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# Idiopathic epilepsy in Finnish Spitz dogs

## Epidemiological, clinical and diagnostic aspects

**Ranno Viitmaa**

Academic dissertation

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*To my parents*

# CONTENTS

CONTENTS ..... 5

ABSTRACT ..... 7

LIST OF ORIGINAL PUBLICATIONS ..... 9

ABBREVIATIONS ..... 10

1. INTRODUCTION ..... 11

2. REVIEW OF THE LITERATURE ..... 13
   2.1. Epilepsy definitions and classifications ..... 13
   2.2. Epidemiology of epilepsy ..... 14
   2.3. Clinical phenotype of epileptic seizures ..... 16
   2.4. Diagnosis of idiopathic epilepsy ..... 18
      2.4.1. Laboratory diagnostics ..... 18
      2.4.2. Magnetic resonance imaging (MRI) ..... 19
      2.4.3. Electroencephalography (EEG) ..... 21
      2.4.4. Positron emission tomography (PET) ..... 24

AIMS OF THE STUDY ..... 28

3. MATERIALS AND METHODS ..... 29
   3.1. General inclusion criteria ..... 29
   3.2. Animals and study designs ..... 29
      3.2.1. Epidemiology, inheritance and phenotype of epilepsy (I) ..... 29
      3.2.2. Structural brain imaging with MRI (II) ..... 30
      3.2.3. Cerebral electrical activity measured with EEG (III) ..... 30
      3.2.4. Imaging cerebral glucose metabolism with FDG-PET (IV) ..... 30
   3.3. Ethical considerations ..... 30
   3.4. Questionnaire and phone interview (I) ..... 31
   3.5. Diagnostic methods ..... 31
      3.5.1. Laboratory analyses (II-IV) ..... 32
      3.5.2. MRI (II, IV) ..... 32
      3.5.3. EEG (II-IV) ..... 33
      3.5.4. FDG-PET (IV) ..... 34
   3.6. Statistics ..... 35

4. RESULTS ..... 37
   4.1. Epidemiology, inheritance (I) and phenotype (I-IV) of epilepsy ..... 37
   4.2. MRI (II, III, IV) ..... 41
   4.3. EEG (III, II, IV) ..... 41
   4.4. FDG-PET (IV) ..... 45

5. DISCUSSION ..... 47
5.1. Methodological issues ..... 47
5.1.1. Animals and study design (I-IV) ..... 47
5.1.2. MRI (II) ..... 48
5.1.3. EEG (II-IV) ..... 49
5.1.4. FDG-PET (IV) ..... 49
5.2. Epidemiology, inheritance and phenotype of epilepsy in FSDs (I) ..... 51
5.3. MRI of epileptic FSDs (II, IV) ..... 55
5.4. EEG of epileptic FSDs (II-IV) ..... 56
5.4.1. Visual evaluation of EEG ..... 56
5.4.2. Quantitative EEG ..... 58
5.5. FDG-PET of epileptic FSDs (IV) ..... 58
5.5.1. Visual evaluation of FDG-PET ..... 58
5.5.2. Semi-quantitative analysis of FDG-PET ..... 59
5.6. Comparisons between MRI, EEG, PET and phenotype (I-IV) ..... 60
5.6.1. Comparison of diagnostic modalities ..... 60
5.6.2. Comparison of epilepsy phenotype in FSDs and humans ..... 61
6. CONCLUSIONS ..... 63
7. ACKNOWLEDGEMENTS ..... 64
8. REFERENCES ..... 67

# ABSTRACT

Epilepsy, a common neurologic disorder in dogs, has also been recognized in the Finnish Spitz dog (FSD) since the 1980s, but scientifically verified data has been lacking. In this thesis, epilepsy in FSDs was characterized. Diagnostic investigations, using tools such as magnetic resonance imaging (MRI) and electroencephalography (EEG), have not been used consistently in veterinary medicine to diagnose epilepsy in dogs. The usefulness of these modalities to diagnose different forms of canine epilepsy needs to be proven. Thus, FSDs with and without focal epilepsy were studied by MRI and EEG. In addition, the novel functional method to investigate epileptic dogs, 2-deoxy-2-[18F]fluoro-D-glucose (FDG) positron emission tomography (PET), was described and results were compared with EEG.

Epidemiological information was based on 2141 FSDs, of which 143 were epileptic, and prevalence on 2069 living FSDs, of which 111 had epilepsy. The prevalence of suspected idiopathic epilepsy (IE) in FSDs was found to be 5.3%; males were more predisposed to epilepsy. The median age at seizure onset was 3 years, seizure frequency was 3 per year, and duration of seizure episode was 12 min. Focal onset seizures, characterized by frequent behavioral and autonomic signs were the main phenotype of epilepsy in FSDs. Although epilepsy in FSDs follows a generally benign course, generalization of seizure indicate a progressive course of epilepsy. The heritability estimate of IE in FSDs (0.22) was best explained by polygenic traits.

Although characterized with focal seizures, FSDs have non-lesional epilepsy based on 1.5T MRI examinations. Infrequent reversible brain changes can be found, as a consequence of seizures.

Visual evaluation of EEG in epileptic FSDs showed interictal epileptiform paroxysmal activity (20%) less frequently than had been described previously. This activity was expressed by spikes, polyspikes, and spike slow-wave complexes in the posterior-occipital derivation. Epileptiform activity, consisting of midline spikes, was recognized in healthy FSDs. Sleep transients, which were frequently found in FSDs from both groups, could be easily misinterpreted as epileptiform activity. Quantitative EEG showed significant difference in various frequency bands related to diseased status or medication.

Cerebral glucose utilization was examined by FDG-PET in 11 epileptic and 8 healthy FSDs. Glucose uptake abnormalities/asymmetries were detected in various brain regions of 82% of epileptic and in 50% of control FSDs; findings in the occipital cortex specifically associated with epilepsy. The epileptic dogs had significantly lower standardized uptake values in numerous cortical regions, cerebellum, and hippocampus compared to the control dogs. The low cortical glucose uptake values found in the occipital lobe in both groups of FSDs is an unique finding and may indirectly reflect the lowered seizure threshold in that region characteristic for this dog breed. Inability to reveal significant difference of white matter normalized uptake values and left-right

asymmetry indexes between epileptic and control groups might be related to the method used to define regions of interest.

Based on these results, epilepsy in FSDs is defined as idiopathic epilepsy, as FSDs lack changes on the brain MRI and epilepsy is genetically determined. EEG and FDG-PET suggest involvement of the occipital region, although also wider posterior cortical areas could be related to epileptogenesis in FSDs.

Visual evaluation of both EEG and FDG-PET can support the diagnosis of IE in dogs. Although diagnostic yield of EEG to diagnose epilepsy seems to be lower than suggested for dogs, it is a method of choice for everyday clinical settings. FDG-PET is a useful research modality for examining epileptic dogs, where visual detection of hypometabolic areas provides the highest sensitivity. Quantitative assessment methods of EEG and FDG-PET can be beneficial, but should be used alongside visual evaluation in epileptic dogs.

# LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the original articles (I-III) and to the unpublished manuscript (IV). These articles are referred to in the text by their Roman numerals:

I. **Viitmaa R,** Cizinauskas S, Orro T, Niilo-Rämä M, Gordin E, Lohi H, Seppälä EH, Bragge H, Snellman M. Phenotype, inheritance characteristics, and risk factors for idiopathic epilepsy in Finnish Spitz dogs. J Am Vet Med Assoc 2013;243:1001-1009.

II. **Viitmaa R**, Cizinauskas S, Bergamasco L-A, Kuusela E, Pascoe P, Teppo A-M, Jokinen TS, Kivisaare L, Snellman M. Magnetic resonance imaging findings in Finnish Spitz dogs with focal epilepsy. J Vet Intern Med 2006;20:305-310.

III. Jeserevics J, **Viitmaa R**, Cizinauskas S, Sainio K, Jokinen TS, Snellman M, Bellino C, Bergamaso L. Electroencephalography findings in healthy and Finnish Spitz dogs with epilepsy: visual and background quantitative analysis. J Vet Intern Med 2007;21:1299-1306.

IV. **Viitmaa R**, Haaparanta-Solin M, Snellman M, Cizinauskas S, Orro T, Kuusela E, Johansson J, Pääkkönen T, Bergamasco L.A, Metsahonkala L. Cerebral glucose utilization measured with high resolution positron tomography in normal and epileptic Finnish Spitz dogs. Submitted to Vet Radiol Ultrasound

# ABBREVIATIONS

| | |
|---|---|
| AI | Left-right asymmetry index |
| ANOVA | Ordinary analysis of variance |
| CBC | Complete blood count |
| CF | Complex focal |
| CFS | Complex focal seizures |
| CI | Confidence Interval |
| CSF | Cerebrospinal fluid |
| CT | Computed tomography |
| EEG | Electroencephalography |
| FDG | 2-deoxy-2-[18F]fluoro-D-glucose |
| FLAIR | Fluid-attenuated inversion recovery |
| FSBC | Finnish Spitz Breeder Club |
| FSD | Finnish Spitz dog |
| HRRT | High resolution research tomography |
| IE | Idiopathic epilepsy |
| ILAE | International League Against Epilepsy |
| im | Intra muscular |
| iv | Intra venous |
| MPR | Multiplanar reconstruction |
| MRI | Magnetic resonance imaging |
| OR | Odds ratio |
| PET | Positron emission tomography |
| ROI | Region of interest |
| RR | Reference range |
| SD | Standard deviation |
| SE | Standard error |
| SF | Simple focal |
| SFS | Simple focal seizures |
| SPECT | Single-photon emission computed tomography |
| SUV | Standardized uptake value |
| T1W | T1-weighted |
| T2W | T2-weighted |
| VOI | Volume of interest |

# 1. INTRODUCTION

Idiopathic epilepsy (IE) is a common canine neurological problem with an estimated prevalence of 0.6% in the general dog population (Kearsley-Fleet et al. 2013). There is remarkable variability in published prevalence figures for different dog breeds (1 to 33%) and study populations (Falco et al. 1974, Famula & Oberbauer 1998, Knowles 1998, Berendt et al. 2002, Casal et al. 2006, Berendt et al. 2009, Gulløv et al. 2011). Breeding studies and pedigree analyses have supported a genetic basis for IE in a wide variety of dog breeds (Srenk et al. 1994, Famula et al. 1997, Jaggy et al. 1998, Kathmann et al. 1999, Patterson et al. 2005, Licht et al. 2007). It is most likely, however, that a number of genetic mutations can determine the IE in different dog breeds, but possibly also for distinct bloodlines within the same breed (Licht et al. 2002). Increasing numbers of genetic studies of canine epilepsy have reported first loci and candidate genes for IE in dogs (Ekenstedt et al. 2011, Seppälä et al. 2011, 2012). Therefore a break-through in our understanding of the pathophysiological mechanisms underlying canine epilepsies may occur in the near future.

Diagnosis of IE in dogs is generally made *per exclusionem* and based on a history of more than two seizures in the absence of other medical problems, normal physical and neurological examination, as well as clinical evaluations (Schwarz-Porsche 1984, Jaggy & Bernardini 1998, Licht et al. 2002). The Neuroimaging Commission of the International League Against Epilepsy (ILAE) suggested that magnetic resonance imaging (MRI) examination should be performed on every human patient with epilepsy (Kuzniecky et al. 2002). The situation in veterinary epileptology differs however, as a limited number of studies documenting MRI findings in dogs with seizures have been available (Kärkkäinen et al. 1991, Kärkkäinen 1995, Mellema et al. 1999, Mariani et al. 2001, Bush et al. 2002). Nonetheless, there is a growing collection of publications exploring that field. In canine IE, similar to humans, structural brain imaging is considered to be normal. (Chandler 2006, Thomas 2010).

Modern electroencephalography (EEG) has unfortunately not become a routine diagnostic procedure when diagnosing epilepsy in dogs (Pakozdy et al. 2012). It is surprising, as interictal EEG changes can be the only positive finding in dogs with IE. In addition to confirmation of epileptic cerebral activity, EEG can supply localization of the epileptogenic foci and play an important role in seizure classification (Commission on Classification and Terminology of the ILAE 1981, 1989, Flink et al. 2002). Diagnostic yield of EEG when examining dogs with epilepsy has been reported to be highly variable however (0% till 86%) (Jaggy & Bernardini 1998, Pakozdy et al. 2012). Thus, multiple methodological questions need to be answered regarding the use of EEG to diagnose epilepsy in dogs.

Positron emission tomography (PET) using different radioligands have been used in epileptic human patients to confirm the epileptic region during presurgical evaluation. The most widely used tracer used with PET is 2-deoxy-2-[18F]fluoro-D-glucose (FDG) which reflects cerebral glucose utilization (Juhasz et al. 2005). Glucose metabolism in

focal epilepsy is usually reduced interictally in the region of seizure onset, and increased during the ictal event. The usefulness of FDG-PET when examining epileptic veterinary patients, however, remains unknown.

The Finnish Spitz dog (FSD) is a relatively rare breed used traditionally in the Northern parts of Scandinavia as a "barking hunting dog" for game birds and also as a guard dog. Since the 1980s there was information available about the incidence of epilepsy in FSDs, but scientifically proved information was missing. This thesis characterizes epilepsy in FSDs and explores the usefulness of diagnostic modalities (MRI, EEG, and FDG-PET) and methods of analysis to investigate epileptic dogs.

# 2. REVIEW OF THE LITERATURE

## 2.1. Epilepsy definitions and classifications

*Epileptic seizure* is defined in human medicine as a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain (Fisher et al. 2005). From the pathophysiological point of view, it is important to appreciate that normal brain can also have an acute seizure as a natural response to transient insult or loss of homeostasis (*provoked seizure*) (Engel 2006). *Epilepsy* is defined for practical purposes as two or more unprovoked seizures occurring at least 24 h apart (Thurman et al. 2011). The conceptual definition of *epilepsy* is rather complex and also includes the neurobiologic, cognitive, psychological, and social consequences of this condition, and is characterized by enduring predisposition of the brain to generate epileptic seizures (requires occurrence of at least 1 seizure) (Fisher et al. 2005). Some parts of the previous definition might be difficult to apply directly to animals, however, the definitions and terminology used in veterinary medicine follow the ILAE 1981–1989 recommendations for the most part (Commission of Classification and Terminology of the ILAE 1981, 1989), and are more recently modified after the 2001–2006 update (Engel 2001, 2006). Epilepsies are classified according to etiology as idiopathic (primary), symptomatic (secondary), and probably symptomatic (cryptogenic) epilepsy (Berendt & Gram 1999). Some authors exclude cryptogenic from the classification and include reactive epilepsy (Bush et al. 2002, Lorenz et al. 2011a). *Idiopathic epilepsy* is defined as recurrent, unprovoked seizures for which no underlying brain abnormalities can be identified and a familial or genetic predisposition may be suspected (Knowles 1998). *Symptomatic epilepsy* refers to a seizure disorder where seizures are a consequence of a structural brain disorder (March 1998). *Probably symptomatic epilepsy* is suspected to be symptomatic, but where etiology is not determined (Berendt & Gram, 1999). Extracranial metabolic or toxic insults are causing *reactive epilepsy* and therefore not considered as true epilepsy (Chandler 2006).

One recent study from the veterinary field has applied the term "genetic epilepsy" (Gulløv et al. 2012), adapted from the latest ILAE proposals (Berg et al. 2010). A modified concept of genetic, structural/metabolic, and unknown cause has been suggested to replace idiopathic, symptomatic, and cryptogenic. Accordingly, *genetic epilepsy* was defined as a direct result of a known or presumed genetic defect(s) in which seizures are core symptom of the disorder; *structural/metabolic* as a distinct other structural or metabolic condition or disease that has been demonstrated to be associated with a substantially increased risk of developing epilepsy and *unknown cause* with neutral meaning that the nature of the underlying cause is as yet unknown. (Berg et al. 2010)

Seizure type classification is based on the clinical signs of seizure onset. Symptomatology of a *focal* (partial) seizure is consistent with initial activation of only a part of one cerebral hemisphere (Blume et al. 2001) whereas involvement of both cerebral hemispheres from the beginning of seizure onset indicates *generalized* seizure

(Blume et al. 2001). Although a descriptive approach of consciousness and seizures is recommended by the newest ILAE guidelines for humans (Berg et al. 2010), dividing focal seizures based on the consciousness level as *simple focal seizures* (SFS) and *complex focal seizures* (CFS) (CFS impaired and SFS preserved consciousness) is still common in veterinary literature. Seizure propagation can occur when SFS progresses to a CFS or focal onset seizure to generalize (Berendt & Gram 1999).

Epileptic seizures can be self limiting or continuous. When the duration of a seizure episode is longer than 30 min or a cluster of epileptic seizures lasts the same time without regaining a normal baseline of function between, it is defined as *status epilepticus* (Bateman et al. 1999, Walker 2005). Also a 5 min (Saito et al. 2001, Brophy et al. 2012) and a 10 min (Monteiro et al. 2012) duration have been used to define SE in the latest literature. The occurrence of more than one seizure episode during 24 h is named a *cluster seizure* (Monteiro et al. 2012).

A seizure episode itself is named *ictus* and the period between the seizure episodes as an *interictal period*. An event shortly prior to the ictus characterized by sensory signs is referred as an *aura* (Berendt & Gram 1999). Behavioral signs without impairment of consciousness and without motor signs which precede hours or days (more than 1h) before the seizure are defined as *prodrome*. A period of behavioral changes, blindness etc., following the seizure episode (lasting minutes to hours) is called the *post-ictal period*. Prodrome and post-ictal signs are not counted as part of ictus, but aura (Berendt & Gram 1999) or short lasting behavioral, autonomic signs preceding ictus (< 1 h) (Licht et al. 2002) are considered to be part of the focal seizure onset.

## 2.2. Epidemiology of epilepsy

A broader spectrum of clinical epidemiological studies of epilepsy deals with classical concepts of incidence, prevalence, basic risk factors, and etiology in addition to a wider scope of socioeconomic questions, co-morbidities, and factors affecting outcome (Thurman et al. 2011).

Epilepsy is the most common chronic neurological disorder in humans; approximately 65 million people suffer from the disease worldwide. The annual incidence of epilepsy is 50 per 100,000 population in industrial countries (100-190/100,000/year in resource-poor countries) and the prevalence of active epilepsy generally 5-10 per 1000 (Sander 2003, Thurman et al. 2011). Lifetime prevalence rates in humans are much higher, as up to 5% of the population may experience seizures at some point in life (Sander 2003). Naturally occurring epilepsy is reported in many animal species including rodents, dogs, cats, horses, cattle, goats, and non-human primates (Chandler 2006). Seizures are also the most frequent neurological problem in dogs, with an estimated prevalence from 0.5 to 5.7% (Licht et al. 2002), comprising 2-3% of canine patients treated at veterinary teaching hospitals (Podell et al. 1995) and involving 10% of neurological problems (Jaggy & Bernardini 1998). IE has been diagnosed in 25% (Berendt & Gram 1999) to 80% of dogs with seizures (Schwarz-Porshe 1994). A number of dog breeds have been

described to have an increased risk of IE with the highest reported prevalence of 33% in the Belgian Shepherd family (Berendt et al. 2009).

The genetic mechanisms of IE are likely to vary not only between breeds, but possibly even within the same breed (Licht et al. 2002). Recently described first loci and candidate genes for IE in dogs (Ekenstedt et al. 2011, Seppälä et al. 2012) represent the first steps to defining the epilepsy genes. LGI2 truncation has been demonstrated to cause benign focal onset juvenile epilepsy in Lagotto Romagnolos (Seppälä et al. 2011). In clinical medicine, approximately 70% of all epilepsy patients lack an obvious extraneous cause and are presumed to have a predominantly genetic basis and 40% are thought to have a complex genetic basis with an unknown number of susceptibility genes (Heron et al. 2007, Dibbens et al. 2007). Some dominant and autosomal recessive epilepsy genes are already known (Heron et al. 2007). Until now, the suggested modes of inheritance for IE in dogs include: autosomal recessive in Keeshonds (Hall & Wallace 1996), Vizsla (Patterson et al. 2003), Standard Poodles (Licht et al. 2007), Belgian Shepherds (Berendt et al. 2009), and Border Collies (Hülsmeyer et al. 2010); partially penetrant autosomal recessive in Irish Wolfhounds (Casal et al. 2006); and polygenic recessive in Golden Retrievers (Srenk & Jaggy 1996), Labrador Retrievers (Jaggy et al. 1998), and Bernese Mountain dogs (Kathmann et al. 1999). In Beagles, autosomal recessive and sex-linked suppressors have been reported (Bielfelt et al. 1971). The male predisposition for IE has been reported for Golden retrievers (Srenk et al. 1994), Bernese Mountain dogs (Kathmann et al. 1999), and Irish Wolfhounds (Casal et al. 2006), when female prevalence was found in Belgian Shepherds (Berendt et al. 2008). Overall 1.5 times higher predisposition to epilepsy in male dogs has been found in one study from the UK which included 539 dogs involving multiple dog breeds encompassing patients collected from veterinary practices (Kearsley-Fleet et al. 2013). Epidemiological studies of human epilepsy indicate a slightly higher incidence of epilepsy in males overall (Kotsopoulos et al. 2002), but gender susceptibility varying for specific epilepsy subtypes (Christensen et al. 2005).

Many predisposing factors for epileptic seizures have been investigated in dogs, including feeding habits, time of day or year, the weather, lunar cycle, and sex cycle, but no significant associations have been made (Berendt et al. 2002, Hülsmeyer et al. 2010, Browand-Stainback et al. 2011). Some authors have found that seizures usually occur at the time of rest or sleep (Langweiler & Jaggy 1998, Morita et al. 2002, Weissl et al. 2012). On the other hand, various nonspecific stress situations have also been reported to trigger seizures in a high proportion of dogs (Heynold et al. 1997, Berendt et al. 2008, Hülsmeyer et al. 2010). High stress levels and significant life events acting as precipitating factors for seizure occurrence have also been documented in both humans (Temkin & Davis 1984, Koutsogiannopoulos et al. 2009) and some animal epilepsy models (Joels 2009).

Numerous predictors of seizure outcome have been suggested in human epilepsy, such as sex, seizures in relatives, prior neonatal seizures, prior febrile convulsions, age at

seizure onset, abnormal neurological status, seizure frequency at onset of seizures, seizure etiology, type and number of seizure types, type of epilepsy syndrome, time prior to the onset of drug therapy, number of seizures prior to the onset of drug therapy, age at the onset of drug therapy, the early effect of drug therapy, and the number seizures during early drug therapy (Sillanpää 2000). In contrast, only a few factors have been found to be predictors of epilepsy outcome in dogs, such as early initiation of treatment, an advanced age at seizure onset, and a high body weight (Heynold et al. 1997, Saito et al. 2001, Berendt et al. 2007, Hülsmeyer et al. 2010). A significantly shorter life span has been reported for dogs in which euthanasia or death was directly caused by epilepsy, and epileptic females live longer than males (Berendt et al. 2007). Some breeds have a shorter life span with IE, including Australian Shepherd dogs (Weissl et al. 2012), Border Collies (Monteiro et al. 2012, Hülsmeyer et al. 2010), and Irish Wolfhounds (Casal et al. 2006). Other breeds, like German Shepherds and Boxers, have been found to have a high occurrence of cluster episodes as a criterion for poor prognosis (Monteiro et al. 2012). On the contrary, no significantly shortened life span has been found in the family of Belgian Shepherds with genetic epilepsy, despite a majority of deaths related to epilepsy (Gulløv et al. 2012). Expected life span in dogs with IE has also been reported in another study, but status epilepticus was found as a factor shortening survival time (Saito et al. 2001). In dogs with juvenile IE, survival time was shortened with a history of status epilepticus. Diagnosis of symptomatic epilepsy and number of antiepileptic drugs used before investigation also shortened survival in dogs with juvenile epilepsy (Arrol et al. 2012). One study found a negative influence of neutering on the occurrence of cluster episodes (Monteiro et al. 2012), whilst others found that neutering may influence seizure frequency and seizure severity in female Belgian Shepherds (Berendt et al. 2008). Genetic studies have demonstrated polymorphism in the *ABCB1* gene to be associated with Phenobarbital treatment and seizure outcome in border collies (Alves et al. 2011) and collies (Muñana et al. 2012), but not in Australian Shepherd dogs (Weissl et al. 2012).

Epilepsy remission rates have been reported to vary from 13.7% in Belgian Shepherds (Gulløv et al. 2012) to 24% in Danish Labrador retrievers (Berendt et al. 2002), but nearly all Lagotto Romagnolos with benign juvenile epilepsy have had remission (Jokinen et al. 2007). However, as the remission has not been determined in a constant way (seizure freedom minimally 1 to 3 years), direct comparison between the studies and breeds may be complicated (Berendt et al. 2002, Berendt et al. 2007, Hülsmeyer et al. 2010). In humans over 70% of epileptic patients will be suspected to enter remission (Sander 2003).

## 2.3. Clinical phenotype of epileptic seizures

An epileptic seizure is a clinical event and can affect motor, sensory, and autonomic function, consciousness, emotional state, memory, cognition, or behavior, depending on location of onset in the brain (Fisher et al. 2005). Some signs from this wide range of epileptic phenomena recognized in humans are complicated or nearly impossible to

assess in animals. Therefore a simplified approach to classify seizures in animals should be more appropriate.

Canine ictus may be evident from motor, behavioral or autonomic signs as a single nominator or in different combinations. Motor signs are isolated to a body part or are unilateral when focal and presented as tremors, tonic or clonic movements, difficulties to walk or stand, or abnormal body deviations. They have been recognized from 45% (Hülsmeyer et al. 2010) to 100% (Licht et al. 2002) of dogs. Psychic or behavioral signs have been occurring from 10% (Licht et al. 2002) to 92% (Gulløv et al. 2011) of dogs and are defined as paroxysmal signs of anxiety, restlessness, unprovoked aggression, and/or out of context behavior. Compulsive tail chasing, fears, hyperactivity, and phobias in Bull terriers might be related to focal seizure activity (Dodman et al. 1996). Epigastric sensations, papillary dilatation, lacrimal secretion, and urinary or fecal incontinence are described autonomic signs and occur in between 23% (Berendt et al. 2004) and 67% (Licht et al. 2002) of dogs. Automatism has been defined in clinical medicine as more or less coordinated, repetitive, motor activity usually occurring with impaired cognition (Blume et al. 2001). Seizure related behaviors such as chewing or swallowing movements, lip smacking, body scratching, as well as changing position or circling when performed repeatedly have been interpreted in veterinary literature as automatisms and therefore indicative to impaired consciousness (Licht et al. 2002). For assessment of consciousness in dogs, responsiveness when owner tries to get their attention and ability to keep attention, in addition to whether the animal is able to navigate in any way (to walk, run or jump) have been applied (Licht et al. 2002).

Focal seizures, with or without secondary generalization, have been reported to be the predominant seizure type of IE for multiple dog breeds, including Dalmatians and Standard Poodles (Licht et al. 2002), Labradors retrievers (Berendt et al. 2002), Vizslas (Patterson et al. 2003), Belgian Shepherds (Berendt et al. 2008), Border Collies (Hülsmeyer et al. 2010), and Petit Basset Griffon Vendeen (Gulløv et al. 2011). Focal seizures with generalization, however, are the most frequent seizure type. A high incidence of generalized seizures (75-90%) has been previously reported in dogs with IE (Schwarz-Porshe 1994, Jaggy & Bernardini 1998). Since more recent veterinary studies have started to pay attention to the initial clinical signs preceding the generalized phase of seizure, seizures have been classified as generalized less frequently in dogs (Licht et al. 2002). Generalized seizures have been reported to be the predominant seizure type in Irish Wolfhounds (Casal et al. 2006), and also involving almost half of the seizures in English Springer spaniels with IE (Patterson et al. 2005).

During generalized seizure, animals may fall to the side and lose consciousness at some point. Motor signs are bilateral and largely symmetrical, most frequently tonic-clonic movements are observed during generalized seizure phase, but tonic, clonic, myoclonic, or atonic signs are also described in dogs. Canine absence seizures are complicated to define, although described when accompanied with myoclonic features (Poma et al. 2010). Automatisms as coordinated paddling of four limbs can be seen. In generalized

seizures, autonomic signs can be observed nearly as frequently as motor signs (Licht et al. 2002). The post-ictal phase in dogs is characterized by restlessness, thirst, hunger, aggression, or brief post-ictal blindness (Hülsmeyer et al. 2010). The duration of the post-ictal phase has been shown to increase with disease progression (Weissl et al. 2012).

A number of characteristics have been shown to be significantly different when comparing seizures in dogs with IE and symptomatic epilepsy, including: age, body weight, presence of partial seizures, cluster seizures, status epilepticus, ictal vocalization and neurological deficits (Pakozdy et al. 2008). IE has been reported to be more probable in dogs that are between 1 and 5 years at the time of seizure onset, if the seizure occurred between 8 am and midnight, or in dogs over 15 kg or if the interval between the first and the second seizure exceeded 4 weeks (Podell et al. 1995). Median seizure onset has been reported to be between 2 and 3 years for numerous dog breeds, but the seizure onset ranged from 2 months to 10 years (Jaggy & Bernardini 1998, Kathmann et al. 1999, Patterson et al. 2003, Patterson et al. 2005, Casal et al. 2006, Hülsmeyer et al. 2010). Dogs with juvenile forms of IE might start to have seizures as early as 5 weeks of age (Jokinen et al. 2007, Arrol et al. 2012).

## 2.4. Diagnosis of idiopathic epilepsy

Diagnosis of epilepsy is primarily based on the history of recurrent seizures, but general physical and detailed neurological examinations are basic assessments that should be performed on every animal with the history of seizures in order to detect changes indicative of symptomatic epilepsy or of general disease conditions (Lorenz et al. 2011a). Dogs diagnosed with IE are supposed to have no neurological deficits when examined interictally. Some neurological deficits (i.e. disorientation, blindness) may persist when the animal is examined during the post-ictal period even longer than 24 h (Hülsmeyer et al. 2012).

### *2.4.1. Laboratory diagnostics*

Laboratory diagnostics, including complete blood count, serum chemistry profile, and urine analysis, are often considered to be the minimal required examinations for the diagnosis of IE in order to exclude extracranial causes for seizures (Thomas 2010). A serum chemistry profile includes glucose, total protein, albumin, globulin, blood urea nitrogen, creatinine, total bilirubin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, creatine kinase, cholesterol, and electrolytes (Na, K, Ca, P, Mg). Paired serum bile acids, collected after 12 h fasting and postprandial, have been used in young animals to rule out a portosystemic shunt (Webster 2005). Specific gravity, chemistry, and sediment examinations should be performed from urine samples. An increasing number of diseases that cause seizures in dogs can also be confirmed from various tissue samples using genetic tests (Lohi et al. 2005, Awano et al. 2006).

Cerebrospinal fluid (CSF) is collected by a cerebellomedullary cisternal puncture under general anesthesia and used to rule out intracranial inflammatory, infectious, or neoplastic diseases. Total cell counts can be measured using a hemocytometer or mirror counting chamber. Differential white blood cell counts and cytological examinations can be performed on samples prepared with a cytocentrifuge or after sedimentation methods to concentrate cells (Lorenz et al. 2011b). For measurements of protein concentration in the CSF, qualitative methods such as the Pandy test, or quantitative analysis can be performed. Increased protein level in CSF is a non-specific indicator of central nervous system pathology. In the dogs with IE, CSF should remain within reference range regarding total nucleated cell count (< 5 cells/ul) and protein level (< 250 mg/l). A mild increase in total cell count of the CSF early after ictus may occur (Goncalves et al. 2010). Electrophoresis is used to quantify the levels of protein fractions or immunoglobulins (Lorentz et al. 2011b) and measurements of C-reactive proteins in CSF in order to exclude inflammatory processes within central nervous tissue (Martinez-Subiela et al. 2011). Levels of neurotransmitters, such as γ-aminobutyric acid, glutamate, and aspartate, may be lower in dogs with IE, but not equally in all dog breeds (Ellenberger et al. 2004). The ability to identify a definitive diagnosis from the CSF can be improved with microbiologic testing, measurement of antibody titers, polymerase chain reaction (PCR) testing, and immunocytochemistry (Lorenz et al. 2011b). CSF analysis has been demonstrated to be of value as it is an indicator of central nervous system pathology; MRI shows changes in only 6% of dogs with seizures and with normal interictal neurological examination and CSF results while MRI abnormalities were present in 43% of dogs with abnormal CSF and normal neurologic examination results and in 97% of dogs with abnormalities detected in both, in CSF and neurologic examination (Bush et al. 2002).

### *2.4.2. Magnetic resonance imaging (MRI)*

The physical nuclear magnetic resonance phenomenon was discovered 1946, but the first applications of medical imaging appeared much later – in the 1980s (Jackson et al. 2005a). MRI is based on the visualization of the hydrogen atoms nucleus using strong magnetic field and radiofrequency pulses (Jackson et al. 2005b, Gavin 2009). For the routine imaging of the neurocranium, T1-weighted (T1W) and T2-weighted (T2W) images in 3 standard planes (i.e. sagittal, transverse, dorsal) are used. Multiplanar reconstruction (MPR) which is 3D of T1W sequence could be used instead of T1W images and reconstructed later in all standard planes. Inversion recovery sequences as fluid-attenuated inversion recovery (FLAIR) can be used to improve delineation of primary lesions from the oedematous changes and distinction between the gray and white matter (Blümcke 2011, Bagley et al. 2009). Diffusion-weighted imaging could be applied when brain infarction is suspected (Major et al. 2012). Repeating T1W images or MPR after injection of paramagnetic contrast agent (i.e. gadolinium - dimeglumine gadopentate) is indicated when inflammatory or neoplastic changes, altering the blood-brain-barrier of the cerebral vasculature are suspected (Bagley et al. 2009). Contrast agents generally cause an increase in T1W signal which is recognized as increased hyperintensity in T1W images (Gavin 2009).

MRI is considered the most valuable diagnostic tool for investigating the etiology of epilepsy *in vivo* because the detection of lesions is an important factor in planning epilepsy management and in predicting the prognosis of individual human patients. Over 80% of humans with epilepsy have a focal epilepsy, and only 43% have a cerebral lesion (Lee et al. 2002). MRI identifies lesions more frequently in patients with temporal lobe epilepsy (76%) than with extratemporal epilepsy (47%) (Casse et al. 2002). Hippocampal sclerosis, the main cause of temporal lobe epilepsy in humans, is detected by MRI in approximately 55% of the patients (Lehericy et al. 1997). The detection of lesions has improved since the application of higher strength magnetic fields and computer-based postprocessing analysis (Kuzniecky et al. 2002, Knake et al. 2005). In addition to visual evaluation of the images, different quantitative measurements can be applied. Hippocampal MRI volumetry is an established technique and performed in epileptic humans with high confidence (Jackson & van Paesschen 2002, Jeukens et al. 2009).

The majority of veterinary MRI studies are performed using low magnetic field equipment (Konar & Lang 2011); although, some recent publications indicate that animals also might benefit from the use of 3 or 7 T MRI machines (Kang et al. 2009, Martin-Vaquero et al. 2011). MRI examinations are used increasingly for epileptic canine patients, but are more frequently performed to examine patients with suspected symptomatic epilepsy. MRI findings from the dogs having seizures as a result of brain tumors (Thomas et al. 1996, Kraft et al. 1997, Bush et al. 2002, Cherubini et al. 2005, Schwartz et al. 2011), inflammation (Sawashima et al. 1996, Kuwabara et al. 1998, Mariani et al. 2001), vascular problems (Kitagawa et al. 2008, Timm et al. 2008), malformations (Kitagawa et al. 2003, Jeffery et al. 2003, Jeffery 2005, Davies et al. 2013), and metabolic diseases (Garosi et al. 2003, Moon et al. 2012) have been described. In dogs, MRI has been approved to be both sensitive and specific for identifying brain lesions and classifying disease as inflammatory or neoplastic, with the exception of cerebrovascular disease in general or specific inflammatory or neoplastic disease, which can frequently be misclassified (Wolf et al. 2012). A low-field MRI study on canine seizures, associated with normal interictal neurological examination and no identifiable metabolic cause, has reported brain changes in 2.2% of dogs younger than 6 years and 26.7% of dogs older than 6 years (Smith et al. 2006). Structural imaging with MRI is suspected to be within normal limits in dogs with IE (Chandler 2006, Thomas 2010). MRI results might also be invariably normal (Bagley et al. 2009) or reveal only mild nonspecific changes (Koie et al. 2004) in dogs with degenerative and metabolic diseases.

Seizure activity may cause structural brain changes that are most likely to occur when MRI is performed within 24 h from the seizure episode. These changes are seen as focal hyperintensive regions on T2W sequences and may be reversible when adequate seizure control is reached. Changes are suspected to resolve between 10 to 16 weeks following seizures.(Mellema et al. 1999) Hippocampal sclerosis is known to be a secondary pathology in humans and dogs, because hippocampal neurons are vulnerable to

excitotoxic damage by intense and prolonged seizure activity (Jackson & van Paesschen 2002, Buckmaster et al. 2002, Fatzer et al. 2000). Secondary hippocampal changes in the brains of dogs with suspected IE have been reported (Hasegawa et al. 2002, Morita et al. 2002). Pseudolesions in the hippocampus region have been described in MRI of seizuring dogs which appear to be artifacts caused by the fat containing petrous temporal bone (Cooper et al. 2010).

An automatic voxel-based morphometry technique is described for the canine model of aging to assess regional gray and white matter brain atrophy and provide higher diagnostic yield compared to the manual ROI drawing method (Tapp et al. 2006). Lateralized hippocampal atrophy in epileptic dogs was demonstrated using MRI in one exceptional study. When hippocampal atrophy was observed in 12% of epileptic dogs using visual evaluation of images, volumetry then described an asymmetry ratio of more than 6% observed in 48% of epileptic dogs. (Kuwabara et al. 2010) Another recent study pointed out difficulties in using hippocampal volumetry in dogs, namely large variation in canine skull size, poor interobserver agreement, and amount of time required to perform volumetry (Milne et al. 2013).

### *2.4.3. Electroencephalography (EEG)*

The EEG is a record of the spontaneous electrical activity of the cerebral cortex. This method has been used in human patients to diagnose and manage epilepsy since 1935 when Gibbs and colleges described spike and wave discharges (Pillai & Sperling 2006). The EEG became commonly used in the 1950s, but the importance of clinical EEG has been diminished during the past 30 years, except for epilepsy (Niedermeier & Schomer 2011). In addition to confirmation of epileptic cerebral activity, EEG can supply detailed localization of epileptogenic foci and thereby play an important role in seizure classification (Commission of Classification and Terminology of the ILAE 1981, 1989, Flink et al. 2002). According to guidelines for EEG methodology in the diagnosis of epilepsy (Flink et al. 2002) the use of "modified combined nomenclature" derived from 10-20 system for electrode location is advised (Klem et al. 1999). Routine montages should include bipolar montages with longitudinal and transverse chains and referential montages. The duration of recording time should include a minimum of 30 min of artifact free recordings. (Flink et al. 2002)

EEG recordings are interpreted on the basis of the frequency and amplitude of background rhythms, the presence or absence and distribution of abnormal events and precise characteristics of abnormal events (Pillai & Sperling 2006).

Background rhythms are distributed to the spectral frequency bands namely defined in humans as delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), and beta (14–40.0 Hz) (Noachtar et al. 1999). The gamma frequency band also described in humans (>40Hz) is not considered in veterinary studies. Epileptiform activity can be found in interictal EEG of epileptic patients and consists of transients clearly distinguishable from background activity, with characteristic spike morphology (Noachtar et al. 1999). These

paroxysms of epileptiform activity represent the summation of excitatory and inhibitory postsynaptic potentials associated with hypersynchronous neuronal firing with paroxysmal depolarization shift and following hyperpolarization (Pillai & Sperling 2006).

In human patients with a history of seizures, focal interictal discharges suggest diagnosis of localization related epilepsy, and the character and location of changes may suggest etiology of the epilepsy syndrome and location of the epileptogenic region (Pillai & Sperling 2006). Generalized epileptic discharges are a hallmark of one of the generalized epilepsy syndromes, including idiopathic and symptomatic generalized epilepsies. (Stern 2005)

Interictal epileptic discharges have been detected by EEG in 29% to 55% of human patients (Pillai & Sperling 2006). When outpatient EEG is repeated up to four times, detection of interictal epileptic discharges could rise up to 90%. EEG sensitivity is highest in children and lowest in elderly patients. (Stern 2005) Epilepsy medication with benzodiazepines and barbiturates decrease the occurrence of interictal epileptic discharges, but only when given acutely. Importantly, not all interictal spikes and sharp waves are associated with epilepsy (i.e. benign epileptiform transients of sleep, wicket spikes, rhythmic midtemporal theta discharges, vertex sharp waves, and midline theta rhythm) and are considered as benign findings unrelated to seizure disorders. (Pillai & Sperling 2006)

Sleep has been known to increase detection of interictal epileptiform discharges and ideally EEG recording should include 30 min of sleep. Sleep recording has been reported to detect epileptic discharges in 40% of humans on whom no epileptic discharges where seen at wakefulness. Sleep deprivation for 24 h increases the detection of epileptic discharges by 20% or more. (Pillai & Sperling 2006)

The effect of hyperventilation is most impressive with absence seizures when it increases generalized spike–wave activity in 50-80% of patients. The hyperventilation may activate focal epileptic discharges in 6% of patients with complex focal seizures and clinical seizures in over 4% of patients. (Pillai & Sperling 2006)

Photic stimulation induces epileptic discharges in many epileptic patients. It usually signifies a variety of generalized epilepsy syndromes. According to some authors, photic stimulation may trigger epileptiform discharges in patients with focal seizures when they arise from the occipital lobe. (Pillai & Sperling 2006)

Interictal epileptic discharges may rarely occur in children (1.9–3.5%) and adults (0.2–0.55) without epilepsy. Interictal epileptic discharges in some locations are less likely to indicate epilepsy, as only 40% of patients with central-mid-temporal spikes, or 50% with occipital spikes, have seizures (Stern 2005, Pillai & Sperling 2006). Therefore EEG findings should always be interpreted in the context of clinical history, physical

examination, and neuroimaging findings, otherwise misdiagnosis is possible. (Pillai & Sperling 2006)

Long-term video-EEG may be the only method to distinguish epileptic from non-epileptic seizures and is performed on hospitalized patients. When suspecting ictal signs, patient responsiveness and muscle tone, etc. could be tested. (Flink et al. 2002) Video-EEG recording could also potentially be employed in the future for animal patients. However, multiple methodological issues need to be resolved before good quality long-term recordings on awake animals could be applied to everyday epileptic canine patients.

In the backwash of human EEG research, veterinary investigators started to publish about the EEG in dogs and cats. Methods of interpreting animal recordings were adapted from human EEG. Despite extensive work done in the 1960s until the mid-80s (Croft 1962, Redding 1964, Klemm 1968a, 1968b, Redman et al. 1973, Knecht et al. 1984), EEG never reached a similar importance in diagnosing epilepsy in dogs, relative to human patients. Early studies were made on conscious animals (Croft 1964). Later numerous restraints were employed when examining dogs with the EEG, associated with a variety of needle placements and montage protocols, but no consensual agreements about standard EEG protocols have been reached (Itamoto et al. 2002, Bergamasco et al. 2003, Pellegrino & Sica 2004, Davis et al. 2011, James et al. 2011).

In sedated dogs, there is a predominance of high frequency and high amplitude background rhythms (δ and θ) of the EEG (Moore et al. 1991, Itamoto et al. 2002, Bergamasco et al. 2003) whilst in conscious animals, low amplitude (<20uV) and high frequency rhythms are more prevalent (Klemm 1989). In visual evaluation of the EEG from sedated dogs, normal sleep transients as spindles, k-complexes, vertex sharp transients, and positive occipital sharp transients can be found (Bergamasco et al. 2003).

Epileptiform paroxysmal discharges can be seen as a focal or generalized pattern consisting of spikes, polyspikes, sharp waves, and spike-and-slow-wave complexes that can be interictally recorded from epileptic dogs (Holliday & Williams 1998, Berendt et al. 1999, Brauer et al. 2011), but these findings do not indicate certain etiology. Some authors have claimed that generalized epileptiform discharges are more indicative of IE and symptomatic epilepsy should be suspected when focal epileptic activity is found on the EEG (Holliday & Williams 1998). Some low frequency focal changes without spikes might be indicative of symptomatic epilepsy (Klemm 1989, Berendt et al. 1999). EEG abnormalities are paroxysmal in dogs with IE whereas in dogs with symptomatic seizures, abnormalities appear nearly all the time (changes in focal background) and might be somewhat helpful (Klemm 1989, Holliday & Williams 1998).

The incidence of interictal paroxysmal discharges on the EEG in dogs with epilepsy has been reported to range from 55% to 99% (Klemm & Hall 1970, Jaggy & Bernardini 1998, Berendt et al. 1999, Pellegrino & Sica 2004). However, recent publications have

suggested a much lower diagnostic yield of EEG (0-25%) when examining dogs with IE interictally (Brauer et al. 2012, Pakozdy et al. 2012).

In canine EEG, the effects of selected activation techniques like photic stimulation and hyperventilation have been shown to be of little value (Holliday et al. 1970, Brauer et al. 2011, 2012). Continuous EEG has been used for monitoring the status epilepticus in dogs and cats (Raith et al. 2010). There is some evidence that interictal EEG would show a decrease of paroxysmal discharge detection rate when following efficacy of antiepileptic medication in epileptic dogs (Wrzosek & Nicpon 2012). EEG has been used in evaluating the efficacy of gold wired implants inserted to acupuncture points in dogs with uncontrolled seizures (with IE); no changes were noted in quantitative EEG despite clinical seizure reduction by at least 50% (Goiz-Marquez et al. 2009). In addition, EEG has been used to distinguish paroxysmal dyskinesia in Chinook dogs from epileptic seizures (Packer et al. 2010). The radio-telemetric method to record EEG in awake dogs to evaluate proconvulsant risk of the medication in safety pharmacology has been also described (Dürmüller et al. 2007).

### *2.4.4. Positron emission tomography (PET)*

PET was developed in the 1970s and has been increasingly used during last couple of decades as a research tool, but also for many clinical fields, e.g. in neurology, oncology, and cardiology (Casse et al. 2002, Juhasz al. 2005, Alavi et al. 2011). PET scanning allows noninvasive measurements of physiological and biochemical processes *in vivo* using compounds of interest labelled with short-living positron emitting isotopes such as $^{15}O$ (half-life 2 min), $^{11}C$ (20 min), and $^{18}F$ (110 min) (Brooks 1991). These positron-emitting radionuclides are produced with accelerators, typically cyclotrons (Saha et al. 1992). The PET scanners are based on detection of the annihilation photons in coincidence. The positron is the antiparticle of the electron and it has the same resting mass, but has a positive charge. Positrons are emitted when a proton in the proton-rich nucleus of an atom becomes a neutron. The positron combines with the electron after it has been traveling a few millimeters in the tissue. After the formation of the extremely short living positronium, the two particles annihilate, converting their mass to energy which is in the form of two 511-keV annihilation photons (gamma ray). (Fahey 2001) These photons move away from each other at the angle of 180 degrees, allowing external detection of them (Phelps & Mazziotta 1985). Circumferentially located radiation detectors of a PET scanner detect the number of emitted photons, but counting only coincidence photons (detected within 10 ns) (Fahey 2001). Spatial resolution of PET is limited by the factor that the scanner detects not the place of positron emission which is question of interest, but the point of annihilation (Phelps & Mazziotta 1985). Accuracy of the PET scanner largely depends on the size and cross sectional geometry of the detectors (Fahey 2001, Juhasz et al. 2005) Therefore spatial resolution of even modern very high resolution small animal PET scanners is close to a millimeter (Park et al. 2007), which is about the distance the positron travels (Fahey 2001).

Data collected with PET is reconstructed to a cross-sectional image of the distribution of the PET tracer in the subject (Phelps & Mazziotta 1985). In static imaging, the tracer uptake is averaged during a determined time in steady-state, whereas in dynamic scanning, series of images are produced showing changes across time.

PET examinations are usually available only from special institutions as they require expensive specialized equipment, generally including a cyclotron on site because of short isotope half-lives (Saha et al. 1992). Importantly, radioactivity exposure may limit the possibility of several PET investigations on the same subject or to control individuals.

The most widely used PET tracer in epilepsy in humans with PET is FDG which reflects glucose utilization. FDG is transported to the tissue and phosphorylated like glucose by hexokinase. Unlike glucose, 2-deoxyglucose-2-fluoro-6-phosphate cannot be further phosporylated and gets trapped in the tissue in proportion to the local rate of glucose utilization. In the brain, this reflects synaptic density and functional activity of the brain tissue (Juhasz et al. 2005). A 3-compartment tissue model was developed by Sokolof to describe the uptake and metabolism of 2-deoxyglucose (Sokolof 1981). This model has been accepted for PET studies using the FDG, which is found to retain the same chemical properties with 2-deoxyglucose. Importantly, this model is applicable only for the steady-state condition.

Cerebral metabolism and blood flow are markedly increased in the epileptic focus during the ictal period, but interictally cerebral metabolic activity declines below normal levels (Alavi et al. 2009). Suggested mechanisms for interictal cerebral glucose hypometabolism include neuronal loss, reduction of synaptic density in pathways involved in seizure onset and spread, and interictal inhibitory processes. In IE, the hypometabolism is most likely an indication of interictal inhibitory processes. (Casse et al. 2002)

In clinical epileptology FDG-PET is mainly indicated for human patients with medically refractory seizures for presurgical evaluation when brain MRI is normal or shows only nonspecific abnormalities (Commission of Classification and Terminology of the ILAE 2000). Interictal FDG-PET has the highest diagnostic yield in patients with temporal lobe epilepsy (up to 90%) (Juhasz et al. 2005). Importantly, FDG-PET has shown correctly lateralized hypometabolism in 80% of patients with temporal lobe epilepsy and with normal MRI (la Fougere et al. 2009) and in more than 50% of patients with non-lateralizing surface ictal EEG (Juhasz et al. 2005). Therefore in many patients, FDG-PET could replace invasive intracranial EEG recordings in presurgical evaluation. However, it has been demonstrated, using ictal intracranial EEG recordings, that the hypometabolic regions seen in FDG-PET are not strictly confined to the seizure onset zone, but commonly extend beyond that and some hypometabolic cortical regions are not involved in seizure onset or early seizure propagation (Juhasz et al. 2005, Alkonyi et al. 2009). Hypometabolic cortical areas in interictal FDG-PET which are distant from

the suspected seizure onset zone may represent the functional deficit zone and be associated with psychiatric comorbidities (Goffin et al. 2008). One possibility to increase the accuracy of the visual findings of hypometabolic regions is to perform additional comparison with a template (based on normal patients) (Goffin et al. 2008, Kumar et al. 2010). Some novel PET tracers, like [$^{11}$C]flumazenil-PET which allow the demonstration of reduced benzodiazepine receptor binding, have been shown to reflect actual brain damage more reliably than FDG in temporal lobe patients (Lamusuo et al. 2000). The sensitivity of interictal FDG-PET for extratemporal epilepsies is almost half that reported for temporal lobe epilepsy (30-60%), depending on the localization of the epileptogenic focus (la Fougere et al. 2009).

Visual evaluation of FDG images is the basic assessment method applied in human epileptology. It is based on detection of regional asymmetrical changes in FDG uptake (Varrone et al. 2009). To minimize bias, it is advised to make the initial visual interpretation without clinical information or structural imaging information. These results are later correlated with clinical information, also comparing images of the patient and healthy controls for interpretation. It is emphasized that scans should be obtained with the same camera. (Neuroimaging Subcommission of the ILAE 2000) Coregistering of FDG-PET data with MRI of the patient is considered optimal for analysis (Neuroimaging Subcommission of the ILAE 2000, Cho et al. 2008).

Numerous quantification methods are used for functional neuroimaging, including FDG-PET. Regions of interests (ROI) on the different brain areas may be drawn on the single two-dimensional plane or on multiple planes which will be consolidated as 3-dimensional volumes of interests (VOI). Standardized uptake value, SUV, is a widely used, simple PET quantifier, calculated as a ratio of tissue radioactivity concentration (kBq/g), and injected dose (MBq) at the time of injection divided by body weight (kg). SUV is the most often used semi-quantitative measure also in veterinary FDG-PET literature (Irimajiri et al. 2010, Lee et al. 2010a, 2010b, Kang et al. 2012). When interpreting SUV values, large variations related to scanners, image acquisition, reconstruction, and ROI methodology should be taken into account (Westerterp et al. 2007, Varrone et al. 2009). To improve this comparison, the relative SUV ratio can be used, where the regional uptake is normalized with the uptake of the whole brain (Kang 2012). Normalization of uptake values with white matter, cerebellar, or brainstem regional activity can be preferred in some situations (Koivunen et al. 2008, Berti et al. 2012).

To semi-quantitatively evaluate lateralization of changes the left-right asymmetry index (AI) for all pairs of homologous VOIs can be calculated. In the formula AI= (L-R) x 100/([L+R]/2)%, positive AI indicates reduced glucose metabolism in the right and negative in the left side VOI. In epileptic human patients asymmetry between 10-15% indicates possible areas of reduced glucose utilization and over 15% indicates clear regions of hypometabolism. (Lamusuo et al. 2001)

Recently some studies have addressed the description of cerebral glucose metabolism measured by FDG-PET in healthy control dogs (Irimajiri et al. 2010, Lee et al. 2010a, 2010b, Hansen et al. 2011). One study has described SUV values for detailed brain regions using coregistration of high-resolution PET and 7T MRI. (Kang et al. 2012) The mean brain SUVs for dogs was ranging from 3.4 to 7.4 with a relative SUV of 1.0. (Irimajiri et al. 2010, Lee et al. 2010, Hansen et al. 2011, Kang et al. 2012). Focal cerebral glucose hyper- or hypometabolism caused by different types of encephalitis using the FDG-PET are also reported in dogs with seizures (Eom et al. 2008, Kang et al. 2010).

Regional brain perfusion, examined with interictal single-photon emission computed tomography (SPECT), another functional imaging modality, has also been reported in epileptic dogs recently (Martle et al. 2009).

# AIMS OF THE STUDY

The aims for this study were as follows:

1. To characterize phenotype, epidemiological characteristics, and inheritance of epilepsy in Finnish Spitz dogs.

2. To define typical findings of advanced diagnostic evaluation methods (MRI, EEG, and FDG-PET) for epilepsy in Finnish Spitz dogs.

3. To compare usefulness of MRI, EEG, and FDG-PET, to evaluate concordance of these modalities, and to compare different analytical methods for evaluating epilepsy in Finnish Spitz dogs.

# 3. MATERIALS AND METHODS

## 3.1. General inclusion criteria

Epidemiological study (I) was based on the Finnish Kennel Club database which contains names of all registered FSDs and contacts of the breeders, and on the Finnish Spitz Breeder Club (FSBC) database containing updated contacts from the dog owners. Dogs which participated in clinical studies (II-IV) were recruited using the FSBC database, collected after announcement where FSDs owners were encouraged to voluntarily inform about their possibility and wish to participate in clinical studies at the locations of Helsinki and Turku (II-IV) or close to their residence (III). The group of control FSDs was selected from the dogs without seizure history and no evidence of diseases known causing seizure-like episodes. Only FSDs with at least 2 seizure episodes and without any interictal neurological abnormalities and no evidence of other disease conditions which could cause seizures were included to the epileptic group in all four studies. Absence of changes in general physical and neurologic examination was confirmed by the European College of Veterinary Neurology diplomate or resident for all FSDs in all clinical studies (II-IV), performed to epileptic dogs interictally. In addition, absence of changes in CBC and blood biochemistry profile (II-IV), urinanalysis (II, IV), and CSF (II) were required for all FSDs for including studies. IE was defined for our studies as recurrent seizures with no underlying structural brain lesion or other neurologic or clinical signs, presumably being genetic.

## 3.2. Animals and study designs

General characteristics of control and epileptic FSDs included in four studies are presented in Table 1.

Table 1. General characteristics of control (C) and epileptic (E) Finnish Spitz dogs (FSDs) included in the investigations (I-IV)

| | Study I C/E | Study II C/E | Study III C/E | Study IV C/E |
|---|---|---|---|---|
| Number of FSDs | 1998/143 | 3/11 | 16/15 | 8/11 |
| Males | 952/86 | 3/10 | 9/11 | 3/8 |
| Females | 1046/57 | 0/1 | 7/4 | 5/3 |
| Mean age at examination (months) | 66/81 | 35/64 | 70/74 | 63/72 |
| Mean age at seizure onset (months) | NA[a]/39 | NA/30 | NA/40 | NA/28 |

[a] not applicable

### *3.2.1. Epidemiology, inheritance and phenotype of epilepsy (I)*

A prospective study via questionnaires and phone interviews was designed. The questionnaires (5960) were sent in the years 2003-2004 to all owners of 1-10 year-old FSDs. Information was obtained from 2299 dogs (38.6%). Some owners also had 10-15 years old living FSDs at home and submitted additional questionnaires about them (dogs were included in the study). Phone interviews (297) to the owners of all epileptic dogs, and when dogs' clinical status remained unclear were planned. All dogs with

insufficient information (117), dogs whose owners could not be contacted by phone (10), whose dogs had only one seizure episode at the time of phone call (24), and whose dogs were younger than one year at the time of answering the questionnaire (5), were excluded from the study (in total 156). Any inconsistencies in the number of dogs (n) included in the estimation of various answers was caused by incomplete filling of the questionnaires or when owners were not confident about the correct answer. Finally, 2141 FSDs of which 143 were epileptic were included in the study. Epilepsy prevalence was calculated for the FSDs who were alive at the time their owners answered the questionnaire (2069 dogs in total, 111 epileptics). For pedigree analysis, closely related families with multiple epileptic dogs over several generations were selected. IE was diagnosed by a veterinarian in 73 of the 141 (51.8%) dogs, based on seizure history in all of the 73 dogs and in addition by CBC and serum biochemistry in 35 (27.8%) dogs (Table 2). Seizure types were classified as SF, CF, focal with secondary generalization, secondary generalized with unknown onset, and primary generalized.

### *3.2.2. Structural brain imaging with MRI (II)*

Control dogs (1 dog was excluded from the initial control group because of seizure episode 1 year later) and FSDs with focal epileptic seizures were studied with MRI for the detection of structural brain changes. EEG was recorded on the previous day and CSF samples from the cerebellomedullary cistern were collected immediately after MRI examination in all dogs (Table 1, 2).

### *3.2.3. Cerebral electrical activity measured with EEG (III)*

EEG in healthy and FSDs with epilepsy was recorded (1 dog was excluded from the initial epileptic group of 16 FSDs because of concurrent diabetes mellitus). A special tour was arranged in 2005 to record EEGs from 18 FSDs situated all around Finland (Table 1, 2).

### *3.2.4. Imaging cerebral glucose metabolism with FDG-PET (IV)*

FDG-PET in FSDs with and without focal epilepsy was examined (Table 1, 2). Median body weight for epileptic and control dogs was 14 (range, 10-19) and 12 (10-15) kg, respectively. Median age at seizure onset was 22 (range, 7-72) months. Median seizure duration and seizure frequency were 10 (range, 0.5-15) min and 3 (0.5-12) per year, respectively. One epileptic FSD had a seizure episode 14 h and one 24 h before PET examination. Four epileptic dogs were receiving treatment with antiepileptics.

## 3.3. Ethical considerations

All studies were approved by the Ethics Committee on Animal Trials at the University of Helsinki.

## 3.4. Questionnaire and phone interview (I)

General information (name, registration number, gender, personality trait, weight, height, food type, feeding habits, living conditions, and activities) was collected from all the dogs. Questions about the age at seizure onset, total number of observed seizures, seizure frequency and severity, existence of clusters or status epilepticus, possible risk factors for seizure occurrence, medical work-up, diagnosis and medication were asked from the owners of epileptic dogs.

Phone interviews followed 1- 2 years later. Interview focused on initial ictal signs and on changes in disease course and treatment. Interviewers provided dog owners with descriptions when needed and with additional list of categories. Specific questions were asked about the very beginning of the seizure episode to confirm the seizure type classification. The owners were asked to report changes in therapy or the course of the disease. The course of epilepsy was defined as stable (where the frequency and length of the seizure episodes remained the same), progressing (where the frequency and/or length of the seizure episodes was increasing or cluster episodes of status epilepticus had appeared), or diminishing (where the frequency and/or length of the seizure episodes was decreasing over time). The time period used to define the course of epilepsy in every affected dog started at the moment of the first seizure and ended either at the time of the owner's interview (if medication had not been initiated) or at the initiation of epilepsy medication. The owners were asked about the appearance of seizures that clearly differed to detect the presence of multiple seizure types in individual dogs. The situations under which seizures occurred were defined as up to two different conditions in the same dog or randomly when the dog experienced three or more conditions under which seizures occurred.

## 3.5. Diagnostic methods

Diagnostic evaluations performed to control and epileptic FSDs included in four studies are summarized in Table 2.

Table 2. Diagnostic evaluations performed to control (C) and epileptic (E) dogs in studies I-IV

| Diagnostic evaluations | Epidemiology (I) C/E | MRI (II) C/E | EEG (III) C/E | PET (IV) C/E | Total C/E |
|---|---|---|---|---|---|
| CBC[a] | 3/35 | 3/11 | 16/15 | 8/11 | 21/41 |
| Chem[b] | 3/35 | 3/11 | 16/15 | 8/11 | 21/41 |
| UA[c] | 3/11 | 3/11 | 3/11 | 8/11 | 10/15 |
| CSF[d] | 3/11 | 3/11 | 3/11 | 2/7 | 3/11 |
| MRI[e] | 7/11 | 3/11 | 3/11 | 7/11 | 8/14 |
| EEG[f] | 7/11 | 3/11 | 16/15 | 6/11 | 24/20 |
| PET[g] | 7/11 | 2/7 | 4/11 | 8/11 | 8/11 |

[a] complete blood count
[b] serum biochemistry
[c] urinalysis
[d] cerebrospinal fluid
[e] magnetic resonance imaging
[f] electroencephalography
[g] positron emission tomography

### *3.5.1. Laboratory analyses (II-IV)*

The following laboratory analyses were performed at the Central Laboratory of the Department of Equine and Small Animal Medicine, University of Helsinki. CBC and serum biochemistry (Na, K, Ca, P, Mg, glucose, total protein, albumin, globulin, cholesterol, blood urea nitrogen, creatinine, total bilirubin, alanin aminotransferase, aspartate aminotransferase, alkaline phosphatase, and creatine kinase) were examined from blood samples using an automatic chemistry analyser (KONE Pro, Konelab, Thermo Clinical Labsystems Oy, Vantaa, Finland). Urinalysis was collected by cystocentesis from sedated dogs. Specific gravity, chemistry, and sediment examinations were performed. CSF samples, collected from the cisternal puncture in anesthetized dogs were analysed [total cell count (RR < 5 leuc/µl); protein concentration (RR < 25 mg/dl)]. Cytology was analyzed using cytocentrifuge (II, III).

Plasma glucose was analyzed by glucose/glucose oxidase method with an Analox GM7 glucose analyzer (Analox Instruments Limited, London, UK) at Turku PET Centre (IV).

### *3.5.2. MRI (II, IV)*

MRI examinations were performed using 1.5 T Siemens Magnetom (Siemens AG, Medizinische Technik, Germany) and 1.5 T Picker Edge (Cleveland, OH) equipment at a private human hospital (Magneettimehiläinen) using standard human knee coils (II). Animals were under general anesthesia throughout the procedure. Dogs were premedicated with butorphanol tartrate (Torbugesic Vet, Fort Dodge Veterinaria SA, Girona, Spain) 0.2 mg/kg intramuscular (im) and atropine sulfate (Atropin, Leiras, Turku, Finland) 0.02 mg/kg im, 30 to 60 min before induction. Induction was performed with propofol (Rapinovet, Shering-Plough Animal Health, Farum, Denmark) 3-5 mg/kg intravenous (iv) and diazepam (Diapam, Orion Pharma, Espoo, Finland) 0.25-0.5 mg/kg iv to effect. Intubated dogs received oxygen through a non-rebreathing system at a rate of 2 L/min. Anesthesia was maintained with propofol infusion 0.3-0.6 mg/kg/min diluted in 0.9% saline solution. Pulse rate, respiratory rate, and end-tidal carbon dioxide were monitored during the procedure. Dogs were placed in the scanner in sternal recumbency.

Routine T1W and T2W images in all 3 standard planes (i.e., sagittal, transverse, dorsal) were recorded. MPR were used instead of T1W images in 4 epileptic and all control dogs, reconstructed later in all 3 planes as T1W images. FLAIR sequence was performed in these 4 dogs in the dorsal plane. T1W images or MPR were repeated immediately after bolus iv injection of gadolinium-diethylenetriaminepenta-acetate dimeglumine (Magnevist, Schering AG, Berlin, Germany) at 0.2 mL/kg (0.1 mmol/kg).

Additional MRI examinations within relation to FDG-PET examinations were performed with 1.5T Philips Integra (Philips, Best, The Netherlands) equipment at the Turku-PET Centre for 4 control FSD (IV). Anesthesia with medetomidine hydrochloride (30µg/kg) and butorphanol (0.2mg/kg) im injection was used.

Approximately 30 min after tracer injection, short MRI scanning protocol (at least T1W-3D and T2W images in transverse plane) was started.

MRI scans were reviewed independently by a board certified veterinary radiologist and a human radiologist. Only findings described by both reviewers were considered significant. The scans of healthy FSDs were examined first to identify any breed-specific differences. The scans were examined for 1) asymmetry and dilatation of fluid spaces, 2) developmental anomalies, 3) hippocampal abnormalities (size or structure), 4) proportions and localization of gray and white matter, 5) focal identifiable changes, 6) presence of inflammation, and 7) other (e.g. post-traumatic) changes.

MRI scans for study IV were reviewed by the author for absence of structural brain abnormalities and used for comparison with FDG-PET images.

### *3.5.3. EEG (II-IV)*

EEG examinations were performed at the Small Animal Clinic of the University of Helsinki (II-IV) and in six veterinary private practices covering different locations of Finland (III), using the same equipment and study protocols. Portable EBNeuro EEG equipment (Galileo Be Light Peripheral Configuration, Firenze, Italy) was used to record EEG under medetomidine sedation (40–60 mg/kg, im). Patients were positioned in sternal recumbency. A method of standardized placement of EEG electrodes that resembled the 10 to 20 international system for humans was used. EEG was recorded in 14-channel reference montage (F7, F3, F4, F8, T3, C3, Cz, C4, T4, P3, Pz, P4, O1, O2; sensitivity, 10 mV/mm; time constant, 0.3 seconds; Hf, 70 Hz; notch filter inserted; reference: on the ridge of the nose between the eyes; ground: caudally to the external occipital protuberance). Sixteen EEG needles (30-gauge 15-mm monopolar stainless-steel needle electrodes, Bionen, S.a.s., Firenze, Italy) were inserted as subdermal active, reference, and ground electrodes. Impedances did not exceed 5 kV. The ECG and respiratory rate were recorded via the polygraphic electrodes (Bionen S.a.s., Firenze, Italy) (for ECG: sensitivity, 70 mV/mm; time constant, 0.3 seconds; Hf, 70 Hz; and for respiratory rate: sensitivity, 20 mV/mm; time constant, 0.3 seconds; Hf, 70 Hz); EEG recording lasted 20 minutes, after which EEG data were stored in the acquisition station (RST Galileo System, EBNeuro, Firenze, Italy) for subsequent analysis.

The EEG records were visually examined (examiner blinded for dogs clinical status) in bipolar montage (III, IV). The sleep stage, possible normal variants, or epileptiform findings, without knowing the clinical status of the dogs were described. Additional review of EEG records was performed on referential montage to eliminate epileptiform patterns and artifacts from further background analysis. For all dogs, 60 replications of 2-second artifact-free epochs were randomly selected from the entire EEG to analyze 2 minutes of recording. For epileptic dogs, unsuppressed epochs without epileptic activity were analyzed. Background activity was analyzed (calculated and averaged for each channel) with the same acquisition station, and with an integrated software program Fast Fourier Transform. The spectral bands of delta (0.5–4.0 Hz), theta (4.5–8.0 Hz),

alpha (8.5–12.0 Hz), and beta (12.5–30.0 Hz) were calculated and expressed as relative power (%).

The EEG records for study II were examined visually relative to clinical information (in referential montage).

### *3.5.4. FDG-PET (IV)*

FDG-PET examinations were performed in the Turku-PET Centre. FDG was synthesized in Radiopharmaceutical Chemistry Laboratory from mannosyl triflate using a nucleophilic method. The radiochemical purity exceeded 95% in every production batch.

Animal preparation and scanning procedures were performed in a quiet and darkened room. Before the PET examination, dogs were fasted for minimum 8 h with water freely available and then sedated with medetomidine hydrochloride 30 μg/kg and butorphanol 0.2 mg/kg im injection. After 10-15 min, an iv catheter was inserted to a cephalic vein and blood samples for baseline glucose measurements were taken. Dogs received oxygen supply through the mask at the rate 2 L/min during the entire sedation period. Pulse rate and oxygen saturation were monitored during the procedure with a pulse-oxymeter. For dogs 5.2 ± 1.1 MBq/kg FDG was administered iv 15-25 min after sedative injection. Forty min after tracer injection an additional dosage of medetomidine 15 μg/kg and midazolam (Midazolam Hameln, Hameln Pharmaceuticals, Hameln, Germany) 0.2 mg/kg was injected im.

PET imaging was performed using a brain dedicated, high resolution PET scanner (ECAT HRRT, Siemens Medical Solutions, Knoxville, TN, USA) (minimal spatial resolution in the reconstructed images 2.5 mm in the 10 cm field of view). Dogs were scanned in sternal recumbency using head fixation foam wedges specially designed for this study. Scanning was started 55 min after injection of the tracer and emission data were collected for 40 min. After emission scan, a 7 min transmission scan for attenuation correction was performed. This protocol was also used for five control dogs. Transmission scan was not used for the three first epileptic dogs and for the sixth epileptic dog (dog moved before the end of scanning). For these four dogs, attenuation correction model was created on the base of the transmission scanning (mu-map image) data set collected from one of the healthy dogs. True events were normalized, corrected for attenuation and scatter (Watson 2000), and then reconstructed by the iterative ordered subsets expectation maximization 3D algorithm (Michel et al. 2000). Images were reconstructed into a volume of 256 x 256 x 207 cubic voxels, size 1.81 $mm^3$.

For three control dogs the same anesthesia protocol, but dynamic FDG-PET scan was used. PET-scanning was initiated simultaneously with the intravenous administration of FDG. Total emission scan duration was 75 min. Transmission scan started immediately after the emission study. For all the dogs, the images were reconstructed to similar time frames. For all epileptics and for 3 of the healthy dogs, a 20 min long frame from the beginning of the scanning-period was reconstructed.

Visual analysis of images was performed independently by the three authors using image analysis software Vinci 2.56 (Max-Planck-Institute for Neurological Research, Cologne, Germany). The scans of healthy and epileptic FSDs were reviewed in a randomized blinded manner. Co-registration with T1W-3D MRI was used when needed for anatomical localization of the findings. Any localized or lateralized visual asymmetries of the glucose uptake which were visible through at least 3 slices were sought. Changes were considered significant when at least 2 reviewers described a similar finding.

For the semi-quantitative analysis, volumes of interest (VOIs) were manually drawn over dorsal (coronal) slices using Imadeus Academic 1.0 software (Forima Inc, Turku, Finland). MRI multiplanar reconstruction 3D images were used as anatomical reference. Bilateral VOIs covered frontal, temporal, parietal, and occipital cortexes, hippocampus, nucleus caudatus, caudal colliculus, thalamus, and cerebellum. Maximal symmetry of bilateral VOIs and homology between individuals was sought after. Additionally unpaired VOIs to white matter, to gyrus cinguli and vermis cerebelli were drawn. For further analysis, VOIs were normalized against the white matter value of the same dog. Standardized uptake values (SUVs) were calculated as a ratio of tissue radioactivity concentration (kBq/g)/ injected FDG dose (MBq) and divided by body weight (kg).

The left-right asymmetry index (AI) for all pairs of homologous VOIs was used to estimate lateralization of changes. In the formula AI= (L-R) x 100/([L+R]/2)% positive AI indicated reduced glucose metabolism in the right and negative in the left side VOI.

### 3.6. Statistics

**Study I** A mixed logistic regression model was used for analyzing factors associated with epilepsy. A stepwise backward elimination procedure was used for fitting the final model. For dogs character description, a binary factor was used (lively or not) because a small number of dogs were characterized differently than lively (phlegmatic, depressed or nervous). The age of the dogs was adjusted for the probability to have epilepsy which increases with age including dogs age and quadratic term of age into the model. The dogs sire was used as a random factor to account for the clustering effect. Univariate analysis was used for evaluating different factors and epilepsy phenotype characteristics associated with disease progression (progressing or stable) or with generalized form of seizures (generalized group included all seizures with the generalized phase of seizure) in diseased dogs. For pairwise comparisons in multi-categorical factors, Bonferroni adjustment was used. Results from logistic regression analyses are presented as odds ratios (OR) with 95% confidence intervals (CI).

The heritability of epilepsy was evaluated by the variance component estimation with restricted maximum likelihood estimation method by using VCE 6.0 software (Neumaier & Groenevald 1998, Groenevald et al. 2008). The linear model used included epilepsy status as the dependent variable, sex, hunting (used or not), personality trait (lively or not), living condition (only outdoors or not), age, and

quadratic term of age as fixed factors. Random factors were additive genetic effect (using dog dam, sire and sire sire information) and error term. Heritability was calculated by dividing the additive genetic variance by the sum of additive genetic variance and residual variance.

**Study III** Gaussian distribution of the study groups was evaluated using Kolmogorov-Smirnov technique for normality, and the Wilk-Shapiro technique of the quantitative EEG data. Ordinary analysis of variance (ANOVA), in combination with the pairwise t-test for multiple comparisons with Bonferroni p correction, was applied first to evaluate the significance of differences. Bartlett's test was used to evaluate the differences among the standard deviations. The Kruskal-Wallis test (nonparametric ANOVA) and Dunn's Multiple Comparison test were used for reevaluating the significance of differences among the groups for delta, theta, and beta bands. Multivariate analyses were performed by using the multiple logistic regression technique with EEG activity type as the dependent variable, and age, sex, weight, heart rate, medetomidine dosage, number of drug administrations, and time from initial injection to the beginning of recording as the independent variables.

All data were expressed as mean ± standard deviation. For all electrodes of a single animal, the relative power mean value was calculated. The values of each derivation were averaged for every study group (14 derivations) and used later to calculate single values for every dog group. For statistical analysis, the epileptic group was divided into two subgroups: dogs with epilepsy without treatment (10 dogs) and dogs with epilepsy under phenobarbital treatment (5 dogs).

**Study IV** To compare epileptic and control FSDs` age and weight Wilcoxon rank-sum test were used and Fisher exact test was used to compare sex distribution of those groups. Fisher exact test was used to compare associations between epileptic status and visual findings of asymmetry in different brain regions. To compare differences between epileptic and control FS dogs in AI index values, SUV and white matter normalized values by brain region non-parametric Wilcoxon rank-sum test were used.

Above described statistical analyses were carried out using Stata versions 9 (I), 11.0 (IV) (Stata Corporation, TX, USA), and R version 2.2.1. (III) (Development Core Team 2005, Vienna, Austria).

The level of significance in all studies was set at 5% ($P < 0.05$).

# 4. RESULTS

## 4.1. Epidemiology, inheritance (I) and phenotype (I-IV) of epilepsy

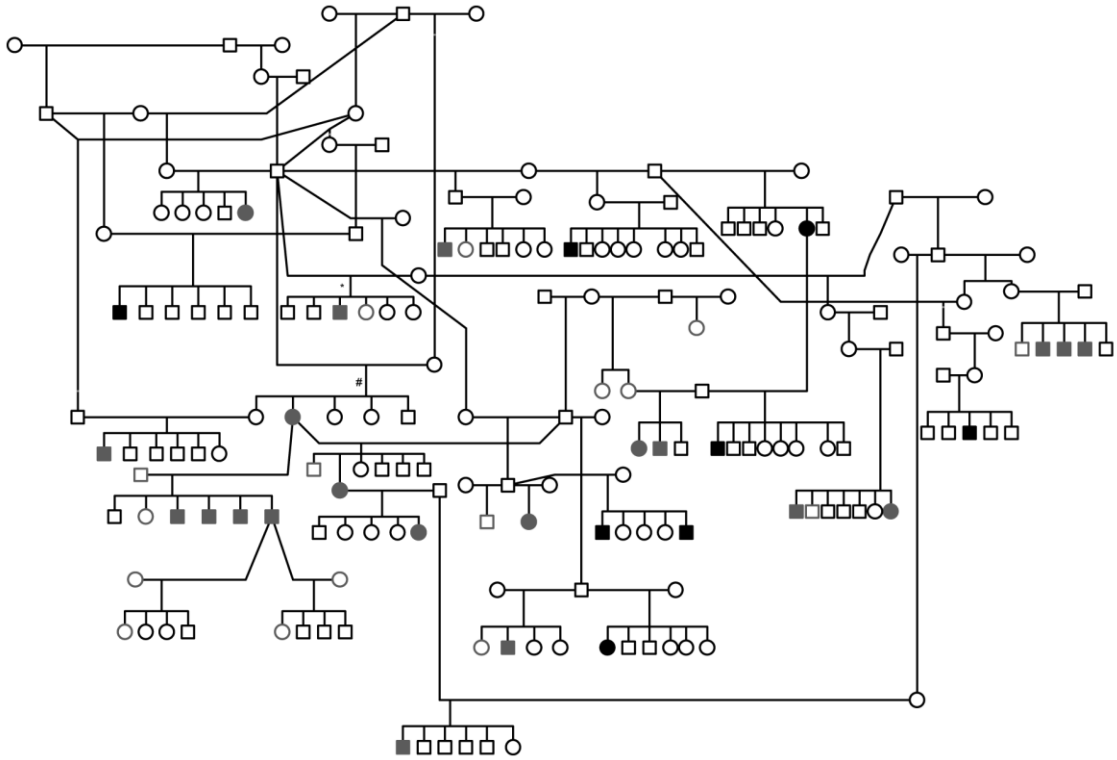
The prevalence of suspected IE in the Finnish population of FSDs was 5.36% (95% CI 4.43-6.43). Epilepsy was more prevalent among males than females (OR 1.7, 95% CI 1.2-2.5; $P = 0.006$). Other personality traits (phlegmatic, depressed, nervous) were more associated with IE than was the characteristic of lively (OR 5.9, 95% CI 2.9-11.7; $P < 0.001$). Being used in hunting and living exclusively outdoors were negatively associated with epilepsy (OR 0.5, 95% CI 0.3-0.9; $P = 0.01$ and OR 0.6, 95% CI 0.4-0.9; $P = 0.01$, respectively). General data used in epilepsy risk factor analyses are presented in Table 3.

Table 3. General data of healthy and epileptic Finnish Spitz dogs (FSDs) in epilepsy risk factor analysis.

| Factor | Healthy FSDs (n = 1998) | Epileptic FSDs (n = 143) |
|---|---|---|
| Median age (IQR[a]) (months) | n = 1998[b] | n = 143[b] |
| | 62 (38 & 87) | 76 (58 & 111) |
| Median height (IQR) (cm) | 46 (43 & 48) | 45.5 (43 & 48) |
| | n =1423 | n = 76 |
| Median weight (IQR) (kg) | 12 (10 & 14) | 13 (12 & 15) |
| | n = 1477 | n = 95 |
| Gender, n (%; 95% CI[c]) | n = 1998 | n = 143 |
| Male | 952 (47.6; 45.4-49.9) | 86 (60.1; 51.6-68.2) |
| Castrated | 37 (1.9; 1.3-2.5) | 5 (3.5; 1.1-8.0) |
| Female | 1046 (52.4; 50.1-54.6) | 57 (39.9; 31.8-48.4) |
| Neutered | 50 (2.5; 1.9-3.3) | 3 (2.1; 0.4-6.0) |
| Feeding times per day, n (%; 95% CI) | n = 1974 | n = 142 |
| Once | 792 (40.1; 38.0-42.3) | 44 (31.0; 23.5-39.3) |
| Twice | 958 (48.5; 46.3-50.8) | 67 (47.2; 38.8-55.7) |
| Three times | 224 (11.3; 10.0-12.8) | 31 (21.8; 15.3-29.5) |
| Food type, n (%; 95% CI) | n = 1913 | n = 134 |
| Commercial food | 531 (27.8; 25.8-29.8) | 35 (26.1; 19.0-34.4) |
| Home food | 282 (14.7; 13.2-16.4) | 16 (11.9; 7.0-18.7) |
| Mixed-food | 1100 (57.5; 55.2-59.7) | 83 (61. 9; 53.2-70.2) |
| Used in hunting, n (%; 95% CI) | n = 1983 | n = 142 |
| | 1784 (89.9; 88.6-91.3) | 110 (77.5; 69.7-84.0) |
| Other animals at home, n (%; 95% CI) | n = 1977 | n = 142 |
| | 1151 (58.2; 56.0-60.4) | 81 (57.0; 48.5-65.3) |
| Personality trait, n (%; 95% CI) | n = 1984 | n = 139 |
| Lively | 1941 (97.8; 97.1-98.4) | 122 (87.8; 81.1-92.7) |
| Phlegmatic | 5 (0.3; 0.1-0.6) | 2 (1.4; 0.2-5.1) |
| Depressed | 10 (0.5; 0.2-0.9) | 4 (2.9; 0.8-7.2) |
| Nervous | 28 (1.4; 0.9-2.0) | 11 (7.9; 4.0-13.7) |
| Living, n (%; 95% CI) | n =1980 | n = 133 |
| Only outdoor | 1446 (73.0; 71.0-75.0) | 80 (60.2; 51.3-68.5) |
| Outdoor and inside | 534 (27.0; 25.0-29.0) | 53 (39.8; 31.5-48.7) |

[a] 25% & 75% quartiles
[b] number of animals where information about evaluated factor was available
[c] confidence interval

The heritability estimate of suspected IE in FSDs was based on the linear variance component model and was 0.22 (SE = 0.07). The median inbreeding coefficient in epileptic dogs was 9 (25% & 75% quartiles: 8 & 11; range: 7-24) and that of healthy dogs was 9 (7 & 11; range: 5-57). The inbreeding coefficient was not associated with epilepsy. The segregation of IE in the sample of FSDs pedigrees is presented in Figure 1.



□ males; ○ females. FSDs that suffered from epilepsy are marked with filled symbols (black or gray), and dogs with no clinical manifestation of seizure episodes are represented by white symbols. Grey-bordered symbols indicate 21 affected and 16 unaffected dogs, which were clinically examined. The sire and dam marked with * were mated twice and produced a total of 11 full siblings. Only one litter is marked on the pedigree. The dogs from the other litter are healthy (not shown). The sire and dam marked with # were mated four times and produced a total of 14 full siblings. Three of these unaffected litters are not shown.

Figure 1. Segregation of idiopathic epilepsy (IE) in the pedigree of a sample of Finnish Spitz dogs.

The situations under which seizures occurred were stress or in connection with exercise (78 cases), during sleep or rest (35 cases), after eating (7 cases), and randomly (49 cases) (total 132 dogs). Other factors possibly related to the occurrence of episodes were the season of the year in 25 of 124 (20.2%), time of the day in 53 of 130 (40.8%), weather conditions in 11 of 134 (8.2%), sexual cycle in 10 of 122 (8.2%), or other disease conditions in 1 of 132 dogs.

The median age of the dogs at the onset of seizures was 3 years (25% & 75% quartiles: 24 & 48; range: 7-120 months). The median frequency of seizures was 2 seizures per year (1.5 & 4; range: 0.5-48). The median duration of ictus, including initial signs, was 12 min (7 & 22.5; range: 1.5-90 min) (n = 138). Initial clinical signs of ictus with a median length of 3 min (1.5 & 5.5; range: 0.5-60 min) were recognized in 87 of 127 dogs (68.5%). These signs were recognized as behavioral (i.e. hiding without reason) (n = 79), automatism (i.e. repeated changing of position or licking movements) (n = 10), motor (i.e. weakness or tremors) (n = 17), and autonomic (i.e. vomiting) (n = 3). The consciousness level at the time of the initial signs was classified as normal in 20 dogs and impaired in 66 dogs. The median duration of the ictal signs (not including the initial signs) was 10 min (5.5 & 20; range 1-80 min). The most important ictal signs were motor (n = 123), recognized as tremors (n = 17), weakness (n = 49), tonic-clonic (n = 40), tonic (n = 16), and clonic (n = 1). The motor signs were localized to the limbs in 59 dogs, to the face in 3 dogs, and involved the whole body of 61 dogs. Behavioral signs were recognized in 20 of the 119 dogs (16.8%). Autonomic signs (n = 105) included salivation (n = 87), vomiting or regurgitation (n = 38), and urination/defecation (n = 29). Automatisms were observed in 49 of 120 dogs, and they mainly involved coordinated paddling of all four limbs at the time of the generalized phase. The level of consciousness was classified in the final stage of ictus as normal in 3 dogs, impaired in 56, and lost in 67 (126 dogs total). Postictal signs including restlessness, impaired responsiveness, thirst, and hunger were found in 117 of 136 dogs. The median duration of postictal signs was 21.3 min (25% & 75% quartiles: 5 & 75). Cluster episodes were present in 22 of 136 (16.2%) dogs.

Of 141 dogs, 120 (85.1%) had focal onset seizures. Seizure episodes were classified as SFS in 3 dogs and as SFS with secondary generalization in 18 (12.8%) dogs. The seizures of 41 (29%) dogs were CFS, and the seizures were CFS with secondary generalization in 58 dogs (41.1%). The seizures of 1 dog were primary generalized, and the seizures of 10 dogs were generalized but with unknown onset. Seizures in 10 dogs remained unclassified. Additionally, 24 of 128 (18.8%) dogs experienced seizure episodes of multiple seizure types. These episodes usually had similar onsets with or without generalization. Differently classified episodes were SFS in 3 dogs, CFS in 13 dogs, and CFS with secondary generalization in 8 dogs.

The only factor associated with a progressive course of epilepsy was the presence of a generalized phase of seizure (OR 2.6, 95% CI 1.0-6.3; $P$ = 0.039). General data regarding the factors associated with focal versus generalized seizures are presented in

Table 4. The age at seizure onset was strongly associated with the seizure generalization ($P$ = 0.009), and a generalized phase of seizure occurred more frequently when the first seizure occurred during the first 3 years of life (OR 2.7, 95% CI 1.3-5.8). When generalized seizures (primarily or secondarily) were compared to focal seizures, they were more prevalent in dogs with a shorter ictal episode. Longer seizures (up to 20 min) tended to be focal seizures rather than generalized ictal events. Seizures longer than 20 min tended to be generalized. This tendency was statistically significant, as generalization was more common with seizures lasting 1-10 min (OR 4.7, 95% CI 1.7-13.0, $P$ = 0.006) or seizures longer than 20 min (OR 3.4, 95% CI 1.4-8.3, $P$ = 0.012) compared with seizures lasting 11-20 min.

Table 4. Factors associated with focal versus generalized seizures in FSDs (n = 131)

| Factors | Focal seizures (n = 44) | Seizures with generalization (n = 87) | *P* value |
|---|---|---|---|
| Disease progression, n (%; 95 CI[a]) | | | 0.039 |
| Progressing | 8 (19.1; 8.6-34.1) | 29 (37.7; 26.9-49.4) | |
| Stable or diminishing | 34 (81.0; 65.9-91.4) | 48 (62.3; 50.6-73.1) | |
| Median (IQR[b]) age at seizure onset (months) | 40.5 (27 & 57.5) | 30 (19 & 48) | 0.022 |
| Age of dogs at seizure onset, n (%; 95 CI) | | | 0.009 |
| ≤ 36 months | 21 (47.7; 32.5-63.3) | 62 (71.3; 60.6-80.5) | |
| > 36 months | 23 (52.3;36.7-67.5) | 25 (28.7; 8.2-32.7) | |
| Length of seizure episode, n (%; 95 CI) | | | 0.004* |
| 1-10 min | 15 (34.8; 21.0-50.9) | 41 (47.1; 36.3-58.1) | 0.006** |
| 10.1-20 min | 20 (46.5; 31.2-62.3) | 16 (18.4; 10.9-28.1) | |
| > 20 min | 8 (18.6; 8.4-33.4) | 30 (34.5; 24.6-45.4) | 0.012** |
| Feeding times per day, n (%; 95 CI) | | | 0.052* |
| Once | 9 (20.4; 9.8-35.3) | 31 (35.6; 25.6-46.6) | |
| Twice | 19 (43.2; 28.3-59.0) | 40 (45.9; 35.2-57.0) | 0.196** |
| Three times | 16 (36.4; 22.4-52.2) | 16 (18.4; 10.6-28.1) | 0.034** |
| Used in hunting, n (%; 95 CI) | | | 0.036 |
| Yes | 29 (65.9; 50.1-79.5) | 71 (82.6; 72.9-89.9) | |
| No | 15 (34.1; 20.5-50.0) | 15 (17.4; 10.1-27.1) | |

[a] confidence interval
[b] 25% & 75% quartiles
* *P* value for multi-categorical factor significance by the Wald test.
** *P* value of pairwise comparison with factor category without p value and corrected with the Bonferroni adjustment coefficient.

Another factor correlated with seizure generalization was the number of feeding times per day. Epilepsy generalization was more frequent among dogs that received food once per day than among dogs that ate 3 times per day (OR 3.4, 95% CI 1.2-9.5; $P$ = 0.034). Similarly, dogs fed once per day had a higher risk of generalized seizures than dogs that ate twice per day. However, this was a non-significant trend (OR 2.1, 95% CI 0.9-5.1; $P$ = 0.196). In addition, hunting was associated with seizure generalization ($P$ = 0.036), as dogs used for hunting had a higher risk of seizure generalization than non-hunting dogs (OR 2.4, 95% CI 1.1-5.6).

Further, 42 of 137 (30.7%) epileptic dogs received epilepsy medication, and phenobarbital was the predominant drug. Treatment was considered to be effective in 30 of 38 (78.9%) dogs (a ≥ 50% decrease in seizure frequency), independently of whether the seizures were focal or secondarily generalized. Most of the treated dogs had a generalized phase of seizure. Fatigue, increased drinking and eating, whilst nervousness and aggressiveness were the possible side effects of the treatment: reported for 16 of 39 (41%) dogs by their owners. Treatment was believed to have a negative influence on the hunting ability of 1 treated dog. Eight out of 33 (24.2%) owners of hunting epileptic dogs claimed that antiepileptic medication would have a negative influence on the dogs' hunting ability, although their dogs had never received any epileptic medication. This reflects the general opinion among the hunting community in Finland. Treatment with diazepam at the time of seizure was used in 13 (9.2%) dogs.

All epileptic FSDs from study II had focal seizures (10 CFS and 1 SFS). Seizures started with focal motor signs in 8 and with sudden behavioral change in 3 dogs. One dog had CFS without generalization or primarily generalized episodes. Other focal seizures had secondary generalization. One dog had SFS as a second seizure type. Mean seizure length was 9 (range: 1-30) min and length of interictal period 4 (range 0.25-12) months.

All epileptic FSDs in study III had focal seizures (11 CFS and 4 SFS) with secondary generalization. Mean seizure length was 10 (range: 1-30) min and length of interictal period 5 (range 0.25-12) months.

All epileptic FSDs in study IV had focal seizures (10 CFS and 1SFS) with secondary generalization and with the mean seizure length of 7 (range: 0.5-20) min and length of interictal period 4 (range: 0.7-12) months.

## 4.2. MRI (II, III, IV)

No MRI changes were noted, except in 1 dog. Mild contrast enhancement after gadolinium injection was observed in the right parietal cortex of this dog. The size of the lesion was 5.5 mm, and it was visible in 2 transverse slices. No changes were present in a repeated MRI examination of this patient performed 13 months later.

Additional MRI scans for study IV revealed no abnormalities.

## 4.3. EEG (III, II, IV)

At the visual examination of the EEG recordings, all dogs exhibited a high-voltage low-frequency background activity. Background activity was superimposed with spindles or focal beta bursts in 8 control dogs and 5 dogs with epilepsy and with benign epileptiform transients of sleep in 13 control and 8 epileptic FSDs. Paroxysmal epileptiform activity was observed in three dogs with epilepsy, and it was characterized by spikes, polyspikes, and spike and slow wave complexes in posterior-occipital derivation in all of them (Figure 2 A, B). One dog with epilepsy exhibited periodic delta activity at right temporal localization, and, in two dogs, delta rhythms were diffuse. One

control dog exhibited midline spikes (Figure 3), and one had occipital theta rhythms. No significant correlations by multivariate analyses between EEG activity type and age, sex, weight, heart rate, nervousness of the patient, medetomidine dosage, number of drug administrations, and time from initial injection to the beginning of recording was found.

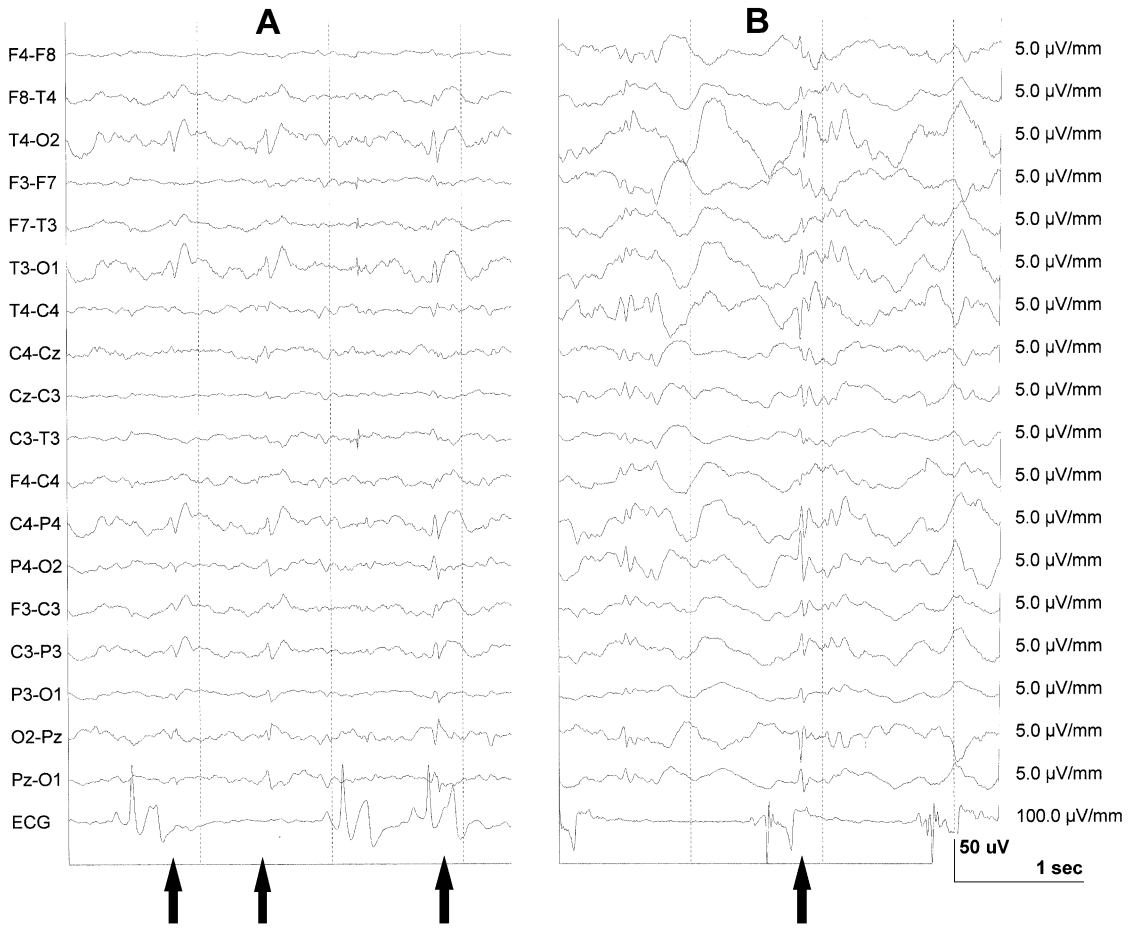



Figure 2. Electroencephalographic traces of an epileptic Finnish Spitz dog. (A) Repeated spike and slow wave complexes in bilateral occipital and parietal derivations. (B) Slow background activity superimposed with spike in bilateral occipital and parietal derivations (arrows). Recording is in bipolar montage, time constant 0.3 second, and high frequency filter 70 Hz; notch filter inserted.

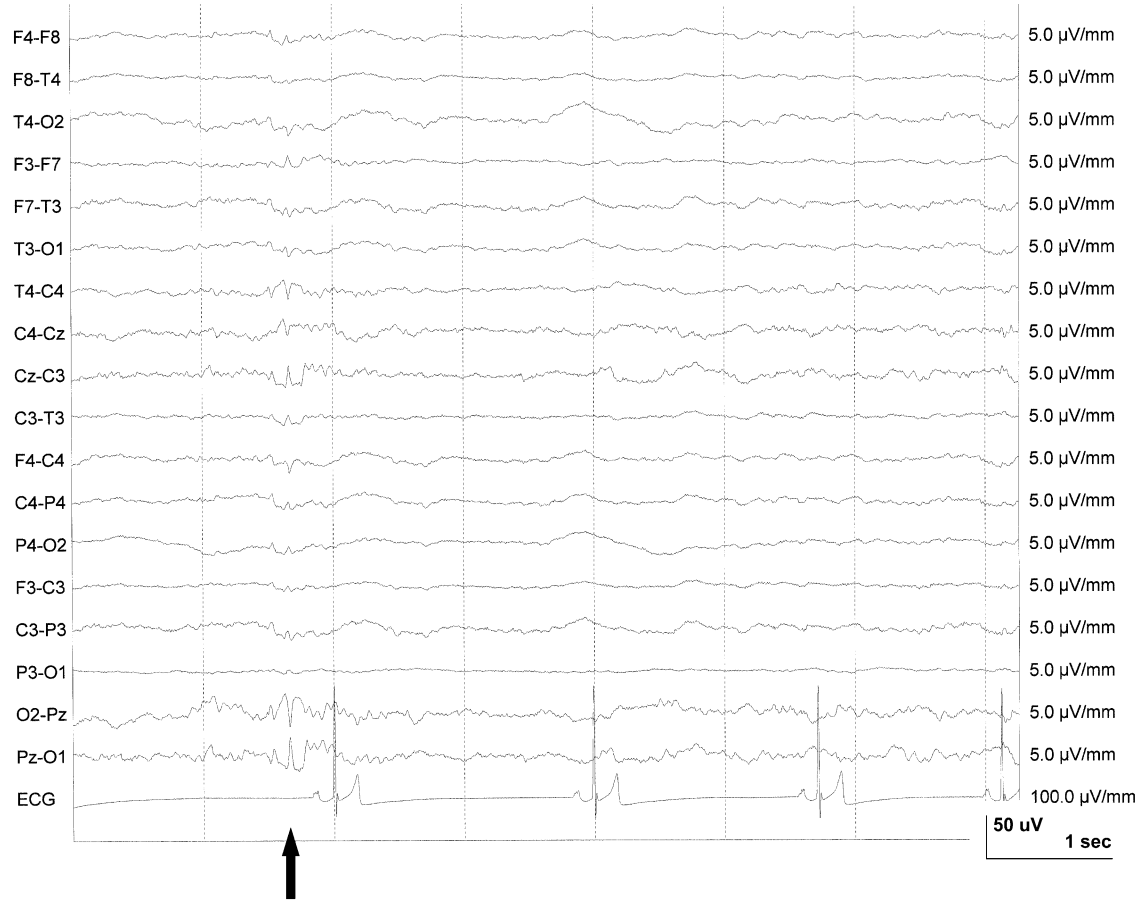



Figure 3. Electroencephalographic traces of a control Finnish Spitz dog showing spikes in midline derivations (arrow). Recording is in bipolar montage, time constant 0.3 second, and high-frequency filter 70 Hz; notch filter inserted.

The results of the quantitative EEG showed a prevalence of slow rhythms (delta and theta) in all groups, whereas fast rhythms (alpha and beta) were poorly represented. All data, but theta band relative power for epileptic FSDs without treatment were sampled after Gaussian distribution and passed a normality test. Differences among standard deviations for mean relative power of delta and alpha bands were significant (Table 5) The control dogs showed significantly less theta and beta activity ($P < 0.01$) on their EEG than did the common group of dogs with epilepsy, although the only significant difference between healthy dogs and dogs with untreated epilepsy was in the alpha band ($P < 0.001$) (Table 6). Phenobarbital treatment increased alpha, beta ($P < 0.001$), and theta ($P < 0.01$), and decreased delta ($P < 0.001$) bands compared with dogs who have untreated epilepsy. All dog groups had similar characteristic relationships for theta and beta frequency bands.

Table 5. Results of mean relative power of electroencephalography background activity bands for different groups of Finnish Spitz dogs expressed as mean and standard deviation (SD).

| Group | Delta band mean (± SD) | Theta band mean (± SD) | Alpha band mean (± SD) | Beta band mean (± SD) |
|---|---|---|---|---|
| FSE[a] | 82.33 (1.10) | 10.46 (0.70) | 3.79 (0.34) | 2.92 (0.20) |
| FSENT[b] | 83.37 (0.90) | 9.97 (0.65) | 3.46 (0.27) | 2.73 (0.17) |
| FSEPh[c] | 80.24 (1.74) | 11.46 (1.00) | 4.44 (0.55) | 3.30 (0.29) |
| FSC[d] | 82.79 (0.61) | 9.64 (0.27) | 4.08 (0.18) | 2.66 (0.14) |

[a] Finnish Spitz dogs with epilepsy
[b] subgroup of Finnish Spitz dogs with epilepsy without treatment
[c] subgroup of Finnish Spitz dogs with epilepsy on phenobarbital monotherapy
[d] control Finnish Spitz dogs

Table 6. Significance in electroencephalography background activity bands between different groups of Finnish Spitz dogs.

| | Delta relative power (%) | Theta relative power (%) | Alpha relative power (%) | Beta relative power (%) |
|---|---|---|---|---|
| FSE[a] verus FSC[d] | NS[e] | ** | NS | ** |
| FSENT[b] versus FSEPh[c] | *** | ** | *** | *** |
| FSENT versus FSC | NS | NS | *** | NS |
| FSEPh versus FSC | ** | *** | NS | *** |

[a] Finnish Spitz dogs with epilepsy
[b] subgroup of Finnish Spitz dogs with epilepsy without treatment
[c] subgroup of Finnish Spitz dogs with epilepsy on phenobarbital monotherapy
[d] control Finnish Spitz dogs;
[e] not significant, significance level $P > 0.05$
* significant difference between compared study groups in the level of $P < 0.05$
** significant difference between compared study groups in the level of $P < 0.01$
***, significant difference between compared study groups in the level of $P < 0.001$ Significance levels for delta, alpha, and beta bands were examined with nonparametric tests. Significance level for theta band was evaluated with parametric test.

Interictal epileptogenic activity in EEG recordings of study II was focal in 7 and generalized in 4 dogs. Focal activity tended to generalize or spread contralaterally in 7 dogs. The epileptic activity occurred in the anterior right hemisphere (1 dog), the central posterior right hemisphere (2 dogs), the posterior areas (2 dogs), the left temporal area (1 dog), or the posterior temporal derivation and the entire posterior derivation (1 dog).

Visual evaluation revealed changes in the EEGs in 4 epileptic and 3 control dogs included to the FDG-PET study (IV). Findings were classified as paroxysmal epileptiform activity in 4 dogs (3 epileptics and 1 control FSD) and epileptiform with uncertain clinical relevance in 2 control and 1 epileptic FSD (midline spikes, Figure 3).

## 4.4. FDG-PET (IV)

There were no statistical differences between the study groups by sex, age, and weight. Visual evaluation revealed 17 significant findings in 13 dogs. Five findings were agreed by three examiners and 12 findings by two examiners. Six dogs had no visible changes (validated in 3 dogs by three and in 3 dogs by two examiners). Findings in epileptic and in control FSDs were classified as mild (5; 3) or obvious (6; 3), respectively. FDG uptake abnormalities/asymmetries appeared in 9 epileptic (82%), and in 4 control FSDs (50%), some findings involved multiple brain regions.

Epileptic and control dogs had findings in the occipital (7 and 0 dogs; Figure 4.), lateral temporal (3 and 3 dogs), frontal (3 and 1 dog), parietal cortex (2 and 3 dogs), caudal colliculum (2 and 2 dogs), cingulate gyrus (1 and 0 dog; Figure 5.) and in the nucleus caudatus (1 and 0 dog). No visual findings were found in thalamus or in cerebellum. Abnormalities were most often considered to be hypometabolic foci. As an exception, one dog which had a seizure episode 24 h before PET examination showed hypermetabolism in the gyrus cinguli. Four out of 8 healthy and 2 out of 11 epileptic dogs had no findings. Visual asymmetry in the occipital cortex was significantly associated with epileptic status ($P = 0.013$).

The regional AI between the control and epileptic dogs did not differ significantly. The AI varied in the control dogs from 0.01 to 2.7 in the cortical regions, from 0.07 to 1.8 in the nucleus caudatus, 0.19 to 1.11 in the thalamus, and from 0.15 to 1.65 in the cerebellum when AIs from the dogs with visual findings were excluded. The highest asymmetry indexes were seen in the caudal colliculus (2.42) in the control dogs. The maximal AI for the hypometabolic cortical regions in the epileptic dogs varied from 0.5 (direction not in agreement) to 0.71 (consensual direction with visual findings). Overall, in 22 of the 28 (14 of 19 in epileptic dogs) areas of visual findings, the direction of AIs agreed with the side of visual finding. All four dogs (two epileptic and two control dogs) with visual asymmetry findings in the caudal colliculus also had the highest AI in that region (from 2.7 to 4.34).

The highest relative uptake of FDG was in tectum (caudal colliculus) and nearly as high an uptake was found in all cortical areas and in the nucleus caudatus. The lowest uptake was in the cerebellar hemispheres and paraventricular white matter, with the exception of cerebellar vermis which had comparable uptake with the hippocampus. The epileptic dogs had significantly lower SUVs in numerous cortical regions (frontal, parietal, temporal, occipital lobes), cerebellum and hippocampus compared to the control dogs ($P \leq 0.05$). White matter normalized values did not reveal any significant difference between epileptic and control group.

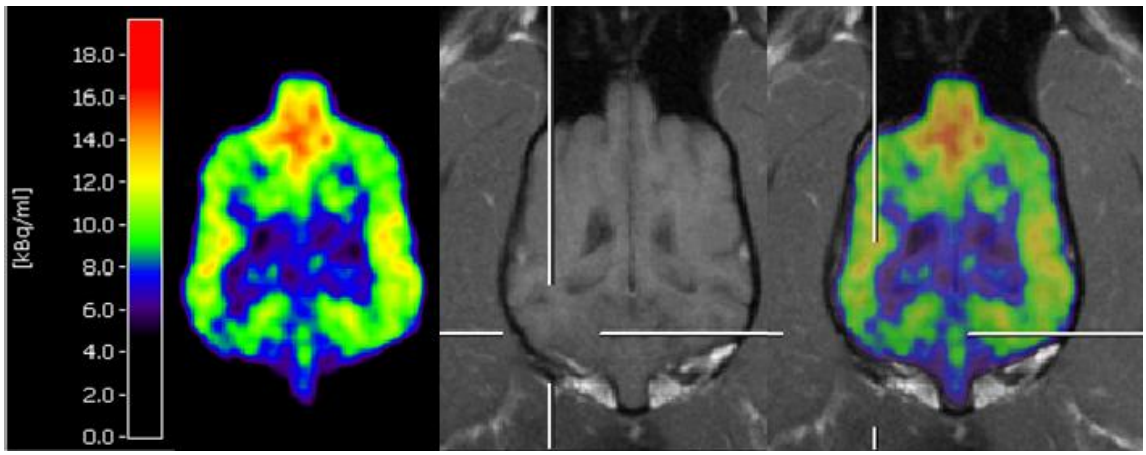



Figure 4. Dorsal plane of FDG-PET (on the left), MRI multiplanar reconstruction image (in middle), and fused FDG-PET and MRI of the Finnish Spitz dog with epilepsy representing decreased glucose uptake in the left occipital lobe. On the left side of figure is in the rainbow scale FDG-PET activity bar.

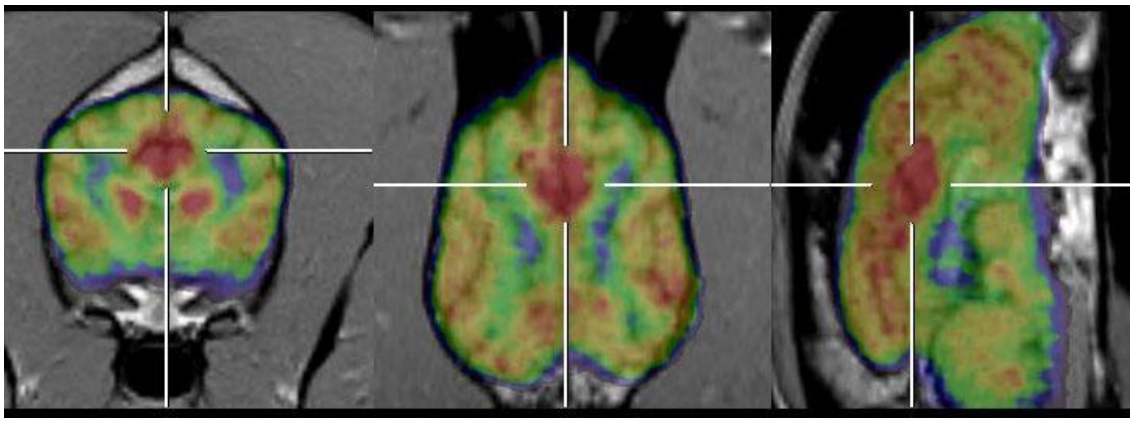


Figure 5. Fused FDG-PET and MRI (in transverse, dorsal, and sagittal plane starting from the left) of the Finnish Spitz dog with epilepsy representing postictal increased glucose uptake in the gyrus cinguli.

In comparison of visual evaluation of FDG-PET and EEG examinations, in two dogs the EEG showed bioccipital spikes and PET showed unilateral occipital hypometabolism (perfect agreement on localization, Figure 4.). In one dog, EEG spikes were localized on the right temporo-occipital region and PET showed hypermetabolism in the cingulate gyrus (Figure 5). The EEG and PET findings did not agree in any of the control dogs.

# 5. DISCUSSION

## 5.1. Methodological issues

### *5.1.1. Animals and study design (I-IV)*

IE seems not to be a distinct disease with consistent pathophysiology and genetic determination in dogs, but consists of multiple disease conditions. Specific seizure characteristics correlated to diagnostic evaluation findings are not defined for dogs. Therefore homogenization of a study population thorough selection of a particular dog breed should provide meaningful results. We selected FSDs with epilepsy as study subjects. Motivated and cooperative dog owners are an ultimate presumption for successful clinical studies. As a consequence of selecting a hunting breed, these dogs might have a nervous character and could behave in an unsuspected way when handled by somebody other than the owner. Therefore protocols of diagnostic evaluations used in our studies should be universally adaptable for different types of dog breeds.

Patient selection is an important issue when examining IE. As no gold- standard method to confirm healthy or epileptic status in dogs exists, evidence of seizure history carries a crucial role when selecting patients. FSDs are kept predominantly outside and are under direct observation of their owners 25% to 50% of the time. Because of low seizure frequency in FSDs, seizures could occasionally occur without owners noticing them. Owners of the FSDs do pay close attention to their dogs, however, spending time with them during some periods (ie. during hunting season) around the clock. Seizures with long duration increase the likelihood of recognizing signs related to seizures (pre-ictal, ictal or, post-ictal signs). A concern regarding control dogs in the MRI study (II) may be that the dogs were young (Table 1.). This is not suspected to interfere with the study results, however. In addition, as these dogs also participated in other clinical studies where they were followed-up for many years without evidence of seizures (1 dog was excluded from the initial control group of MRI study because of seizure onset 1 year later). All owners of control dogs (I-IV) were instructed to contact researchers if seizures were witnessed in their animals. Although we cannot completely exclude that an epileptic dog was erroneously included as a healthy control, the possibility that it would be a dominant trend interfering with the common findings in multiple control dogs is considered highly unlikely.

The prospective epidemiological investigation (I) was designed to cover the largest possible FSD population in Finland. Therefore the questionnaires were sent to all owners of FSDs in Finland (age range 1-10 years) whose contacts were available. Life span for FSDs is suspected to range from 10 to 13 years, but we were not able plausible to validate information older than 10 years. Cumulative occurrence of epilepsy should be highest within older dogs, but as our database included some dogs up to 15 years of age, the population curve followed a normal distribution. The aim was to mimic the natural population of FSDs in Finland in the prevalence study. To use a randomized design for an epidemiological study would be optimal for larger populations, but would lead to a remarkable decrease in this study sample which contradicts with one of the

aims, to collect information about as many new epileptic FSDs as possible. Standards for epidemiologic studies and surveillance of epilepsy for humans are proposed by the ILAE (Thurman et al. 2011). Such criteria are seldom applied for epidemiological studies of epilepsy in dogs (Berendt et al. 2002).

Actual randomization of patients for clinical studies was not used, although some randomness occurred when dog owners volunteered to clinical studies. The majority of the FSDs population is located in Northern Finland whereas MRI and FDG-PET examinations were performed in Southern or Southwestern parts of Finland (500-1200 km distant). Therefore patients situated closer to examinations were more frequently included, as their owners were more likely able to participate in clinical studies. It could be argued that results of our clinical studies are therefore not reliably representative of all epileptic FSDs. However, as study populations (I-IV) had similar general characteristics (Table 1) and total amount of clinically examined epileptic FSDs reached approximately 20% (Table 2) from the living epileptic FSDs, and results of different diagnostic modalities were accordant, hereby findings should most likely reflect different aspects of epilepsy in FSDs as reliable. Owners of control dogs having some epileptic relatives had clearly higher motivation to participate. Therefore some clinical findings in control dogs might reflect close genetic relationship to epileptic dogs. Further, special selection of control patients from the bloodlines with low incidence of epilepsy or reinterpreting current data within the light of results from genetic studies would be of great interest.

Visual evaluation of EEG (III, IV) and FDG-PET (IV) was performed under conditions where reviewers were blinded for the animal's clinical status. It is surprising that our EEG study has been first and only which has applied blinding, despite so many controversies related to that topic in veterinary medicine. A rough discrepancy of diagnostic yield by EEG was found, not only for epileptic FSDs (III) and what has been reported previously from other dog breeds, but also for FSDs depending whether the reviewer was blinded (III, IV) or not (II). Randomization and blinding was thus also applied for visual evaluation of the FDG-PET images (IV).

### *5.1.2. MRI (II)*

Although MRI allows diagnosis of various cerebral pathologies *in vivo* with high sensitivity, changes are not seen in a considerable number of canine patients with seizures. Whilst high-field MRI examinations are routinely employed for epileptic human patients (Neuroimaging Subcommission of the ILAE 2000), low-field examinations, remain the principal approach used in veterinary medicine: despite the fact that improved spatial resolution, contrast, and shorter imaging time can be achieved with higher magnetic field equipment (Vaquero et al. 2010, Konar & Lang 2011). The application of 1.5T MRI examinations for FSDs in this study ensured the likelihood of detecting structural brain changes. Nonetheless, continued progress in diagnostic imaging techniques in human medicine suggests that the epileptogenic foci of what we

now consider to be idiopathic may someday become visible (Kuzniecky et al. 2002, Casse et al. 2002).

### *5.1.3. EEG (II-IV)*

Despite a promising introduction of EEG to veterinary medicine, its role has diminished over time, mainly because advanced diagnostic imaging methods are applied when symptomatic epilepsy is suspected. Nevertheless, EEG should be clearly indicated for animals with seizures when IE is suspected, being the only examination in everyday clinical use that is able to supply positive findings with this diagnosis.

There are a number of limitations regarding the use of EEG in veterinary medicine (Pakozdy et al. 2012). It remains a challenge to obtain artifact free ictal/interictal recordings without sedation, and extensive muscles over the calvarium impair recording from lateral and ventral areas of the canine brain (James et al. 2011); but the development of radio-telemetric EEG recording techniques and new electrodes (Davis et al. 2011) may help to overcome these concerns in the future. Other limitations, related to the need for universally accepted needle placement, recording, and montages protocols requires urgent cooperation between institutions and specialists working in the field of veterinary EEG. Otherwise, data collected from canine EEG recordings will be fragmented and yield few benefits. Finally, veterinary doctors need to address their limited knowledge in the interpretation of EEG findings. This limitation will be easiest to eliminate through cooperation with human electrophysiologists and with emphasis on EEG interpretation in neurology specialty training in veterinary medicine.

This study was the first to employ medetomidine as a solo agent for the sedation of epileptic dogs during EEG recording. Sedation with medetomidine, an alpha-2 adrenergic agonist, is easy via im injection even for dogs behaving aggressively. Despite controversial reports about alpha-2 agonist pro- and anticonvulsive properties (Mirski et al. 1994, Jolkkonen et al. 1999, Miyazaki et al. 1999), dexmedetomidine, the enantiomer of medetomidine, is increasingly used for the sedation of pediatric human patients when examining EEG (Talke et al. 2007, Mason et al. 2009, Aksu et al. 2011).

### *5.1.4. FDG-PET (IV)*

PET imaging of epileptic dogs has not been described previously to our knowledge. High resolution research tomography (HRRT)-PET scanner with high spatial resolution and superior to SPECT scanners (Peremans et al. 2001, van Velden 2009, Kang et al. 2012) was used in this study to maximize the likelihood to detect changes. FDG was used as a PET tracer because of easy availability and because FDG has been used widely when examining epileptic human patients with PET. FDG-PET reflects glucose metabolism at the time from the tracer injection till the uptake reaches a steady-state (at least 20 min). Therefore making the patient feel comfortable for this time-period is crucial (Neuroimaging Subcommission of the ILAE 2000, Varrone et al. 2009). Taking into account breed selection (FSD), injecting the FDG without sedation, which would be optimal, was not an option and possible sedation effects were considered better than

non-homogeneous and hardly interpretable study data. Sedation influences brain glucose metabolism and blood perfusion (Veselis et al. 1997, Lee et al. 2010a, Schlünzen et al. 2012). However, no consistency exists between anaesthesia protocols and sedatives used for veterinary PET/SPECT studies (Peremans et al. 2001, Irimajiri et al. 2010, Lee et al. 2010a, 2010b, Hansen et al. 2011, Waelbers et al. 2011, 2012,). Combined medetomidine, butorphanol, and midazolam sedation was used in this study, when initial sedation was given prior to tracer injection. Data is lacking, however, regarding the use of such combined sedation protocol for functional imaging studies; but a quantitative EEG study in dogs showed no significant differences between low dose medetomidine and combined medetomidine-butorphanol-midazolam sedations (Itamoto et al. 2002). Therefore we expect that the influence of sedation on global cerebral glucose utilization was mainly determined here by medetomidine (increased metabolism), but similarly for all dogs examined. No statistical difference on SUVs was found when epileptic dogs with and without antiepileptics treatment were compared, as has been supposed in humans (Casse et al. 2002).

Because FDG-PET has not been previously applied for canine epileptic patients, the main goal was to define visual findings of glucose hypometabolism, through a method that was not highly observer-dependent. Therefore 3 examiners with different backgrounds (veterinary radiologist, human children epileptologist with FDG-PET experience, and a scientist working with small animal PET imaging) were used. There was complete agreement (3 examiners) on 5 findings and partial (2 examiners) agreement on 12 findings. Despite a large total number of non-confirmed findings (22), single evaluators detected findings consensual with other evaluators more frequently. Therefore changes in cerebral glucose uptake can be detected by visual evaluation of canine FDG-PET images with reasonable consistency, however it is crucial to avoid over-interpretation of minor changes in cerebral FDG uptake. Nonetheless, in clinical patients, findings of FDG-PET should be interpreted in the context of the clinical picture and results compared with findings from other diagnostic modalities.

Although the PET scanner was used here with high spatial resolution, fusion with the anatomical imaging methods would improve detection of fine anatomical structures (Peremans et al. 2001, Irimajiri et al. 2010, Lee et al. 2010, Hansen et al. 2011, Kang et al. 2012). Unfortunately, for technical reasons, it was not possible here to apply fusion of 1.5T MRI and HRRT-PET images for all of the dogs and to draw VOIs on the MRI. The VOIs were drawn with maximal inter-individual similarity and bilateral VOIs were done symmetrically and not following possible visual findings of FDG-PET. VOIs covered the maximal volumes of the selected brain structures. The relatively low sensitivity of semi-quantitative methods compared to visual findings in this study is largely related to the ROI defining method. To draw ROIs to the template as described for SPECT (Peremans et al. 2003) would be another option to diminish influence of examiner. However, epilepsy syndromes have seldom well characterized suspected homologous epileptic foci in dogs. Therefore escaping visual analysis and concentrating only on quantitative measuring defined by a template is likely to mask possible findings

on an individual basis. To draw ROIs strictly to the hypometabolic region was not sought after in the scope of this study and the question of how much a hypometabolic region can differ from the cerebral cortex with normal function between control and epileptic dogs unfortunately remains unanswered.

To improve comparison of SUV between different studies and individuals, the relative SUV ratio (where the regional SUV is normalized with SUV of the whole brain, with white matter, cerebellar, or brainstem regional activity) can be performed (Koivunen et al. 2008, Berti et al. 2012, Kang et al. 2012). In order to also reveal global or extensive differences in the glucose uptake of epileptic dogs, normalized VOI values with the regional activity of the white matter (minimal uptake) were used. We considered it likely that normalization of SUV with the global cerebral activity which has been reported for healthy dogs examined with FDG-PET (Kang et al. 2012) would mask regional changes to be higher.

## 5.2. Epidemiology, inheritance and phenotype of epilepsy in FSDs (I)

Epileptic seizures are considered to be a response of the brain to a large variety of stimuli. Genetic factors that play an important role in the development of IE may influence the individual basic level of epileptogenicity. These factors may also be causative or influence the moderation of the disease development. (Lüders et al. 2009) A complex pattern of inheritance such as polygenic recessive or autosomal recessive with incomplete penetrance seems to fit best with the heritability estimate of 0.22 in FSDs obtained in our study. Even if the first pedigree analysis supported the hypothesis of an autosomal recessive mode of inheritance the high incidence of IE in many litters could also be explained by a high homozygosity level in this dog breed. We believe that using pedigree drawing or segregation analysis of the general non-pre-selected breed population makes a difference in inheritance estimates. In pedigree analysis, litters were found with several epileptic littermates, but some bloodlines also completely lacked epileptic dogs. Therefore, an IE prevalence of 5.36% in the Finnish population of FSDs serves as a general estimate and can differ significantly depending on the bloodline. Moreover, initial results from genetic study indicate that no single locus is clearly related to epilepsy in FSDs. This finding agrees with veterinary and human literature, where idiopathic epilepsies have been suggested frequently to have a complex genetic basis (Srenk et al. 1994, Kathmann et al. 1999, Heron et al. 2007, Dibbens et al. 2007).

In addition to issues with study design, some other factors may influence the estimate of epilepsy prevalence. Although the owners of all dogs were encouraged to complete a questionnaire regardless of the clinical disease status of the dogs, were considered the owners of epileptic dogs less likely to respond. Many dog owners are ashamed about epilepsy in their animals, although this attitude is changing slowly. The episodic nature of epilepsy facilitates this denial. In any case, based on recent literature, the observed prevalence estimate for IE in FSDs seems to be clearly higher than that of the general dog population (0.6%) (Kearsley-Fleet et al. 2013), but not reaching the prevalence (18-33%) reported from some pedigrees with a high accumulation of epilepsy (Casal et al.

2006, Berendt et al. 2009). Caution should be used when comparing prevalence estimates, however, due to inconsistency in the study populations or in methods used in veterinary studies.

A statistically significant predisposition for epilepsy was found in male FSDs, in that the incidence of epilepsy in males was 1.7 times higher than that of females. The predisposition of males for IE is not reported for all dog breeds, however results of one study suggests that male dogs overall might be at a higher risk for epilepsy (Kearsley-Fleet et al. 2013) and FSDs appear to share this general predisposition. In addition, the pedigree data presented here demonstrates that IE in FSDs appears not to be sex-linked, as was supposed for beagles (Bielfelt et al. 1971).

Results presented here indicate that some personality traits such as being phlegmatic, depressed, or nervous could be risk factors for IE in FSDs. These personality traits were 5.9 times more often associated with IE than lively-character. Reliable statistical analysis without considering all other personality traits than lively was not possible, however. Personality characteristics were not established for epileptic animals for two time points, before seizure onset and at the later course of disease in this study, which is a limitation. Therefore, it is impossible to distinguish whether some canine characteristics can predict later seizure onset or whether it is more indicative of changed personality caused by the epilepsy. Moreover, some bias introduced by the owners could not be excluded (tendency to classify non-epileptic dogs as lively). Our own experience with epileptic FSDs is that many of them can be described as nervous and often have such behavior before seizure onset. Some prove that dogs with IE may also experience neurobehavioral comorbidities together with the development of epilepsy (Shihab et al. 2011), similar to that described in epileptic humans (Hermann et al. 2008, Kerr et al. 2009).

Factors found in the epilepsy risk analysis were the use of the dogs for hunting (OR 0.5) and the housing of dogs exclusively outdoors (OR 0.6). A likely explanation for the fact that healthy dogs were used more often for hunting is that dogs that started to have seizures were not subsequently taken on hunts. Interestingly, a significant portion of epileptic dogs were still being used for hunting. Historically, it has been recognized that some epileptic FSDs may have extremely good hunting skills (very good senses and a high level of alertness). Therefore, it seems that epilepsy was systematically ignored for many years when breeding decisions were made. The majority of FSDs still live outdoors in the Northern parts of Finland. Living only in outdoor conditions was associated with a decreased risk of epilepsy. The suggestion that outdoor dogs are healthier because they live in conditions which are natural for FSDs is somewhat naive. As the mean seizure frequency reported for FSDs living outdoors was very low, it could be argued that the owners may not always witness seizures in dogs living entirely outdoors and may therefore falsely classify them as healthy. Based on our experience, we suggest that the most common reason is that after the owners realize a dog is prone to seizures, they keep the animal under observation as much as possible. However,

despite reaching the level of statistical significance, biological justification could not be provided for any of the factors found to be associated with the occurrence of IE in this study, except for the genetic predisposition.

Seizures occurred here most often (> 50%) under stressful conditions or with exercise, although the patient material is far too limited to offer reasonable factors to apply in the clinical situation. It seems that precipitating factors that trigger a seizure are multifactorial and may vary between individuals that many animals have more than one risk factor. For example 8% of FSD owners reported their dogs' seizures were being triggered by the breeding sexual cycle. Nonetheless a majority of them were male dogs which had seizures more frequently in stress situation when female dogs were in "heat". The same dogs also had other excitation triggers. In contrast, some female FSDs were exclusively having seizures when on "heat". Therefore, relying more on what the owner observes for every individual dog could facilitate the provision of strategies for better control of seizure by avoiding recognized situations associated with epileptic episodes.

This study represents the most extensive epidemiological study of IE concentrating on one dog breed to our knowledge: including 143 epileptic dogs and 2141 control dogs. Importantly, unlike previous studies, an estimate was made for the native disease course. The generalized phase of seizure was found to be the only significant risk factor for the progression of epilepsy. A clear benefit of such a disease progression predictor is that a generalized phase of seizure can be easily recognized by the owners. In this analysis, generalized phase was associated with a progressive course of epilepsy in every context in which it was observed, but trustworthy applicable to the phase of secondary generalization (91.6%). The disadvantage of ignoring the context of the generalized phase is that it makes harder to find a generally meaningful and adaptable pathophysiological explanation. Evidence provided mainly by experimental models such as kindling studies suggests that neuronal death depends on seizure spread and that propagated generalized seizures may by more harmful than partial seizures (Pitkänen & Sutula 2002). The presence of secondary generalized seizures has been reported to be one of the factors associated with a poor outcome in human epileptic patients (Jackson et al. 2005b). Drawing direct comparisons with our data is difficult because of the different preselection of patients. Our population represents patients with epilepsy, a genetic predisposition, no detectable brain lesions, and different seizure types. However, we found that secondary generalization, used as the pathophysiological hallmark of seizure spread within the brain, could be used to predict disease progression. This result seems to be biologically meaningful and is in accordance with the literature. As most representative seizure types in this study were CFS and focal onset seizures with secondary generalization, the results could be applied most reliably to dogs with IE and to those with similar seizure types.

Some factors were correlated with the generalized phase of seizure. Interestingly, the age at seizure onset was not directly correlated with disease progression, but was correlated via a generalized phase of seizure. The majority of seizures were focal,

although secondary generalization occurred more frequently when the first episode occurred during the first 3 years of life. The age at seizure onset is an important factor in human epilepsy, and many age-specific epilepsy syndromes have been recognized. Among the childhood epilepsies, IE has a strong negative association with focal seizures (Beilmann et al. 1999). However, the exact prognosis for outcome is usually syndrome dependent, therefore direct comparison with this data is not sensible.

A bimodal relationship between the seizure length and the generalized phase of seizure was also demonstrated here. Seizures that lasted up to 10 min were 4.7 times more likely to be generalized than seizures lasting 11-20 min. However, seizures longer than 20 min were more likely (OR 3.4) to be generalized than those lasting 11-20 min. Evidence from human medicine can be found that this bimodal tendency might be related to variation of seizure type and depend on the seizure locus (Jenssen et al. 2006, Afra et al. 2008). It is impossible to confirm this reasoning to our results, as groups of all seizure types were not representative for statistical analysis and comparison.

Regarding seizure length, it is poignant to ask at what point do seizures with a long duration become life-threatening. Although various criteria (seizure frequency, quality of life, and no need for medication) suggest a benign course of IE in FSDs, long-lasting seizures are classically considered to be a sign of a non-benign course of epilepsy. It is remarkable that seizures of up to 30-40 min were self-limiting in the FSDs and seldom needed special treatment at the time of the episode. In human patients, different patient groups may have seizures of different durations which can be considered life-threatening; Morbidity and mortality varying for different age groups and for different epilepsy syndromes (Metsäranta et al. 2004). Therefore, we suggest rather a syndrome approach ("benign" or "not-benign") for some canine epileptic patients, as a simple cut-off point for the inclusion of status epilepticus to estimate seizure outcome risks.

Several factors correlated here with the occurrence of a generalized phase of seizure, such as the number of meals per day and whether the dog was used for hunting. Consistent with a biological meaning, the correlation between the number of meals per day was linear. More frequent eating should stabilize the blood glucose levels. Eating could also be connected to the use of dogs for hunting or to excitement levels. Physiological hypoglycemia due to exercise in hunting dogs has previously been suggested as a cause of seizures (Lord et al. 1975, Leifer 1986), but hypoglycemia has not been proven to be an etiological factor. Blood sample analyses from a larger number of FSDs in connection with an ongoing study have identified no abnormalities in the blood glucose levels except in one dog, where hyperglycemia was associated with a later onset of diabetes mellitus (III). Eating just once per day and excessive physical activity due to hunting may cause a reduction in glucose levels and may therefore serve as a metabolic trigger to induce seizures of greater magnitude. However, in addition to constituting excessive physical activity, hunting also causes high levels of excitement via the activation of the autonomic nervous system.

Epilepsy was characterized by focal onset seizures in 85% of FSDs. This is in line with the latest veterinary literature where focal seizures have been reported to be the main seizure type for many dog breeds with IE (Licht et al. 2002, Berendt et al. 2004) and contradicts earlier literature that epileptic seizures in canine idiopathic (primary) epilepsy are generalized, whereas symptomatic (secondary) epilepsy usually is characterized by partial seizures (Schwarz-Porshe 1984, de Lahunta 1983, Oliver et al. 1997, March 1998). Changes in attitude have occurred after observation of the initial ictal signs crucial for seizure type classification by some researchers (Berendt & Gram 1999, Licht et al. 2002). CFS with secondary generalization was the most frequent seizure type (41%) detected in FSDs, similar to what is reported for many other breeds with IE (Licht et al. 2002, Berendt et al. 2002, Patterson et al. 2003, Berendt et al. 2008, Hülsmeyer et al. 2010, Gulløv et al. 2011), detailed descriptions of seizures are likely to differ, however.

## 5.3. MRI of epileptic FSDs (II, IV)

No brain changes were evident on the MRI of FSDs with focal onset seizures, except in 1 dog with changes as a suspected consequence of seizures. There are only a few studies published in veterinary medicine about MRI findings in dogs with focal epilepsy (Podell et al. 1995, Patterson et al. 2003, Seppälä et al. 2012). Therefore, the opinion that focal epileptic seizures are caused by a focal structural brain lesion is most probably based on a pathophysiologic understanding of epilepsy. Focal IEs are well defined in human epileptology, however (Callenbach et al. 2003, Michelucci et al. 2003, Combi et al. 2004). The pathogenesis of some focal IEs in humans has also been described recently, with suggested mechanisms related to changes in the nicotinic acetylcholine receptor subunit or in leucine-rich glioma-inactivated factor 1 (epitempin) (Morante-Redolat et al. 2002, Rodrigues-Pinguet et al. 2003, Combi et al. 2004). The reason why changes, which are expressed ubiquitously in the central nervous system, are causing focal epilepsy is unknown (Gourfinkel-An et al. 2004). Described changes are not visible with imaging methods available at this time, but can be detected with immunohistochemistry (Morante-Redolat et al. 2002).

In certain canine populations, focal IE without visible MRI changes can be detected, as has been demonstrated here. Therefore, despite the fact that a higher prevalence of focal seizures has been demonstrated for dogs with symptomatic epilepsy (Pakozdy et al. 2008), the results presented here suggest that classification of canine patients into idiopathic or symptomatic categories should be based on the patient's diagnostic evaluation rather than on the seizure pattern alone. Furthermore, both, focal and generalized seizures can be idiopathic in origin. The classification of all non-lesional canine focal epilepsies as cryptogenic, and only generalized seizures as idiopathic, has been suggested (Berendt & Gram 1999); however, the use of a cryptogenic category for canine epileptic patients is not uniform. Although they experience focal seizures, we believe that the FSDs studied here best represent IE. Veterinary medicine might benefit from further adaptation of a new ILAE concept of classification (Berg et al. 2010), consequently IE in FSDs categorization into genetic epilepsy could follow.

MRI changes are most frequently detected in human patients with temporal lobe epilepsy (Casse et al. 2002), where hippocampal sclerosis is the main finding (Leherecy et al. 1997). No hippocampal structural changes were observed in any of the FSDs with focal epilepsy studied here, including the 2 dogs with EEG changes in the temporal area. The number of dogs was small, however, and pre-selection according to clinical signs and EEG pattern was not performed to state that temporal lobe epilepsy in FSDs does not exist. These findings do support a previous report that canine epilepsies may be mainly extra-temporal (Buckmaster et al. 2002), but disagree with another study which found lateralized hippocampal atrophy in a relatively high proportion of epileptic dogs examined with MRI (Kuwabara et al. 2010). The description of both focal temporal and focal extra-temporal epilepsies in humans with proven genetic background and no MRI changes is also relevant in veterinary epileptology. These results, however, show that identification of epileptic lesions in dogs with MRI might be even lower than in humans, at least for some canine populations.

Reversible MRI abnormalities in the piriform lobe, temporal lobe or both of 3 dogs after seizures have been reported previously (Mellema et al. 1999). These changes completely or partially resolved on reevaluation. Of our dogs, only the 1 in which MRI changes were detected had a 2-min generalized seizure episode 3.5 h before imaging. A contrast-enhanced area in the right parietal lobe was noted in this dog, but these changes were not observed in repeated MRI 13 months later. The time period between seizure occurrence and MRI seems to play a critical role. Lesion identification might improve if examinations were done on the same day as seizure occurrence (Mellema et al. 1999). There was no evidence of brain changes in the other 10 dogs in our study, including a dog which had a 45-min period of status epilepticus 21 days before MRI examination. It may indicate that despite long-lasting seizure episodes, changes may not occur or may resolve earlier as suggested by the literature (Mellema et al. 1999). In addition to long duration of seizure, seizure frequency may also be an important factor in induction of MRI changes, but FSDs represented dogs with relatively low seizure frequency.

## 5.4. EEG of epileptic FSDs (II-IV)

### *5.4.1. Visual evaluation of EEG*

We described benign epileptiform transients of sleep and sleep spindles, findings well recognized in healthy and epileptic humans (Westmoreland 1996, Beun et al. 1998, Radhakrishnan et al. 1999, Zumsteg et al. 2006), in 68% of dogs included in this study when standard descriptions used in human neurophysiology were adapted (Noachtar et al. 1999). One study on humans reported 93% of sharp transients in the sleep EEG of healthy volunteers, with an incidence of 13% of true epileptiform discharges (Beun et al. 1998). Although these findings have been described previously in dogs (Klemm 1989, Holliday & Williams 1998, Bergamasco et al. 2003), we believe that high occurrence of sleep transients, when epileptiform paroxysms were seldom in our study, could indicate that misinterpretation of these changes as true epileptic discharges is a relevant risk in veterinary medicine.

Epileptiform activity in healthy dogs is an ignored phenomenon in the veterinary literature. We detected midline spikes in four control dogs, and in one epileptic dog (III, IV). In humans, this finding is described to present epileptiform activity with uncertain clinical significance (Jabbari et al. 2000, Stern & Engel 2005). In addition, one control FSD had paroxysmal epileptiform discharges characterized by lateralized frontal spikes occurring simultaneously with midline spikes. Therefore, our results suggest that midline spikes, despite representing epileptiform activity, should be rather considered as a finding not characteristic for epilepsy in FSDs. Within the wider healthy human population, interictal epileptic discharges are present in up to 2.6% of individuals (Stern & Engel 2005). We found epileptiform paroxysms in 1 (5%) of the 19 control dogs or in 4 dogs (21%) when midline spikes were included. Because of low seizure frequency in FSDs, some mild ictal events could be missed by their owners and dogs falsely classified as healthy. The control dogs with epileptiform paroxysmal activity showed no closer relationship to the epileptic relatives as dogs from control group on average. The majority of control dogs had epileptic relatives in their pedigrees at least in the 2nd or 3rd generations. The literature on humans clearly defines the higher prevalence of epileptic discharges (up to 50%) on the EEG recordings of the siblings of epileptics (Degen et al. 1991). At present, we are unable to unequivocally answer whether epileptiform activity in healthy individuals may have some predictive value for the further development of epilepsy or could be associated with suspected polygenic inheritance which we have found with initial genetic investigations.

All the epileptic dogs included in this study, according to information obtained from owners, suffered from focal seizures, and some with generalization. In 3 epileptic FSDs (1 dog with midline spikes from study IV not included) out of 17 dogs (III, IV), focal epileptiform activity was noted upon visual examination of the EEG records. Spikes, polyspikes, and spike slow wave complexes were located in the posterior and occipital areas in all of them. Spikes and spike slow wave pattern is recognized as a specific finding in many epileptic syndromes in human medicine (Stern & Engel 2005, Pillai & Sperling 2006). Interictal focal epileptiform paroxysms were found in the EEG of 18% of the epileptic FSDs (24% when dogs with midline spikes were included) and that contradicts the general viewpoint (Klemm & Hall 1970, Jaggy & Bernardini 1998, Berendt et al. 1999, Pellegrino & Sica 2004). Our study (III) was the first which pointed out that interictal epileptiform activity in dogs might be a less frequent finding than described. More recent publications have confirmed our results (Brauer et al. 2012, Pakozdy et al. 2012). For blinded EEG evaluation, a medical electrophysiologist was employed (III, IV), who strictly applied standards adapted from human medicine (Noachtar et al. 1999). Another EEG evaluation which detected epileptiform discharges in all epileptic dogs was performed by a veterinary specialist working with animals who was aware of dogs' epileptic status (II). Interestingly, some findings described as epileptiform by a non-blinded observer and not assured by the medical electrophysiologist had the perfectly same location as described by FDG-PET findings. This might indicate some variations between EEG patterns in dogs and humans. However, diagnostic yield of EEG for epilepsy would also rise up to 41% for first

evaluator when summarizing all findings (3 dogs with changes in background rhythms and 1 dog with midline spikes). In humans, epileptiform activity is found in 29-55% of epileptic patients at first EEG examination, but can be increased up to 90% in repeated examination with the help of sleep deprivation, hyperventilation, or photic stimulation (Jabbari et al. 2000, Pillai & Sperling 2006).

EEG results indicate that FSDs could experience focal IE originating from occipital or other posterior areas. Epileptic discharges in the occipital lobe are a relatively common finding in human patients, especially in children (Taylor et al. 2003), but occipital epilepsies account for 5-10% of all human epilepsies (Adcock & Panayiotopoulos 2012).

### *5.4.2. Quantitative EEG*

We found low delta and theta rhythms to be the dominant background activity, but alpha and beta bands were poorly represented in background frequency analysis of quantitative EEG in all FSDs. This seems to be a common EEG background pattern in sedated dogs (Srenk & Jaggy 1996, Itamato et al. 2001, Bergamasco et al. 2003, Pellegrino & Sica 2004). Slow theta and fast beta frequency bands appeared significantly more frequently in epileptic dogs than in control dogs, but only alpha activity showed statistical difference between epileptic FSDs without treatment and control dogs.

An increase in the beta band was observed in a previous EEG study of healthy human volunteers after the oral administration of phenobarbital, and in a study of epileptic patients, although the results failed to reach statistical significance (Sannita et al. 1980, Herkes et al. 1993). A dose-dependent increase in beta and theta bands after phenobarbital administration has been reported in rats as well (Sato 1980). Theta rhythm is associated with the use of sedatives, such as barbiturates or neuroleptics, in humans (Stern & Engel 2005). We observed highly significant differences in all background bands between epileptic dogs without treatment and under medication. The phenobarbital influence described here agrees with the literature. Phenobarbital seems to increase the theta, alpha, and beta bands, and to decrease the delta band. Our results indicate that treatment with antiepileptic drugs should be considered when interpreting the data from quantitative EEG. Despite the fact that multiple findings from quantitative EEG in this study reached a level of significance (Table 7), applying these results to clinical settings might be complicated. However, in epileptic patients whose visual evaluation of interictal EEG remains normal, information from quantitative analysis of EEG might be complementary.

## 5.5. FDG-PET of epileptic FSDs (IV)

### *5.5.1. Visual evaluation of FDG-PET*

This is the first report where cerebral glucose metabolism in dogs diagnosed with IE is examined alongside FDG-PET results that indicate focal seizures in FSDs. Visual focal abnormality/asymmetry in FDG-PET demonstrated high sensitivity (82%) but low

specificity (50%) for epilepsy as several healthy dogs also had focal findings in FDG-PET. Visual findings in FSDs involved a variety of cortical areas, but the most consistent focal abnormalities in the epileptic dogs were seen in the occipital region (with sensitivity of 64% and specificity of 100%). Therefore we summarize that visual evaluation of FDG-PET is able to detect hypometabolic foci in FSDs in a variety of cortical areas while changes in the occipital lobe are specifically related to epilepsy. In epileptic human patients interictal FDG-PET identifies epileptic foci related to neocortical epilepsy (temporal lobe epilepsy excluded), including occipital lobe epilepsy, in a relatively small proportion (< 50%) of patients (Casse et al. 2002, Juhasz et al. 2005, Lee et al. 2005). However, depending on the subgroups of patients or the use of a high resolution PET scanner, a remarkably higher diagnostic yield of FDG-PET can be expected (Hong et al. 2002, Juhasz et al. 2005).

Cortical hypometabolic areas were also detected in half of the control dogs. Epilepsy not recognized by the owner in control dogs cannot completely be excluded, as they had close epileptic relatives. However, follow-up interviews 5 years after PET examination did not change the disease status of any of the control dogs. High frequency of cerebral hypometabolic changes in control dogs can be explained similarly to what was described previously for EEG findings in control dogs for disease with polygenic inheritance.

### *5.5.2. Semi-quantitative analysis of FDG-PET*

The range of the asymmetry indexes was quite wide in the control dogs and the AIs on the visually identified hypo/hypermetabolic regions did not exceed the normal range. In epileptic human patients, asymmetry between 10-15% indicates possible areas of reduced glucose utilization and over 15% indicates clear regions of hypometabolism (Lamusuo et al. 2001). In study IV, AI of control and epileptic dogs had no significant variance in any regions and the highest value was 4.3. The highest AI was detected in the caudal colliculus, a minor structure where it also had a perfect match with asymmetry findings detected in the visual evaluation. The lack of asymmetry findings is suspected to be related to the VOI defining method. Partial hypometabolic findings can be masked with higher uptake of neighboring cortical areas when the VOI covers the wider cortical structures. Therefore to define the validity of asymmetry characteristics for cerebral glucose metabolism in epileptic dogs, new studies examining dogs with homologous clinical epilepsy manifestation with well defined ROIs guided by the results of visual analysis and drawn on the high resolution MR images would be optimal.

Median SUV for whole brain for our control dogs was in compliance with results previously reported for dogs (Irimajiri et al. 2010, Lee et al. 2010, Hansen et al. 2011, Kang et al. 2012). In Study IV the highest regional uptake of FDG was found in the tectum (caudal colliculus). This has also been described in earlier reports and may be explained by the auditory reflex, which is also active in sedated dogs and is more prominent in some dog breeds (Irimajiri et al. 2010, Kang et al. 2012). The highest

cortical SUV was found in the temporal and parietal lobes, and the lowest in frontal and occipital lobes. This is in discrepancy with previous publications where the highest uptake in dogs was detected in nearly opposite order (Lee et al. 2010, Hansen et al. 2011, Kang et al. 2012). A similar trend was also noticed in control FSDs, however there was no significant variability in FDG uptake of these cortical structures. Interestingly, dogs studied by SPECT have also shown a perfusion index that is highest in the occipital lobe, with a significant rostro-caudal perfusion gradient, and epileptic dogs were not significantly different from the healthy controls (Peremans et al. 2001, Martle et al. 2009). Therefore it is possible that such relative reduction of FDG uptake in the occipital lobe in the FSDs is a unique finding and may indirectly reflect the lowered seizure threshold in the occipital lobe, possibly characteristic of the breed or group of dog breeds. The lowest FDG uptake in dogs is reported to be in the brainstem, but this area was not measured in this study (Lee et al. 2010). In our study, FDG uptake in the cerebellar hemispheres was lower than in the vermis. Therefore the uptake variations within the substructures of larger anatomical structures should be cautiously considered when interpreting quantitative data (Kang et al. 2012).

## 5.6. Comparisons between MRI, EEG, PET and phenotype (I-IV)

### *5.6.1. Comparison of diagnostic modalities*

MRI results suggest that FSDs represent patients with non-lesional epilepsy and that MRI should be considered as a diagnostic modality for excluding other possible cerebral diseases causing seizures and not to confirm etiological background for seizures in our population. The conventional brain histopathology of 2 control and 3 FSDs with epilepsy that were examined also failed to detect abnormalities (results not presented). Therefore no method to confirm validity of functional cerebral examinations in FSDs with epilepsy exists at the moment. We are the first to contrast EEG and FDG-PET results in dogs (IV). EEG was less sensitive (36%) than FDG-PET (82%) and equally specific (both detected changes in 50% of control dogs) when all possible changes with epileptogenic nature were included. Both diagnostic modalities detected changes in the occipital region with highest specificity (100%) where EEG detected changes in 3 (27%) and FDG-PET in 7 (64%) out of 11 epileptic FSDs. All occipital hypometabolic changes in FDG-PET were described as lateralized. In 2 epileptic dogs which had consensual occipital findings, EEG localized but didn't lateralize the changes. In the third epileptic dog with lateralized occipital EEG findings, FDG-PET detected a hypermetabolic region that was suspected to be related to postictal changes. Therefore these results suggest that FDG-PET is a superior functional modality to detect changes in epileptic dogs originated from the occipital region.

Complementary semi-quantitative analysis of FDG-PET determined the lowest SUV on the epileptic dogs in the occipital region. Moreover, control dogs also had the lowest SUV in the occipital lobe, contradictory with previous reports. Therefore we hypothesize that this finding may reflect the seizure susceptibility in this cortical region characteristic for the FSDs. Regional distribution of background activity bands was not investigated in the EEG study, therefore no supportive information was gathered from

other diagnostic modalities to validate this hypothesis arising from semi-quantitative results of FDG-PET. Despite the fact that FDG-PET demonstrated a higher diagnostic yield than EEG for detection of epilepsy related changes in FSDs, the use of PET for everyday clinical animal patients is impractical. EEG on the contrary is relatively easy to perform, and equipment is available at many veterinary institutions. Special training is also required, however, when performing the EEG and interpreting the results.

The weakness of this study is that although EEG and FDG-PET results were largely in concordance, these findings were based on a small number of dogs. Therefore future studies will be needed to assert these findings. The association of changes in regional cerebral metabolism with the clinical signs of epilepsy and evolution of the cerebral metabolic pattern over time would be of great scientific interest.

### *5.6.2. Comparison of epilepsy phenotype in FSDs and humans*

Characterizing epileptic seizures solely by seizure type lacks accuracy when type and time of ictal clinical signs is not specified. Special attention was paid to initial clinical signs of ictus when ictal phenomenology in FSDs was evaluated, to reveal the brain region generating these signs. Owners recognized initial signs preceding classical ictus in 68.5% of dogs. Behavioral signs like restlessness or anxiety were most consistently reported initial signs of the seizure. Named ictal signs are hard to link to some particular brain region in dogs as they might have multiple interpretations. However, other behavioral signs, like staring at one point with fixed eyes as described by some dog owners, might be more likely linked to visual signs, suspected with occipital-lobe epilepsy. Visual signs described in humans with occipital epilepsy include elementary visual hallucinations, visual illusions, blindness, or visual field defects (Lee et al. 2005). As there is no chance to obtain direct feedback from animal patients, the linkage of these behavioral signs commonly occurring in FSDs to the occipital region (suggested by functional examination), could not be confirmed nor excluded with confidence.

Automatisms, motor or autonomic signs, or combination of these signs were also described as initial ictal signs in some FSDs. Autonomic signs like vomiting, observed in FSDs, have been reported to also occur in Border collies (Hülsmeyer et al. 2010). They have been recognized as a predominantly ictal phenomena in some human epilepsy syndromes (ie. Panayiotopoulos syndrome), that most typically arise from the occipital lobe. Ictal autonomic signs, however, seem to pertain to any epileptogenic cortical onset zone where ictal discharges activate the lower threshold autonomic network (insular cortex, medial prefrontal cortex, amygdale, hypothalamus, and ventrolateral medulla (Panayiotopoulos et al. 2008). Similar to FSDs, these autonomic seizures frequently have a long duration, but are still considered to present benign epilepsy. A different pathophysiological background for FSD epilepsy and Panayiotopoulos syndrome is likely, however as FSDs have an onset of epilepsy at an adult age, whereas maturation-related susceptibility of the central autonomic network specific for childhood epilepsy is the probable underlying mechanism of Panayiotopoulos syndrome (Panayiotopoulos et al. 2008). Autonomic signs, like

vomiting occur in adult humans rarely and usually when consciousness is impaired and following other focal mainly temporal symptoms (Koutroumandis 2003). Contrary autonomic signs following initial ictal signs were commonly observed in FSDs, salivation was the most frequent autonomic sign, but vomiting or regurgitation occurred in quarter of seizures. Motor signs which were also observed as initial ictal signs in some FSDs and were the most frequent symptom during further seizure progression usually indicate involvement of the frontal cortical structures (Foldvary-Schaefer & Unnwongse 2011).

We conclude that although functional examinations most consistently indicate occipital lobe involvement, seizure phenotype of epilepsy in FSDs seems not to be exclusively uniform for that cortical region. Functional examinations in FSDs inconsistently showed involvement of other cortical regions. When combining these findings with the epilepsy phenotype, is likely that this breed experiences epilepsy with a genetic background which determines susceptibility to epileptogenesis in wider cortical regions, foremost posterior.

Although many similarities between epilepsy in FSDs and humans can be shown, no human epilepsy syndrome was in line with FSDs epilepsy. This study indicates, however, that epilepsy in FSDs represents a fairly uniform phenotype and diagnostic characteristics to define it as a novel epilepsy syndrome in dogs, posterior focal IE. Further studies should assert whether posterior focal IE is typical for FSDs or if it might have a wider appearance in a variety of dog breeds.

# 6. CONCLUSIONS

The major conclusions of the work presented in this thesis are:

- Epilepsy in FSDs is characterized by focal seizures. Multiple criteria suggest a generally benign course of epilepsy, although the occurrence of a generalized seizure phase is a risk factor for the development of a progressive disease course in FSDs. Prevalence of epilepsy in the FSDs population in Finland is 5.3% and IE is inherited via polygenic traits.

- Based on the current MRI study (1.5T), epilepsy in FSDs is non-lesional. Focal cerebral glucose hypometabolism in FDG-PET and paroxysmal epileptiform activity in EEG, both detected in the occipital region, were characteristic for epilepsy in FSDs. Although not reaching a significant level, EEG and FDG-PET findings in wider posterior cortical areas could be related to epilepsy in FSDs.

- Visual evaluation of interictal FDG-PET is a sensitive method, but hypometabolic findings are highly specific for IE in FSDs only when detected in the occipital region. Diagnostic yield of interictal EEG for IE in FSDs remains lower. Results from two functional modalities (EEG and FDG-PET) used in control and epileptic FSDs were in concordance. Concomitant use of visual and quantitative analysis of EEG and FDG-PET in epileptic dogs is advised as complementary information can be achieved. Further research on the larger cohort of epileptic dogs will be needed to evaluate concordance of the EEG and FDG-PET findings, and results interpreted in light of the clinical signs.

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Ranno Viitmaa

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