and damage-associated molecular patterns (DAMPs) associated with components released from host cells during damage or death (Hanke & Kielian, 2011; Pascual et al., 2021). Following insult, all TLR signaling pathways converge in the activation of NF-κB, regulating the expression of various inflammatory cytokine genes. The release of these pro-inflammatory cytokines promotes epileptogenesis (Kawai & Akira, 2007; Kumar, 2019). Notably, the research by Costello & Lynch (2013) performed in mice showed the significant impact of TLR3-secreted cytokines, particularly Interferon-beta (IFNβ), in increasing hippocampal excitability, thereby triggering seizure activity. Furthermore, mice studies conducted by Wang et al. 2015 revealed alterations in the cortical and hippocampal sub-regional expression of TLR1 during epileptogenesis, suggesting a close association with the process of epilepsy.

**High mobility group box 1 (HMGB1)**

HMGB1 serves as both a nuclear factor and a secreted protein. Within the cell nucleus, it participates in maintaining chromatin architecture and transcriptional regulation (Voll et al., 2008). However, following CNS damage, it can promptly migrate towards the cytoplasm before being released into the extracellular space. HMGB1, discharged from immune cells, glia and neurons in the CNS, serves as DAMP molecule, signaling cellular distress. HMGB1 binds to PRRs such as TLR2, TLR4 and to the receptor for advanced glycation end-products (RAGE) (Walker & Sills, 2012).

Increasing evidence indicates that in the absence of pathogens, TLR signaling can be initiated by DAMPs, with a higher action of HMGB1. The binding between HMGB1 and PRRs triggers the production of pro-inflammatory cytokines like IL-1β, fostering neuronal hyperexcitability (Maroso et al., 2010). Thus, HMGB1 behaves as a cytokine. Its ability to induce the release of HMGB1 from the very same cells it activates initiates a positive-feedback autocrine loop, thereby maintaining the inflammatory cascade (Müller et al., 2004).

**Cluster of differentiation 44 (CD44)**

Cluster of differentiation 44 (CD44) is a versatile glycoprotein involved in cell-cell and cell-matrix adhesion, as well as in cell migration and signaling. It is expressed in various neural cell types, including glial cells and neurons, where it extends into dendrites, mediating dendritic morphology (Roszkowska et al., 2016). The proper Golgi structure and positioning are intricately linked to dendrite morphogenesis, with actin cytoskeleton dynamics playing a crucial role in Golgi maintenance. Under stress conditions, such as glutamate-induced neuronal hyperexcitability, CD44 triggers Src kinase activation, inducing actin remodeling that defines Golgi structure and alters dendritic morphology (Skupien et al., 2014). The link between alterations in dendritic morphology, including changes in dendritic spines, branching patterns, and the formation of axonal sprouting, with epileptogenesis has been established by Tejada et al. (2014).

Additionally, Wang et al. (2022) showcased that CD44 regulates TLR activation, acting as a co-receptor for TLR4 and modulating TLR4 signaling. These findings suggest that CD44 inhibition has a beneficial role in mitigating neuroinflammation, potentially by suppressing the TLR4/NF-κB axis, leading to a reduction in pro-inflammatory cytokines and subsequent alleviation of neuroinflammation.

**Matrix Metalloproteinase-9 (MMP-9)**

Astrocytes and microglia cells located in the brain produce matrix metalloproteinase-9 (MMP-9), an enzyme acting as a mediator of inflammation and contributing to structural modifications within the brain. MMP-9 levels rise during neuronal depolarisation and the upregulation of other inflammatory mediators. Chronic elevation of MMP-9 cause thinning and elongation of dendritic spines, leading to morphological changes in synapses that are linked to impaired synaptic plasticity (Bronisz & Kurkowska-Jastrzębska, 2016). Additionally, MMP-9 plays a role in inducing cell death through various mechanisms, indicating a greater susceptibility to epileptogenesis (Hoehna et al., 2012).

Furthermore, MMP-9 can damage the BBB by affecting adherens and tight junction proteins, influencing the integrity of the endothelial barrier. An evident disruption of adherens junctions, such as vascular endothelial cadherin (VE-cadherin), and tight junctions, including occludin, claudin-5 and zonula occludens-1 (ZO-1), was observed by Qin et al. (2019).

**Neurological Disorders and Systemic Inflammation**

Neural inflammation, as outlined by Rana & Musto (2018), can be initiated by various neurological insults such as TBI, stroke, hypoxia, or febrile seizures. These conditions are associated with subsequent neuronal damage, potentially leading to epileptogenesis. The comorbidities induced by these disorders stimulate the production of pro-inflammatory mediators, contributing to the mediation of seizures in a multifarious manner.

In addition, systemic inflammation plays a pivotal role in provoking brain inflammation. Inflammatory agents originating from systemic infections or comorbidities infiltrate the brain by breaching the BBB, triggering a sequence of inflammatory responses that activate pro-inflammatory elements within the brain (van Vliet et al., 2007). This inflammatory process exacerbates barrier damage, allowing the further penetration of harmful substances and systemic inflammatory mediators (Choi & Koh, 2008). To sum up, as earlier noted, Cerri et al. (2016) found that CCL2/CCR2 signaling plays a crucial role in linking systemic inflammation with seizure susceptibility.

**Insights into Idiopathic Epilepsy**

It is evident from the research made, that the emphasis is placed on identifying neuroinflammatory mediators. While experimental findings outline these mediators and their roles, there's a gap in explaining their intricate interactions in the genesis of idiopathic epilepsy. This study holds paramount significance, addressing this crucial gap by exploring overlooked neuroinflammatory mediators and molecules in symptomatic epileptic seizures by demonstrating how these mediators interrelate, contributing to heightened recurrence in idiopathic epilepsy in dogs.

## CHAPTER 3: METHODOLOGY

### 3.1 Overview

The study's basis, emphasized by Al-Busaidi (2018), rests on its methodology. Methodological decisions are pivotal in ensuring research generates reliable, valid, and practical findings. Conversely, as highlighted by Goundar (2019), unreliable methods and inaccurate data can undermine the credibility of study conclusions.

This thesis extensively addresses methodological considerations, drawing primarily from empirical data found in peer-reviewed veterinary and medical journals. The inclusion of a mix of empirical studies and literature review is intentional, aimed at providing a comprehensive and nuanced understanding of the topic. Empirical studies contribute concrete evidence, offering direct insights into the relationship between idiopathic epilepsy and neuroinflammation. Additionally, literature reviews are incorporated to provide a broader context, theoretical insights, and a synthesis of existing knowledge in the field. The investigation centers on the relationship between idiopathic epilepsy and neuroinflammation in dogs.

To provide a thorough overview, this section covers the criteria for selecting secondary sources, data retrieval strategies, data sources, and the methodology for extracting relevant information from these empirical sources. It also delves into quality assurance mechanisms for the selected data and the steps taken to synthesise the established outcomes.

### 3.2 Information Sources and Search Strategy

This comprehensive analysis involved accessing a vast array of recent publications, numbering at least 700, spanning from 2013 to 2023. Various reputable medical research sources, including but not limited to authorised Government websites, CAB abstracts, Cinahl, Cochrane Library, Google Scholar, MEDLINE, NCBI, Nature, PubMed, Science Direct, Veterinary Research, Wiley Online Library, and relevant conference proceedings sites, were consulted.

The selection criteria focused on source relevance, with sources with publication dates between 2013 and 2023, and the search terms included "idiopathic epilepsy," "neuroinflammation," and "therapy for canine epilepsy." Importantly, all sources accessed adhered to copyright regulations, as they were publicly available and peer-reviewed.

### 3.3 Data Extraction Process and Quality Assessment

Employing standardised checklists ensured precision and facilitated the extraction and examination of relevant information pertaining to neuroinflammation in canines, particularly idiopathic epilepsy. The quality assessment incorporated three key tools: Assessment of Multiple Systematic Reviews II (AMSTAR II) (Shea et al., 2017), Critical Appraisal Skills Programme (CASP) (Chotaliya, 2022), and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Page et al., 2021).

AMSTAR II and CASP were employed to evaluate the methodological quality of the researched studies and to assess the trustworthiness, quality, relevance, and applicability of the selected published papers. Additionally, the PRISMA tool not only enhanced the recording and crosschecking of electronically-searched studies but also played a crucial role in designing the research process, from the electronic search to full-text selection, as depicted in the selection criteria flow chart in Figure 3.

The combined use of AMSTAR II, CASP and PRISMA, along with the inclusion of studies adhering to predetermined standards, ensured the systematic review's highest quality with minimal error feasibility.

### 3.4 Sources' Eligibility and Selection Criteria

Eligibility criteria for this study were aligned with its objectives, focusing on neuroinflammation and idiopathic epilepsy in dogs. Selected studies focused on idiopathic epilepsy and neuroinflammation from the past 10 years, prioritising experimental research. Adhering to veterinary medical research standards, exclusion criteria filtered out sources with inconclusive or unusable data. Out of over 700 prospective scholarly materials, only eight published articles met the criteria. Notably, the selected papers exclusively addressed idiopathic epilepsy and neuroinflammation. The PRISMA model flow chart in Figure 3 outlines the study's selection process.

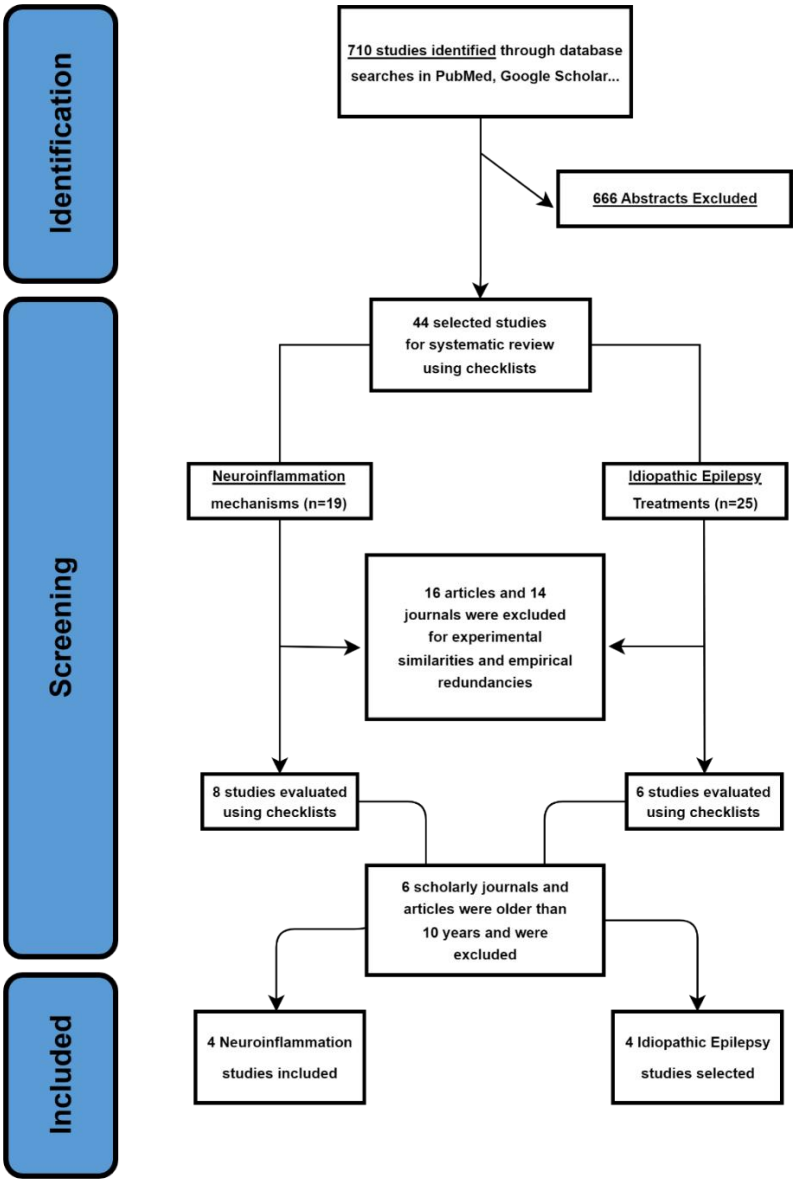


*Figure 3. PRISMA flow diagram for the selection process for quality experimental articles and empirical journals* (Self-generated).

### 3.5 Synthesis of Results and Findings

According to Noyes et al. (2019), secondary informational extraction and combinational analysis are crucial stages in synthesising data, especially in clinical or medical studies. For this systematic review, a descriptive synthesis approach was applied for qualitative analysis. This method was chosen to articulate explicit relationships among key variables. These include the recurrence of symptomatic epilepsy in dogs, idiopathic epilepsy aetiologies, and their connection with neuroinflammation.

## CHAPTER 4: RESULTS AND FINDINGS

### 4.1 Overview

As detailed in the methodology chapter, this study included a total of eight empirical studies within the systematic review. The selected studies engaged in clinical research, investigating the intricacies of neuroinflammation and idiopathic epilepsy. The eight studies encompassed a combination of in vivo and in vitro experimental approaches. They were systematically divided into two primary categories, with four studies dedicated to neuroinflammation and four to idiopathic epilepsy. Importantly, all entries were not only scholarly papers but also underwent rigorous peer review. This section subsequently presents the empirical data gathered from these eight studies, aligning them with the study's objectives, aims, and research inquiries.

### 4.2 Findings from Studies on Neuroinflammation Mechanism

In the experimental investigation conducted by Hanael et al. (2019), 46 dogs experiencing recurrent seizures were enrolled, with 56% being males and the remaining females. The age of these dogs ranged from 1 to 18 years (median = 7.7 years). The body weight range was 2.5 to 53 kg (median = 20.7 kg). Notably, 14 dogs were diagnosed with idiopathic epilepsy, a condition correlated with occurrences of neuroinflammation. The research team systematically examined the integrity of the BBB in dogs diagnosed with idiopathic epilepsy. A BBB score below 20.7 was considered normal, with all tests being two-tailed and significance set at a p-value of less than 0.05.

Dogs experiencing seizures displayed a mean whole-brain BBB score of 21.26 ± 12.63, contrasting with the mean of 15.2 ± 6.74 observed in control dogs. However, this difference was not statistically significant ($p$ = 0.14, t test). Furthermore, the assessment of BBB integrity in dogs with idiopathic epilepsy indicated a mean whole-brain BBB score of 22.52 ± 6.16 compared to 15.2 ± 6.74 in the control group. Despite this disparity, the difference was also not statistically significant ($p$ = 0.3, t test). However, upon separate analysis of each brain region, the BBB score was notably higher, particularly in the analysed piriform cortex of the idiopathic epileptic dogs compared to the control group, with values of 36.44 ± 9.50 versus 27.61 ± 5.95, respectively, and a p-value of 0.03. Moreover, 71% of idiopathic dogs exhibited a BBB score exceeding the threshold when exclusively evaluated for the piriform region.

From the group of dogs enrolled in the study, three idiopathic epileptic dogs were euthanised due to uncontrolled seizures and underwent necropsy. Immunofluorescence staining of the piriform cortex

uncovered the presence of albumin and the astrocytic marker, glial fibrillary acidic protein (GFAP), indicating activation of the TGF-β pathway and the presence of reactive astrocytosis.

The second selected journal stems from an experimental investigation conducted by He et al. (2018). This study aimed to explore the effects of curcumin, a naturally derived active molecule known for its anti-inflammatory efficacy, on epilepsy and its underlying mechanisms of neuroinflammation by examining 40 rats with induced epilepsy. The rats were systematically divided into four groups: a control group, a Kainic Acid (KA)-induced epilepsy group, a group treated with curcumin, and a group subjected to curcumin pretreatment before KA induction. The animals underwent the Morris water maze test for evaluation of spatial learning and memory. Additionally, the researchers extracted the rats' hippocampus and subjected it to Western blot analysis coupled with immunohistochemistry to elucidate the mechanisms at play.

He et al. (2018) revealed that rats with KA-induced epilepsy spent significantly more time escaping the maze, whereas those treated with curcumin exhibited a notable improvement in cognitive functions related to spatial learning and memory compared to rats solely exposed to KA. The study also observed a considerable neuronal loss in KA-induced rats, accompanied by increased production of nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3 (NLRP3), caspase-1 and IL-1β expression. Furthermore, the research demonstrated that curcumin significantly suppressed the protein expression of the inflammasome NLRP3, cleaved caspase-1 and IL-1β in the hippocampus, acting as an inhibitor of neuroinflammation. Notably, NLRP3 and IL-1β are identified as a key player in inflammation activation, potentially triggering rapid Neuroinflammation.

The third and fourth investigations incorporated in this study derives from the works of Cullen et al. (2019) and Wofford et al. (2017). Their focus revolves around elucidating the acute drivers of the neuroinflammation process in TBI. The studies employed a porcine model of closed-head rotational velocity/acceleration-induced TBI. Through a comprehensive single-cell quantitative analysis of the brain, Wofford et al. (2017) findings revealed that in areas free of traumatised neurons, microglial density and morphology were comparable, whether in control animals or those experiencing mild or severe TBI. However, in close proximity to individual injured neurons, where trauma-induced plasma membrane disruption occurred, microglia density increased, and morphology shifted to towards a more reactive state, activating pro-inflammatory pathways.

### 4.3 Results of Studies About the Recurrence of Idiopathic Epilepsy and Therapy

In this segment, four studies were assessed. To begin with, a study by Chen et al. (2023) which focused on the main mediators involved in recurrent idiopathic epilepsy, was reviewed. According to

the researchers, neuroinflammation manifest through a series of inflammatory responses in the CNS, typically triggered by neuropathological conditions. This process involves the activation of microglia, astrocytes and endothelial cells at the BBB, which in turn, upregulate a wide spectrum of pro- and anti-inflammatory mediators. Consequently, initial brain insults, such as TBI, CNS infection, cerebral ischemia, brain tumor, and SE, trigger acute immune and inflammatory responses within the brain, which can become chronic and maladaptive and, in turn, contribute to subsequent development of unprovoked seizures and ultimately to the progression of epileptogenesis. The authors collectively suggest that IL-1β, IL-6, TGF-β, TNF-α, HMGB1, chemokines and PGs are the major mediators responsible for neuroinflammation, as illustrated in Figure 4.

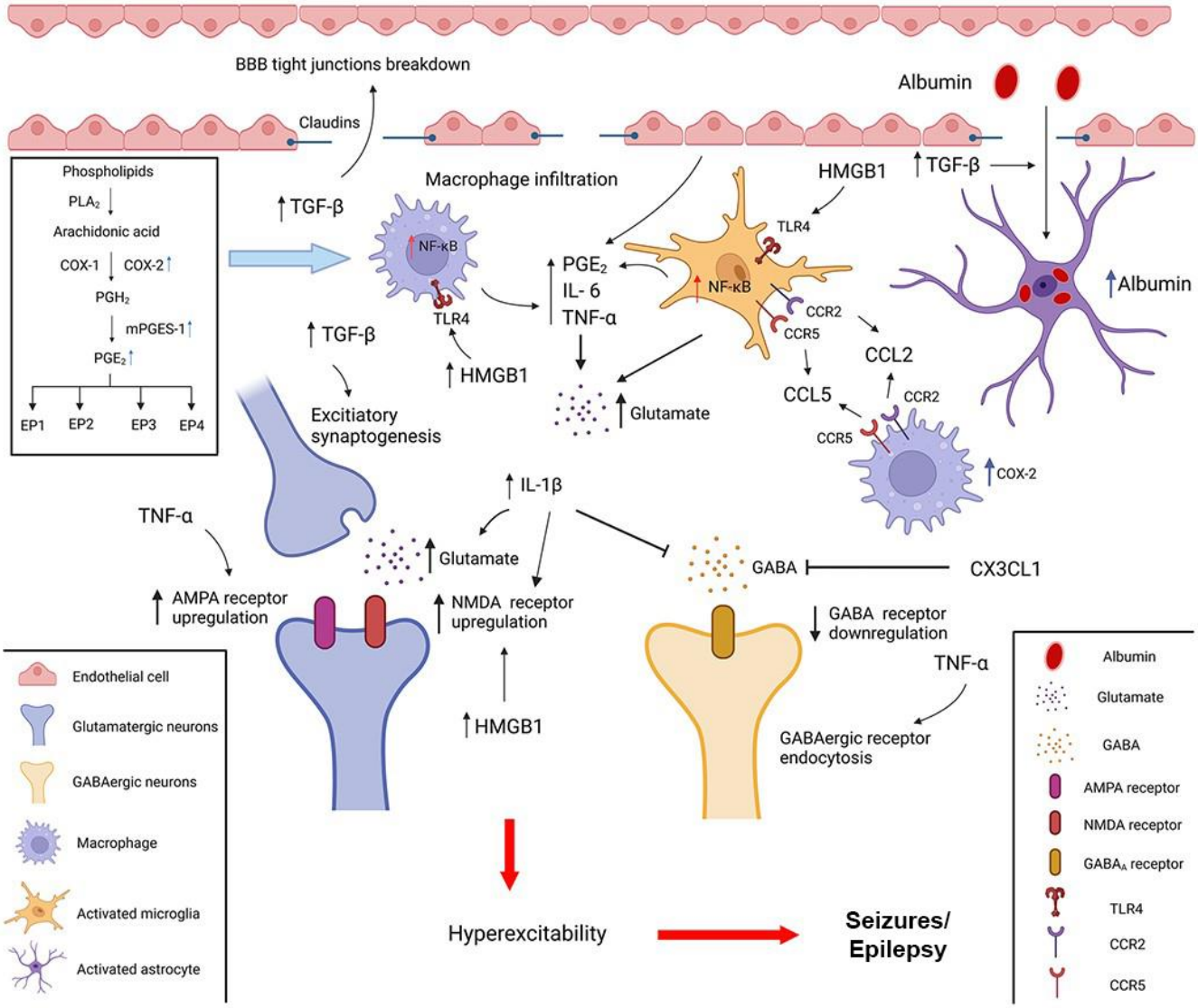


*Figure 4. Neuroinflammatory pathways associated with epileptogenesis* (image adapted from Chen et al., 2023).

The second study, conducted by von Rüden et al. (2020), involved a systematic collection and processing of cerebral specimens from a cohort of 48 canines aged 2 months to 15 years. The canines were categorised into three groups for analysis: a patient control group (CTRpat, n=18) comprising owner-kept dogs without CNS diseases, an experimental control group (CTRexp, n=10) featuring dogs without such diseases, and a group of epileptic animals classified by etiology. The epileptic group included dogs with structural epilepsy (n=12) and idiopathic epilepsy (n=8). Categorization of epileptic dogs was based on clinical diagnoses derived from anamnesis, neurological examinations, and pathological evaluations. Notably, statistical analysis was performed using one-way analysis of variance (ANOVA), with a p-value lower than 0.05 considered statistically significant. The study introduced a distinctive feature by incorporating two control groups, a patient control group and an experimental control group. The latter was characterised by enhanced homogeneity in breed, age, environmental exposures, and dietary provisions.

TLR4 overexpression was observed in the Cornu Ammonis 3 (CA3) subfield of the hippocampus in the brain of canines exhibiting structural epilepsy. Comparison with control dogs revealed a 32% increase in optical density (OD), with a p-value of 0.0535. The study also provided evidence for upregulation of HMGB1 expression, a TLR4 ligand, in the CA1 regions of dogs with idiopathic and structural epilepsy. Quantitative analysis confirmed elevated OD in dogs with idiopathic epilepsy and an 81% increase ($p$ = 0.069) in the HMGB1-positive area in animals with structural epilepsy compared to control dogs. The overexpression of heat shock protein 70 (HSP70), another TLR4 ligand, and HMGB1 was observed in the piriform lobe of canines with idiopathic and structural epilepsy. In this region, the HMGB1-positive area in dogs with idiopathic epilepsy exceeded that in dogs suffering from structural epilepsy by 88% ($p$ = 0.1204). Further analysis in other brain areas indicated that receptor and ligand expression rates were either within the normal range or decreased below the typical range.

Finally, the comprehensive overview of the current treatment options for canine idiopathic epilepsy drew upon two studies conducted by IVETF. In the study by Bhatti et al. (2015), the primary objective was to establish a consensus on managing canine idiopathic epilepsy, addressing key questions regarding the purpose of antiepileptic drug (AED) treatment.

The ultimate goal of AED therapy is to strike a balance between minimising epileptic seizures and enhancing the patient's quality of life. Achieving complete seizure eradication is often unrealistic, making more pragmatic objectives such as reducing seizure frequency, duration, and severity, as well as the overall number of seizures within a short timeframe, more attainable. Additionally, the aim is

to achieve these outcomes with minimal and acceptable AED adverse effects, aiming to optimise both the dog's and owner's quality of life.

Despite the absence of evidence-based guidelines for selecting AEDs in dogs, numerous factors come into play. Considerations include AED-specific factors like safety, tolerability, regulatory aspects, adverse effects, drug interactions, and administration frequency. Dog-related factors, such as seizure type, frequency, and etiology, as well as underlying pathologies like kidney, hepatic, or gastrointestinal issues, and owner-related factors like lifestyle and financial circumstances, are also critical. However, the ultimate choice of AED is typically made on a case-by-case basis.

Clinicians are advised to follow a systematic approach in epilepsy management, including determining when to initiate AED treatment, selecting the most suitable AED and dosage, monitoring serum AED concentrations, adjusting treatment as needed, and knowing when to consider adding or changing to a different AED. As a general guideline, the decision to commence treatment is recommended under specific criteria. These include having two or more epileptic seizures within a six-month period, experiencing SE or cluster seizures, observing notably severe postictal signs, or signs lasting over 24 hours, such as aggression or blindness. Additionally, the early commencement of suitable AED therapy in the disease course is thought to improve the effectiveness of long-term seizure management, particularly in dogs with a high seizure density and breeds predisposed to severe epilepsy.

The second study addressing the treatment of idiopathic epilepsy, conducted by Charalambous et al. (2014), focuses on the diverse use of AEDs in managing canine idiopathic epilepsy. The study involved a systematic review to assess the existing evidence regarding the effectiveness of AEDs in presumptive canine idiopathic epilepsy. In canine epilepsy, there are limitations in treatment of idiopathic epilepsy due to the rapid elimination of most AEDs, with only a few, such as phenobarbital and potassium bromide, having a sufficient half-life. To establish a hierarchy of AEDs based on the quality of evidence and efficacy a pyramid was proposed, as seen in the Figure 5.

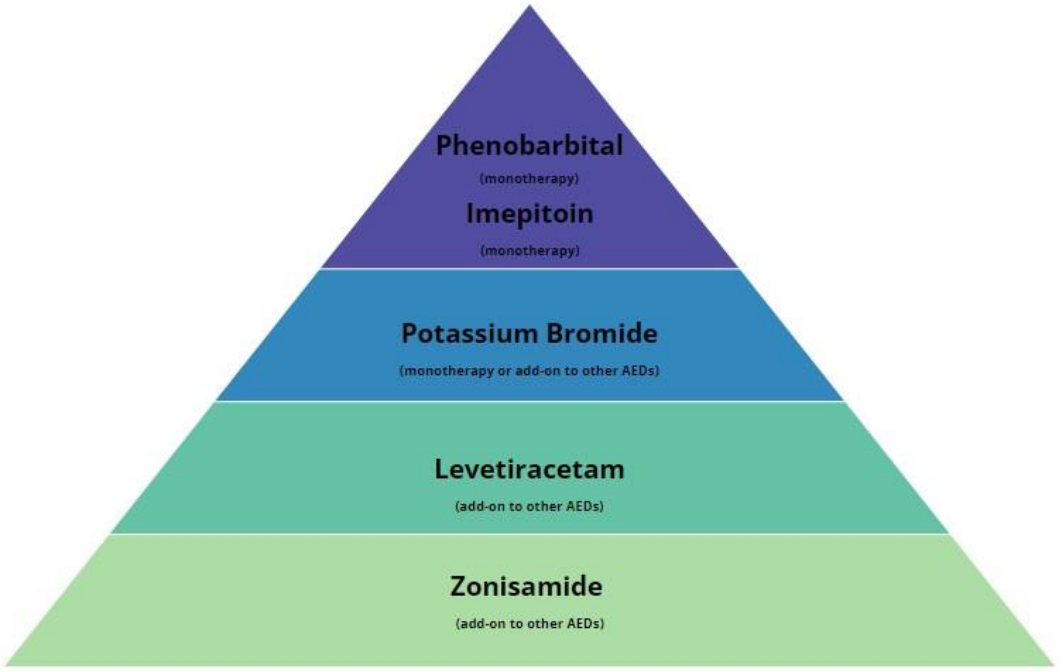


*Figure 5. Pyramid of hierarchy describing the recommendadtions of AEDs, established through the assessment of their efficacy and the quality of evidence* (image adapted from Charalambous et al., 2014).

The systematic review revealed compelling evidence supporting the efficacy of oral phenobarbital and imepitoin, with a fair level of evidence supporting oral potassium bromide and levetiracetam for idiopathic epilepsy treatment. However, for other AEDs like zonisamide, primidone, sodium valproate, gabapentin, pregabalin, felbamate, and topiramate, positive results regarding efficacy were reported, but their use lacks sufficient evidence due to a shortage of studies.

## CHAPTER 05: DISCUSSION AND ANALYSIS

### 5.1 Aetiological Mechanisms of Neuroinflammation that Lead to Idiopathic Epilepsy in Dogs

The research by Hanael et al. (2019) gives support to the fact that BBB dysfunction plays a role in the development of epilepsy in dogs. Notably, epileptic dogs exhibited a significantly elevated BBB permeability score in the piriform lobe compared to the control group. This indicates a vulnerability to vascular issues in the piriform lobe, not only in experimental epilepsy models but also in dogs with idiopathic epilepsy. This finding is particularly relevant due to the piriform cortex's strong connections with other epileptogenic brain regions, such as the amygdala and hippocampus (Aroniadou-Anderjaska et al., 2008). Immunohistochemistry of the piriform cortex further revealed the activation of the TGF-

β pathway and reactive astrocytosis, supporting the hypothesis that these mechanisms contribute to epileptogenesis in dogs.

Furthermore, the collective findings from various studies within this systematic review highlights the intricate relationship between BBB dysfunction and neuroinflammation. The complex interplay between BBB damage, neuroinflammation and epilepsy raises questions about whether it acts as a trigger for epilepsy or is a consequence of neuroinflammation (Marchi et al., 2012). Evidently, BBB dysfunction is revealed to be both a cause and a result of seizure activity, contributing to a nuanced understanding of this complex relationship.

Cullen et al. (2019) suggests that neuronal damage serves as an initial trigger for neuroinflammatory responses in non-penetrating TBI. The implication is that neuronal trauma intensifies microglial activity, subsequently destabilising neurons and causing pathophysiology leading to neuroinflammation. When microglia-mediated neuroinflammation signaling pathways are activated after TBI, DAMPs such as HMGB1 can promote priming of the NLRP3 inflammasome through NF-κB signaling in microglia, which subsequently leads to the outflow of pro-inflammatory cytokines including IL-1β. Furthermore, TLR signaling initiates acute inflammatory response by promoting the release of pro-inflammatory cytokines. It is essential to note that this pathway represents just one of the numerous neuroinflammatory pathways associated with TBI. (Shao et al., 2022).

### 5.2 Predominant Neuroinflammatory Mediators/Molecules in Epileptic Dogs

Drawing from He et al. (2018) findings, it becomes evident that pivotal elements in the neuroinflammatory cascade elucidated in the study encompass NLRP3, caspase-1 and IL-1β. This cascade is initiated through the activation of inflammasomes, intricate intracellular protein complexes that culminate in the activation of inflammatory caspase-1, a pivotal pathway in inflammation. As a critical component of the innate immune system, the NLRP3 inflammasome, belonging to the nucleotide-binding oligomerization domain-like receptors (NLR), assumes the role of a PRR by discerning PAMPs or DAMPs (Barczuk et al., 2022). Additionally, the activation of NLRP3 responds to a spectrum of stimuli and molecular events, including ionic flux, mitochondrial dysfunction, ROS production, and lysosomal damage. Upon activation, NLRP3 undergoes oligomerization, binding to the adaptor protein apoptosis-associated speck-like protein with a caspase recruitment domain (ASC) through their pyrin domain (PYD). This oligomeric structure is then referred to as the NLRP3 inflammasome. ASC, in turn, recruits pro-caspase-1 via its caspase activation and recruitment domain (CARD) domain and activates the effector caspase through proteolytic cleavage. Caspase-1, once

activated, mediates the cleavage of pro-IL-1β into their active and secreted form, IL-1β, initiating inflammatory signaling (Kelley et al., 2019).

In line with this perspective, Chen et al. (2023) literature review proposes a myriad of events contributing to idiopathic epilepsy, ranging from the activation of microglia and astrocytes to the breakdown of the BBB. The review also encompasses various subtle mediators, including but not limited to IL-1β, IL-6, TGF-β, TNF-α, HMGB1, chemokines and PGs, which collectively stimulate and promote neuroinflammation, leading to idiopathic epilepsy. It is important to note that these molecules have been extensively discussed in preceding chapters.

Moreover, the review emphasises the importance of identifying and monitoring these molecules or biomarkers to track the progression of epileptogenesis accurately. Such monitoring process is crucial for achieving a more precise diagnosis of epilepsy, considering the common occurrence of misdiagnosis due to the absence of suitable biomarkers (Kothur et al., 2019). Ultimately, the development of a biomarker for brain inflammation would prove invaluable in monitoring the effectiveness of anti-inflammatory therapy, and determining the appropriate point to cease treatment (Ma & Lin, 2021). A noteworthy example is HMGB1, which has been proposed as a predictive biomarker for epilepsy. Recent research findings indicate dynamic changes in HMGB1 and its isoforms in both the brain and blood of animals exposed to acute brain injuries and undergoing epileptogenesis (Koo et al., 2020; Ravizza et al., 2018). Similarly, patients with drug-resistant epilepsy exhibit significantly higher levels of blood HMGB1 compared to those with drug-responsive epilepsy (Walker et al., 2021). These insights underscore the potential of HMGB1 as a valuable biomarker in understanding and diagnosing epilepsy.

Similarly, as suggested by von Rüden et al. (2020), the study uncovers intricate variations in TLR4 and its ligands among different types of canine epilepsy, delineating unique patterns. TLR4 overexpression in structural epilepsy may indicate a lasting consequence of an initial epileptogenic insult. The research also highlights the upregulation of HMGB1 and HSP70 in both idiopathic and structural epilepsy, emphasising the intricate molecular landscape. HMGB1 acts as a DAMP and is one of the main ligands and activators of TLR4. TLR4 activation results in enhanced generation and release of pro-inflammatory cytokines, including IL-β and TNF-α. While the study did not attain the conventional significance threshold ($p < 0.05$), the obtained results provide valuable insights into the molecular intricacies of canine epilepsy, warranting further exploration and consideration in the broader context of neurological research.

### 5.3 Advancing Treatments for Neuroinflammation in Canine Idiopathic Epilepsy

This systematic review highlights the crucial involvement of inflammation and immune mechanisms in seizure activity. Consequently, targeting specific inflammatory molecules to interrupt epileptogenesis is a conceivable approach. Studies like He et al. (2018), demonstrate the potential of anti-inflammatory substances like curcumin in addressing neuroinflammation. Additionally, Zhu et al. (2014) illustrated that curcumin administration within a 24-hour window post-TBI, may enhance patient prognosis by mitigating microglia activation and reducing neuronal apoptosis through the inhibition of the TLR4/NF-κB signaling pathway in the brain.

Other substances such as cannabinoids like Delta-9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD) have demonstrated anticonvulsant and neuroprotective effects in both rat and human models of epilepsy (Devinsky et al., 2014; Jones et al., 2012). These compounds interact with the Endocannabinoid System (ECS), a critical axis in the CNS that modulates synaptic activity—both excitatory and inhibitory (Demuth & Molleman, 2006). The effects of cannabinoids on epilepsy may be attributed to their interactions with the ECS, including the downregulation of pro-inflammatory prostaglandins and leukotrienes, as well as alterations in endogenous cannabinoid (endocannabinoid) levels. Moreover, modulation of ECS receptors can influence a wide range of intracellular and intercellular signaling pathways, including ion channels, intracellular calcium concentrations, and inflammatory responses. By regulating these processes within the CNS, the complex interplay between the ECS, cannabinoids, and neuroinflammatory pathways can play a pivotal role in mitigating the incidence and severity of epilepsy (Kwan Cheung et al., 2019).

Chen et al. (2023), however, contend that while anti-inflammatory agents may offer broad benefits, ranging from neuroprotection to functional recovery, they are typically less effective than AEDs in directly controlling acute seizures, as they do not directly act on ion channels. Consequently, developing anti-inflammatory treatment as a sole therapy for epilepsy may be unrealistic. Future translational efforts should focus on assessing polytherapy involving anti-inflammatory agents and current AEDs to investigate whether targeting neuroinflammation can enhance AED sensitivity in drug-resistant epilepsy.

In a similar perspective, Löscher et al. (2020) acknowledges the intricate nature of epilepsy treatment, suggesting that success may not be attained solely by targeting a single mechanism. Instead, a more promising approach involves simultaneously modulating multiple mechanisms, aligning with the concept of multi-targeting strategies, which holds the potential for greater effectiveness compared to a single-specific targeting approach.

Within this framework, clinicians may consider drugs inhibiting pivotal inflammatory pathogenic mechanisms. Notably, this strategy aligns with the existing use of inflammation-targeting drugs in human medicine for the management of autoimmune or auto-inflammatory pathologies. Examples include Anakinra, an interleukin-1 receptor antagonist (IL-1Ra) used to treat rheumatoid arthritis, and Infliximab, an anti-TNF-α monoclonal antibody, used to treat a number of autoimmune diseases. Novel compounds, such as TLR3 or TLR4 blockers, have also demonstrated therapeutic efficacy in pre-clinical studies (Vezzani et al., 2015).

Building on this, Rawat et al. (2019) propose pharmacological inhibition of the COX-2 enzyme using selective and non-selective COX-2 inhibitors in rat studies. This not only resulted in reduced seizure recurrence and disease severity but also increased the efficacy of administered AEDs. Amidst the various prostanoids produced upon COX-2 activation, PGE2 emerges as a key factor in the signaling pathway that mediates seizure-induced up-regulation of P-glycoprotein (Pgp) expression though EP1 receptor (EP1R) activation (Pekcec et al., 2009), as demonstrated in rat studies. Pgp, an important cell membrane protein expressed in different cell types, among which are capillary endothelial cells composing the BBB, plays a vital role in pumping out foreign substances from cells. It significantly contributes to drug efflux at the BBB and may be the underlying factor in drug-resistant epilepsy (Wang et al., 2015). Pharmacological EP1R blockade in a rat kindling model of epileptogenesis resulted in anticonvulsive effects of a phenobarbital dose that was otherwise ineffective in the absence of EP1 blockade (Pekcec et al., 2009).

Löscher et al., 2020 also underscores the importance of restoring the BBB. Changes in BBB permeability can alter the pharmacokinetics of AEDs, diminishing their effectiveness by reducing delivery to cellular and molecular targets in the brain. Consequently, approaches to address BBB dysfunction, such as anti-inflammatory drugs that target cytokine and COX-2 signals to restore permeability, may enhance the response to specific therapeutic drugs for seizures.

Ultimately, the challenge lies in designing an intervention that addresses the detrimental harm of brain inflammation without interfering with the homeostatic mechanisms. Moreover, preventing inflammation proves difficult due to its swift onset and amplification following the initial triggering event. This aligns with Chen et al.'s (2023) perspective that establishing a biomarker for brain inflammation would significantly aid in assessing the efficacy of an anti-inflammatory intervention and determining the optimal point to conclude the treatment (Vezzani et al., 2015).

## CHAPTER 6: CONCLUSION AND LIMITATIONS

This systematic review supports the activation of the innate immune system and consequent neuroinflammation link with canine epilepsy. Various neurological insults activate brain cells, triggering a cascade of inflammatory events. Prolonged stimulation of proinflammatory molecules and pathways is sufficient to induce neurological changes, including impaired BBB, neuronal loss, and excitotoxicity. These pathological events have the potential to convert a normal brain into an epileptic one. Notably, neuroinflammation is not only responsible for morphological and physiological brain alterations but systemic inflammation can also impact the brain by disrupting the BBB.

While AEDs such as phenobarbital and potassium bromide stand out as effective therapeutic options for idiopathic epilepsy in dogs, employing multi-targeting strategies to modulate neuroinflammatory cascades could become crucial for more effectively managing epilepsy symptoms and addressing drug-resistant epilepsy. Moreover, exploring the potential of biomarkers linked to neuroinflammatory processes, such as HMGB1, holds promise for precise epilepsy diagnosis and monitoring the efficacy of anti-inflammatory therapy.

However, the current lack of reliable neuroinflammatory treatments or biomarkers for dogs underscores the need for more research. Addressing the present contradictory and incomplete data situation requires additional studies to delve into the underlying neuroinflammatory pathophysiological alterations. Given that canines serve not only as potential patients and pets in veterinary medicine but also as a premedical model for human medicine, further experimental studies and empirical research on the correlation between neuroinflammation and idiopathic epilepsy in this species are warranted. This approach would benefit both veterinary and human medicine.

Concerning the limitations of this study, the systematic review needed an array of previously collected, potentially contradictory data, making it challenging to trust or verify all sources. Additionally, when examining difficult to study conditions, such as the connection between neuroinflammation and idiopathic epilepsy that have not yet been confirmed in the literature, systematic reviews are restricted to the available published material. This constraint may introduce inherent biases and systematic errors, potentially leading to biased assertions. Finally, using animal models, particularly rat or mice, for epilepsy research has its challenges. While these models offer valuable insights, their translational potential is somewhat limited. Unlike naturally occurring conditions in humans and dogs, induced models often involve artificial methods like chemical induction or electrical stimulation. This can result in discrepancies in how epilepsy develops and progresses, making it difficult to extrapolate findings to dogs and humans (Potschka et al., 2017).

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