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# The role of the insula in heart rate variability

*Par*

**THI PHUOC YEN TRAN**

**Département de Neurosciences**

**Faculté de Médecine**

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# The role of the insula in heart rate variability

*Présenté par*

**Thi Phuoc Yen Tran**

*A été évalué par un jury composé des personnes suivantes*

**Michel Panisset**
Président-rapporteur

**Dang Khoa Nguyen**
Directeur de recherche

**Mark Keezer**
Co-directeur

**Elsa Rossignol**
Membre du jury

# Résumé

Des preuves cumulatives soutiennent le rôle de l'insula dans la régulation autonomique cardiaque et son dysfonctionnement pourrait être impliqué dans la physiopathologie de la mort subite et inexpliquée en épilepsie (MSIE –SUDEP en anglais). La variabilité de la fréquence cardiaque (VFC) est un outil simple et fiable pour évaluer la fonction autonomique; il est également considéré comme un prédicteur potentiel de la tachycardie ventriculaire et de la mort subite chez les patients après un infarctus du myocarde. Au cours des deux dernières décennies, la VFC a suscité beaucoup d'intérêt dans le monde de l'épilepsie. Toutefois, même si plusieurs études ont tenté d'évaluer les changements de VFC dans différentes formes d'épilepsie, les résultats ont été hétérogènes voire paradoxaux de sorte que son utilité en tant que marqueur de la MSIE est loin d'être concluant. Notons que la majorité des études ont porté sur l'épilepsie temporale. Aucune étude n'a étudié les changements de la fonction autonomique cardiaque dans l'épilepsie insulo-operculaire (EIO). Il est encore incertain si une chirurgie d'épilepsie insulaire peut accélérer la dysfonction autonomique inhérente. Dans cette étude, nous visons à étudier les changements de la VFC interictale chez les patients avec EIO. Nous avons en outre évalué l'effet de la chirurgie insulo-operculaire sur ces modifications de la VFC.

Quatorze patients avec une EIO et un bon résultat post-chirurgie insulo-operculaire (Engel I-II) ont été recrutés pour cette étude. Quatorze patients appariés pour l'âge et le sexe atteints d'épilepsie du lobe temporal (ELT) et exempts de crise après une lobectomie temporale antérieure et 28 individus en bonne santé appariés selon l'âge et le sexe ont également été identifiés pour les besoins de l'étude. La VFC dans le domaine temporel [RMSSD (root mean square of successive RR interval differences, pNN50 (percentage of successive RR intervals that differ by more than 50ms)] et le domaine fréquentiel [LF (low frequency) et HF (high frequency)] ont été étudiés dans les périodes préopératoire et postopératoire (6-204 mois). La VFC avant la chirurgie des patients épileptiques fut calculée à partir des enregistrements EKG obtenus simultanément aux enregistrements vidéo-EEGs effectués dans le cadre de leur évaluation préchirurgicale. La VFC après la chirurgie fut calculée chez tous les patients et les sujets sains à partir d'un EKG de repos d'une durée d'une heure au laboratoire. Le score d'inventaire des risques

de MSIE (le score SUDEP-7) a été calculé à partir des données cliniques obtenues dans le dossier médical de chaque patient.

Les résultats n'ont montré aucune différence statistiquement significative dans toutes les mesures de VFC entre les groupes de patients avec EIO, de patients avec ELT avant la chirurgie et de sujets sains. Chez les patients avec EIO, le score SUDEP-7 variant de 1 à 6 (moyenne de 2,9; SD :1,2) était positivement corrélé avec le pNN50 ($r = 0,671$; $p = 0,009$ et Ln (RMSSD) ($r = 0,591$; $p = 0,026$). En postopératoire, les mesures de la VFC n'étaient pas statistiquement différentes des valeurs préopératoires ou de celles des témoins. Nous avons mené une analyse exploratoire dans laquelle nous avons stratifié les patients avec EIO en deux sous-groupes : un premier groupe (1a) dont les valeurs préopératoires de Ln (RMSSD) étaient inférieures à 3,52 (valeur moyenne de notre échantillon sain) et un second groupe (1b) dont les valeurs préopératoires étaient au-dessus. En préopératoire, dans le groupe 1a, toutes les valeurs du domaine temporel et fréquentiel (LnRMSSD, pNN50, LnLF et LnHF) étaient significativement inférieures à celles du groupe témoin ($p <0,01$), tandis que dans le groupe 1b, seules les valeurs du domaine temporel (LnRMSSD et pNN50) étaient significativement plus élevées que ceux du groupe témoin ($p <0,01$). Dans les deux groupes, les valeurs de la VFC avaient tendance à se normaliser après l'opération. En revanche, la lobectomie temporale antérieure des patients avec ELT n'a pas modifié les valeurs de HRV.

Ces résultats préliminaires suggèrent que dans les EIO réfractaires, la VFC peut être soit diminuée au niveau du tonus sympathique et parasympathique, soit augmentée au niveau du tonus parasympathique. L'augmentation du tonus parasympathique est possiblement inquiétante puisqu'elle était corrélée positivement avec le score SUDEP-7. Une operculo-insulectomie n'a pas affecté négativement la VFC; au contraire, une chirurgie réussie semble entraîner une certaine 'normalisation' de l'HRV. Une confirmation avec un échantillon plus grand est nécessaire.

**Mots-clés** : Variabilité de la fréquence cardiaque, fonction autonomique, épilepsie insulo-operculaire, épilepsie du lobe temporal, mort subite inattendue dans l'épilepsie.

# Abstract

Cumulative evidence supports the role of the insula in cardiac autonomic regulation whose dysfunction may be involved in the pathophysiology of sudden unexpected death in epilepsy (SUDEP). Heart rate variability (HRV) is a simple and reliable tool to assess autonomic function; it is even considered a potential predictor of ventricular tachycardia and sudden death in patients after myocardial infarction. Over the last two decades, heart rate variability (HRV) has also received much interest in epilepsy research. Several studies have tried to assess HRV changes in different epilepsy types but the results have been heterogeneous and sometimes contradictory; its role as a marker of SUDEP remains uncertain. Of note, most studies involved TLE patients and TLE surgeries; none have looked at HRV changes in insulo-opercular epilepsy (IOE) and how insular resection can affect autonomic function. In this study, we aimed to investigate changes in interictal HRV in IOE. We further evaluated the effect of insulo-opercular surgery on these HRV changes.

Fourteen IOE patients who had a good outcome (Engel I-II) after an insulo-opercular surgery were enrolled in this study. Fourteen age- and sex-matched patients with temporal lobe epilepsy (TLE) who were seizure-free after temporal lobectomy and 28 age- and sex-matched healthy individuals were also included. HRV measurements including time domain [root mean square of successive RR interval differences (RMSSD) and percentage of successive RR intervals that differ by more than 50ms (pNN50)] and frequency domain [low-frequency (LF) and high-frequency (HF)] parameters were carried out in pre- and post-operative periods (6-204 months). Presurgical HRV values for epileptic patients were calculated using EKG obtained simultaneously with video-EEG recordings during the presurgical evaluation. HRV of healthy individuals and post-surgical HRV from all operated epileptic patients were calculated from a 1-hour resting electrocardiogram at the laboratory. We also collected the patients' presurgical data to calculate the SUDEP-7 risk inventory score.

Findings showed no statistically significant differences in all HRV measurements between groups of IOE patients, TLE patients before the surgery, and healthy controls. In IOE patients, the SUDEP-7 score ranged from 1 to 6 (mean 2,9; SD: 1,6) and was positively correlated with pNN50

(r=0,671; p<0,009) and LnRMSSD (r=0,591; p<0,026). Postoperatively, HRV measurements were not statistically different from either preoperative values or those of controls. We conducted exploratory analyses where we stratified IOE patients into those whose preoperative LnRMSSD values were below (Group 1a) versus above (Group 1b) a cut-off threshold of 3,52 (mean value of our healthy sample). Preoperatively, in Group 1a, all time and frequency domain values (LnRMSSD, pNN50, LnLF, and LnHF) were significantly lower than those of controls (p<0,01) while in Group 1b, only time-domain values (LnRMSSD and pNN50) were significantly higher than those of control subjects (p<0,01). In both groups, HRV values tended to normalize postoperatively. In contrast, anterior temporal lobectomy for TLE patients did not alter HRV values.

Our preliminary results suggest that in refractory IOE, HRV may be either decreased globally in sympathetic and parasympathetic tones or increased in parasympathetic tone. The increase in parasympathetic tone observed preoperatively may be of clinical concern as it was positively correlated with the SUDEP-7 score. The insulo-opercular resection did not affect the HRV; successful surgery might even have a good impact on HRV changes. Confirmation with a larger sample size is necessary.

**Keywords:** Heart rate variability, autonomic function, insulo-opercular epilepsy, temporal lobe epilepsy, sudden unexpected death in epilepsy.

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# List of Acronyms

ASM: Anti-seizure medication

ApEn: Approximate entropy

ATL: Anterior temporal lobectomy

CWE: Children with epilepsy

DFA: Detrended fluctuation analysis

EKG: Electrocardiogram

EEG: Electroencephalography

DS: Dravet syndrome

fMRI: Functional magnetic resonance imaging

GTCS: Generalized tonic-clonic seizure

HF: High frequency

HRV: Heart rate variability

HR max – HR min: Average difference between the highest and lowest heart rates during each respiratory cycle.

HTI: HRV triangular index

LF: Low frequency

LF/HF: Ratio of LF to HF power

Ln: Natural logarithm

IOE: Insulo-opercular epilepsy

MCA: Middle cerebral artery

N-N: Normal to normal

pNN50: the proportion derived by dividing number of interval differences of successive NN intervals greater than 50 ms by the total number of NN intervals

RMSSD: Root mean square of successive difference between normal heartbeats

RRI: R-R interval

SCN1A: Sodium voltage-gated channel alpha-1 subunit

SDNN: Standard deviation of the NN interval

SDNN index: Mean of the standard deviations of all the NN intervals for each 5-min segment of a 24-hour HRV recording

SD1: Poincaré plot standard deviation perpendicular to the line of identity

SD2: Poincaré plot standard deviation along the line of identity

SDANN: Standard deviation of the average NN intervals for each 5 minute-segment of a 24-hour HRV recording.

SUDEP: Sudden unexpected death in epilepsy

TLE: Temporal lobe epilepsy

TP: Total power

TWA: T-wave alternans

VLF: very low frequency

ULF: ultra-low frequency

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# Chapter 1 – Introduction

## 1.1. Epilepsy and sudden unexpected death in epilepsy

### 1.1.1. Epilepsy

#### 1.1.1.1. Definition, epidemiology and classification

Epilepsy is characterized by “an enduring predisposition to generate spontaneous epileptic seizures”(1). It is estimated that 45,9 million people around the world have epilepsy (2). A recent meta-analysis study showed that the prevalence of active epilepsy was 6,38 per 1000 persons, while the overall lifetime prevalence was 7,6 per 1000 persons. The annual cumulative incidence was 67,77 per 100 000 persons, and the incidence rate was 61,44 per 100 000 person-years (2). This condition has numerous neurological, cognitive, and psychosocial consequences (3). In 2010 epilepsy was ranked as the second most burdensome neurologic disorder worldwide in disability-adjusted life-years (4). According to the 2016 Global Burden of Disease Collaborators, epilepsy is still an important cause of disability and mortality representing 0,56% of the total disability-adjusted life-years globally (5).

The classification of epilepsy, when possible, is made at three different levels (“seizure type,” “epilepsy type,” and “epilepsy syndrome”) and also based on etiology (6). Depending on initial ictal manifestations, seizures are classified as “focal onset,” “generalized onset,” or “unknown onset” (7). Epilepsy types include “focal epilepsy,” “generalized epilepsy,” “combined generalized and focal epilepsy,” and “unknown epilepsy” (6). An epilepsy syndrome is defined as “a cluster of features incorporating seizure types, electroencephalographic (EEG), and imaging features that tend to occur together.” Factors contributing to epilepsy syndrome include age at onset, remission, triggers, diurnal variation, comorbidities (e.g., intellectual and psychiatric dysfunction), imaging studies, and etiology (6).

#### 1.1.1.2. Etiology

The etiology of epilepsy can broadly be divided into structural, genetic, infectious, metabolic, immune, and unknown. A patient may have more than one cause (6).

A visible structural abnormality on neuroimaging refers to a structural etiology of epilepsy when it is concordant with the electroclinical assessment (6). Structural abnormalities may be genetic (e.g., malformations of cortical development such as lissencephaly or a focal cortical dysplasia, tubers and tuberous sclerosis complex), acquired (e.g., head trauma, stroke, or infection), or both (e.g., polymicrogyria may result from a mutation in *WDR63* and *PIK3R2* genes or due to intrauterine cerebral injury, infection, or hypoxia-ischemia) (8).

A genetic etiology refers to a known or presumed genetic mutation in which seizures are a core symptom of the disorder. A genetic mutation may be inherited or de novo (6). The majority of genetic epilepsies, such as the genetic generalized epilepsies (childhood absence epilepsy and juvenile myoclonic epilepsy) and familial temporal lobe epilepsy, do not show Mendelian inheritance (9). These epilepsies are thought to have a complex inheritance pattern and polygenic architecture (10). A number of genes known to be responsible for different specific epilepsy syndromes have been identified (6). For example, mutations in *CHRNA4* and *CHRNB2*, coding for the α4 and β2 subunits of the neuronal nicotinic acetylcholine receptor, have been linked to familial sleep-related hyper-motor epilepsy (previously known as autosomal dominant nocturnal frontal lobe epilepsy) (11). Mutations in *SCN1A* are found in 80% of patients with Dravet syndrome, 90% of which occur *de novo* (12). In some cases, the underlying genes are not yet identified but presumed based notably on epidemiological data (6).

The most common etiology worldwide is brain infections. Some common infectious causes include neurocysticercosis, tuberculosis, cerebral toxoplasmosis, and cytomegalovirus (6).

A metabolic disorder is often combined with a genetic etiology because many metabolic epilepsies have known genetic mutations (6). For example, mutations in the *SLC2A1* gene can cause Glucose transporter type 1 deficiency syndrome (13).

An autoimmune etiology is increasingly recognized, especially with the development of antibody testing (6). Leucine-rich glioma-inactivated protein 1, N-methyl-D-aspartate receptor,

and glutamic acid decarboxylase 65 antibodies are some common antibodies associated with autoimmune epilepsy (14).

It is not always feasible to find an etiology for every patient, and sometimes, the prevalence of each category can vary across countries and continents (6).

#### 1.1.1.3. Pathophysiology and the consequences of refractory epilepsy

Epileptogenesis is the gradual process by which a non-epileptic brain is converted into one capable of generating spontaneous, recurrent seizures (15). Epileptic seizures represent an imbalance between excitatory and inhibitory currents or neurotransmission within the neuronal network (15). Two types of ion channels mediate these currents (16). The first type is the voltage-gated ion channels activated by the inward and outward sodium, calcium, chloride, and potassium conductance. The second type is the ligand-gated ion channels activated by binding glutamate or gamma-aminobutyric acid to the postsynaptic membrane's receptor (16). Microglial cells also have an essential role in maintaining synaptic and neuronal homeostasis during normal and abnormal brain conditions (17).

The consequences of epilepsy are multiple and multifactorial, depending on the etiology, epilepsy syndrome, seizure types, frequency, and epilepsy duration. Patients with epilepsy face numerous psychosocial and medical challenges. About 40% of epileptic children aged between 4 to 15 years have at least one additional neurological problem, such as intellectual disability, speech and language difficulties, or other specific cognitive disabilities (18). Patients with other neurological disabilities (e.g., feeding or swallowing difficulties, inability to walk) will require more healthcare support than those without these problems (18). Psychiatric disorders are relatively frequent comorbidities in epilepsy, with a lifetime prevalence of up to 35% with mood and anxiety disorders being the most frequently reported (19). Depression has been identified as an essential predictor of lower quality of life, increased seizure severity, drug-resistance, and a poor surgical outcome (20). Suicide is a severe complication of untreated and undiagnosed depression. The risk of death by suicide is about three times higher in people with epilepsy than in the general population (21). Epileptic patients may die from other causes such as sudden unexpected death

in epilepsy, accidents, vascular disease, and factors directly related to the underlying causes (e.g., brain tumors) (22).

### 1.1.2. Sudden unexpected death in epilepsy

Sudden unexpected death in epilepsy (SUDEP) is a fatal complication of epilepsy; definite SUDEP is defined as a "sudden, unexpected, non-traumatic, non-drowning death in an individual with epilepsy, witnessed or unwitnessed, in which autopsy does not reveal an anatomic or toxicologic cause for the death" (23). Probable SUDEP must meet the definite SUDEP criteria in the absence of an autopsy (23). Recent findings indicate that SUDEP affects 1 in 4500 children and 1 in 1000 adults with epilepsy per year (24).

#### 1.1.2.1. Risk factors

The precise causes and underlying pathophysiological mechanisms of SUDEP are still unknown, but some consistent risk factors include poor seizure control, frequent generalized tonic-clonic seizures (GTCS), especially during sleep, and long-standing epilepsy (25).

The first SUDEP inventory (26), named the SUDEP-7 inventory, was developed from seven validated SUDEP risk factors identified in a prospective study (27). In this study, the authors prospectively enrolled 4578 epileptic patients evaluated at three epilepsy centers (MINCEP Epilepsy care, Minneapolis, MN; Mayo Clinic Epilepsy Division, Rochester, MN; and Marshfield Clinic Epilepsy Section, Marshfield, WI) from June 1991 to December 1996 and followed for a total of 16 463 patient-years. This cohort was screened for deaths annually and was pivotal in determining SUDEP incidence and risk factors. One hundred eleven patients died during this period and SUDEP accounted for 18% of these deaths. The seven core risk factors that were identified for SUDEP consisted in the following: (1) more than three tonic-clonic seizures in the last year; (2) one or more tonic-clonic seizures in the last year; (3) one or more seizures of any type over the last 12 months; (4) more than 50 seizures of any type per month over the last 12 months; (5) duration of epilepsy ≥ 30 years; (6) current use of three or more antiseizure medications (ASMs); (7) mental retardation, IQ<70, or too impaired to be tested (27). DeGiorgio et al. (2017) conducted a meta-analysis from studies published in the core clinical and epilepsy journals from 1987 to 2017 (28). The "weighted log of adjusted odds ratio (OR) or relative risk

ratio (RR)" was used to rank the risk factors. The top ten leading risk factors of SUDEP ranked from highest to lowest were (1) ≥ 3 GTCS/years; (2) ≥ 13 seizures in the last year; (3) no ASM treatment; (4) ≥ 3 ASMs; (5) ≥ 3 GTCS in the past year; (6) 11-12 GTCSs in the last three months; (7) age at onset 0-15 years old; (8) IQ < 70; (9) 3-5 ASM changes in the last year; (10) ≥ 3 ASMs (28). Risk factors "more than three GTCS" and "more than 3 ASMs" from separated sources occurred twice. Also, patients who carried ion channel gene mutations (e.g., *SCN1A, SCN2A, SCN5A, KCNQ1*…) had an increased risk of SUDEP (29-31).

Despite these identified risk factors, there has been no robust way to predict the occurrence of SUDEP, and the only and the most effective preventive strategy against SUDEP remains achieving seizure control, although this may prove arduous in refractory epilepsy (32). The American Academy of Neurology and the American Epilepsy Society recommend that clinicians inform their patients that seizure freedom, particularly freedom from GTCS, is strongly associated with a decreased SUDEP risk (level B guidelines) (24). For drug-resistant focal epilepsy, evaluation for potential epilepsy surgery is highly recommended as epilepsy surgery is associated with a high chance of seizure freedom for well-selected patients. It has been shown that successful epilepsy surgeries significantly reduce the risk of SUDEP and mortality. Sperling et al. consecutively evaluated 1110 patients between 1986 and 2013 with a total follow-up of 8126,62 person-years: 909 patients had focal resection or subpial transection, 97 patients had anterior or complete corpus callosotomy, and 104 patients did not have brain surgery (33). Of 89 observed deaths, the causes of death were SUDEP or probable SUDEP in 15, cancer in seven; trauma in three, suicide in three, cardiovascular causes in three; various causes in three, and unknown etiology in 55 patients. The authors found that patients treated surgically had a significantly lower mortality rate (8,6 per 1000 person-years [95% confidence interval: 6,58-11,15]) than patients without surgical treatment (25,3 per 1000 person-years [95% confidence interval: 14,5-41,17) (p<0,001).

Recently, Casadei et al. retrospectively analyzed all-cause mortality and SUDEP in a cohort of 590 epilepsy surgery patients and 122 refractory focal epilepsy patients without surgery at Columbia University Medical Center between 1977 and 2014 (34). In the surgical group, there were 34 deaths, 14 of which were SUDEP. The standardized mortality ratio and SUDEP rate were 1,6 and 1,9 per 1000 patient-years. In the non-surgical group, five SUDEP out of 13 deaths were

observed. Both standardized mortality ratio and SUDEP rate were significantly higher than those of the surgical group. The authors found that the time to SUDEP in the surgical group was significantly longer than in the non-surgical group (10,1 years versus 5,9 years; p<0,05). They hypothesized that there was an early benefit of surgery on the occurrence of SUDEP (34).

#### 1.1.2.2. Cardiorespiratory dysregulation in SUDEP

The mechanisms underlying SUDEP are currently unknown. Surges et al. suggested that SUDEP's pathophysiology is multifactorial. It includes several possible pathological pathways, including cardiorespiratory dysregulation, dysfunction in systemic and cerebral circulation physiology, seizure-induced hormonal and metabolic changes (35).

Most SUDEP cases are seizure-related, suggesting that seizure-induced cardiorespiratory changes are the primary mechanisms. In the Mortality in Epilepsy Monitoring Unit Study (MORTEMUS), an appraisal of cardiorespiratory arrests occurring incidentally in epilepsy monitoring units demonstrated generalized tonic-clonic seizures-induced severe respiratory and cardiac alterations (apnea/hypoventilation and bradycardia/asystole) leading to SUDEP and near SUDEP (36). The authors suggested that the SUDEP's primary mechanism is an early postictal autonomic nervous breakdown induced by generalized tonic-clonic seizures. The link between brain and heart is further supported by the discovery that mutations in channel genes responsible for the cardiac arrhythmias (e.g., *SCN5A* for Brugada syndrome; *KCNQ1* for long QT syndrome) are also expressed in the brain as well as associated with seizures and sudden death (30, 31).

Repetitive seizures can initiate molecular signals and cellular processes that ultimately result in brain structure alterations, including the autonomic nervous system and respiratory control centers. Indeed, voxel-based morphometric studies have shown tissue loss in cortical and subcortical structures that modulate cardiorespiratory functions such as the thalamus, frontal cortex, including the medial and lateral orbitofrontal cortex, and brainstem both in SUDEP patients as well as patients at high risk (37-39). A recent study by Allen et al. showed that SUDEP patients had significant tissue loss in brain areas, which are essential for cardiorespiratory recoveries, such as the medial and lateral cerebellum, the periaqueductal gray area, left posterior and medial thalamus, left hippocampus, and bilateral posterior cingulate areas (40). There was

also an increase in volumes in areas that trigger hypotension or impede respiratory patterns, including bilateral amygdala, entorhinal, parahippocampal, and sub-callosal cortices (40). Functional studies using resting state-fMRI in patients at high risk of SUDEP showed reduced functional connectivity between the brainstem and thalamus, anterior cingulate, thalamus, and bilateral putamen (41, 42). Besides, there were enhanced connectivity patterns from the frontal medial or orbital frontal cortex to the insula and limbic cortices (hippocampus and amygdala) (42). These studies demonstrated the significant inhibitory role of the medial and orbital frontal cortices in autonomic regulation (43, 44), suggesting that these regions are involved in blood pressure regulation (45). Therefore, the increased connectivity between the insula and the orbital frontal cortex/medial frontal cortex found in the high-risk SUDEP patients may be linked with blood pressure regulation during ictal periods.

## 1.2. Insular cortex

The insular cortex, also known as the island of Reil, is a pyramid-shaped area located below the Sylvian fissure, fully covered laterally by the frontal, parietal and temporal opercula. Owing to the widespread connections between the insula and other cortical (in the frontal, temporal and parietal lobes) as well as subcortical (thalamus, amygdala, hippocampus, putamen, globus pallidus, caudate nucleus and nucleus accumbens) structures (46, 47), the insula plays a vital role in multiple functions including sensorimotor processing (viscerosensory, somatosensory, gustatory, olfactory, auditory sensations), socio-emotional processing (emotional experience, empathy, and social cognition, risky decision making), cognitive functions (attention and salience processing, speech), and autonomic control (48). In this chapter, we will mainly focus on the role of the insula in autonomic function.

It is now well established that the insula is part of the central autonomic network (which includes the anterior cingulate, prefrontal cortex, amygdala, and subcortical structures such as the periaqueductal gray matter, parabrachial nucleus, and nucleus of the solitary tract) (49) and that the insula is one of the primary components of the brain-heart association (50). However, there is still some controversy about the side and specific subregions of the insula involved in sympathetic and parasympathetic autonomic control (51, 52).

The insula's implication in autonomic regulation is supported by both animal and human studies. In rats, electrical micro-stimulation of the insular cortex has induced two distinct patterns of autonomic response: (a) an increase in arterial pressure and tachycardia from the stimulation of the rostral half of the posterior insular cortex or (b) a decrease in arterial pressure with bradycardia from stimulation of the caudal part of the posterior insular region (53, 54). Researchers have also found connectivity differences between pressor and depressor sites by injecting the anterograde axonal tracer Phaseolus Vulgaris leucoagglutinin (PHA-L). Pressor sites of the insular cortex (the rostral posterior insula) strongly connected with other limbic regions, including the infralimbic cortex, amygdala, bed nucleus of the stria terminalis, medial dorsal and intralaminar nuclei of the thalamus, while depressor sites of the insula (the caudal part of the posterior insula) connected with sensory areas of the brain, including the primary somatosensory cortex, a peripheral region of the sensory relay nuclei of the thalamus, and the caudal spinal trigeminal nucleus (53). Similarly, in humans, changes in heart rate and blood pressure have been elicited by insular electrical stimulation (51, 55). Chouchou et al. provided a functional mapping of the insula's autonomic cardiac activity through the intracortical stimulation of 47 epileptic patients. Findings indicated symmetric parasympathetic and sympathetic responses between the right and left insula (51). However, sympathetic responses predominated in the posterior insula while parasympathetic responses were more anterior in the insula's medial part, in line with animal studies. More recently, combining the instantaneous estimation of heartbeat dynamic and brain resting-state fMRI, Valenza et al. (56) confirmed the critical role of the insula (as well as other structures such as the cingulate, frontal/prefrontal cortices, and thalamus) in the central autonomic network (57-59). Macey et al. used three autonomic challenges (the Valsalva, handgrip, and foot cold pressor) with fMRI procedures to examine the topographic organization for autonomic regulation in the human insula (60). The authors showed that all autonomic challenges elicited sympathetic responses bilaterally in both the anterior and posterior insula with the predominance in the right anterior insula for the Valsalva maneuver, both posterior insulae for handgrip, and the right anterior/posterior insula for the cold pressor.

Apart from cortical stimulation and functional MRI studies, lesion-based studies can also provide additional insight into the role of the insula in autonomic control. Indeed, the insula may

be implicated in several neurological conditions such as insular ischemic and hemorrhagic strokes, tumors, or epilepsy.

### 1.2.1. Cardiac autonomic changes with insular strokes

The arterial supply of the insula originates from the middle cerebral artery (MCA). The anterior insula and the posterior insula are supplied predominantly by the superior division and the inferior division of the MCA's second branch (M2), respectively (61). Apart from the insula, the M2 branch supplies other areas, including the extreme capsule, the claustrum, the external capsule, the corona radiata, and the operculum's medial surface (61). Due to its blood supply nature, small insular infarcts (e.g., limited to insula only) are rare while larger MCA stroke with insular damage is common. The insula is vulnerable to ischemia due to thromboembolic occlusion of the proximal MCA (62). Indeed, insular involvement occurs in nearly 50% of patients with non-lacunar ischemic MCA territory strokes (63). On the other hand, isolated insular strokes occur only in 0,1-8% of acute strokes (62, 64, 65).

In general, autonomic dysregulation is frequent after acute ischemic strokes (66). Following acute ischemic strokes, there is an activation of the hypothalamic-pituitary-adrenal (HPA) axis resulting in increased cortisol production and circulating cortisol levels, stimulating the adrenal gland to secrete catecholamines (67, 68). Several studies have revealed higher sympathetic hyperactivity and cardiovascular complications in patients suffering from strokes that involved the insula compared to those without insular involvement.

In 2004, Meyer et al. prospectively assessed the evolution of autonomic dysfunction during the acute post-ischemic stroke period (from admission, 4-hour after admission, then every day up to the fifth day) in nine patients with complete insular infarction versus 20 non-insular infarction patients (69). The authors showed significantly higher plasma norepinephrine and epinephrine concentrations in patients with strokes involving the insula. Notably, in non-insular infarcts, plasma norepinephrine levels declined significantly after five days, while in insular involvement infarcts, a sustained pathological norepinephrine increase was observed. Also, in patients with strokes involving the insula, both norepinephrine and epinephrine levels were significantly higher in right-sided compared to left-sided strokes (this phenomenon was not seen

in patients without insular involvement infarcts). These findings suggest sustained sympathetic overactivity over the first five days after strokes involving the insula with more severe and excessive disturbance observed in right insular infarcts (69).

Several studies have also shown that new EKG abnormalities (such as atrial fibrillation, atrioventricular block, inverted T wave, prolonged QT dispersion) were more frequent after strokes involving the insula than those without insular involvement (70-72). For example, in a series of 1228 ischemic stroke patients (250 with insular involvement), insular involvement proved to be an independent predictor for new atrial fibrillation in a multivariable regression analysis. Indeed, 45,6% of patients with newly paroxysmal atrial fibrillation during in-hospital EKG monitoring had insular cortex involvement while only 17,8% of patients without paroxysmal atrial fibrillation had insular cortex involvement (70).

Other studies have reported significantly suppressed HRV in patients with acute ischemic stroke, especially when the insula is involved in the infarct (73-75). These studies have also concluded that right hemispheric strokes involving the insula were more vulnerable to developing cardio-autonomic dysfunction, which was correlated with sudden death and three- or twelve-month post-stroke cardiac mortality (72, 74-77). However, these findings conflict with some other studies that showed no association between strokes involving the insular cortex with fatal cardiac events (78, 79). Insular infarction was not a predictor of acute cardiovascular events (63), and there was no difference between right-side or left-side stroke (80).

The main limitation of the studies cited above is that strokes were not limited to the insula to allow isolating the contribution of the insula itself. Also, these studies did not control for the infarction volume which is strongly associated with catecholamine levels after stroke. In 2013, Walter et al. analyzed serum metanephrine and normetanephrine levels after hospital admission in 384 adult patients with an acute MCA ischemic stroke (of whom 149 patients had insular involvement infarcts) (81). When considering only patients with ‘small’ infarcts (<33% of MCA territory), those with insular involvement (n=56) had higher levels of normetanephrine than those with non-insular involvement (n=114). In this group with ‘small’ MCA infarcts, blood pressure, heart frequency, and HRV did not differ between patients with insular and non-insular

involvement. In the group with lesions affecting >33% of MCA territory, normetanephrine and metanephrine levels were significantly higher compared to the group with lesions affecting <33% of MCA territory. There was no difference between patients with and without insular involvement in this group. This study suggests that with massive infarctions, systemic effects appear to overweight the effect of an insular infarction.

There are a few case studies which have reported cardiac complications after an isolated insular stroke. Strokes restricted to the right posterior insula may present with hypertensive episodes (62) or heart failure (82). Mandrioli et al. reported a patient with a left isolated insular stroke who presented persistent neurogenic T wave alterations (83). In 2020, Giammello et al. identified 13 isolated insular stroke patients and reported a high-frequency detection of cardiac disturbances in 11/13 patients (eight left and three right), including newly-detected atrial fibrillation, hypotensive or hypertensive episodes, atypical atrial flutter, bradycardia or tachycardic episodes, and extrasystoles (84).

Overall, most studies in stroke patients suggest a role of the insula in autonomic regulation.

### 1.2.2. Cardiac autonomic changes with insular tumors

Very few studies have looked at cardiac autonomic function in the context of other types of insular damage. Autonomic changes after resection of the insula were first reported by de Morree et al. who examined the alteration of HRV and cardiac rhythms during insular resection (15 minutes before and 15 minutes after the removal of the insular tumor) in two patients with insular low-grade glioma (one right and one left) (85). Following right insular tumor resection, a significant increase in high frequency (HF) power value (which reflects the parasympathetic activity) and bradycardia (resolved with atropine) were observed. The authors hypothesized that the right insular tumor compressed autonomic regulatory tissues leading to parasympathetic suppression and that its removal abolished this suppressive effect by increasing the parasympathetic index. In contrast, left insular tumor resection did not induce any HF power changes although relative tachycardia and high systolic blood pressure were noted both before and after surgery. From this observation, the authors assumed that the sympathetic overactivity

was unaffected by resection of the left insula. Obviously, these observations were limited by the low number of cases.

Subsequently, a study on HRV in 50 patients with newly diagnosed insular glioma (86) showed significantly lower parasympathetic parameter values (all time-domain and HF power indices) and higher sympathetic parameter values (low frequency (LF) power and LF/HF ratio) compared to healthy controls. The meaning of each HRV value will be discussed in more details in section 1.3. Patients with left insular glioma had significantly lower HF values and higher LF and LF/HF ratio values than patients with right insular glioma. These findings suggested a shift toward an increased sympathetic tone in patients with left insular glioma. There were no significant differences related to the size and extent of the tumor. Interestingly, patients who presented seizures (31 out of 50 patients) had more significant suppression in parasympathetic components as compared to patients without seizures. Note that most of these patients had acute seizures in the context of vasogenic edema rather than chronic epilepsy. In this study, the authors did not assess the effect of surgery or the changes in HRV over time.

### 1.2.3. Cardiac autonomic changes in the insular epilepsy

Recently, the role of the insula in drug-resistant epilepsy has been increasingly recognized with the report of several cases of insular, insulo-opercular, or temporo-insular epilepsy successfully treated by resection, radio-frequency thermocoagulation, or laser ablation (87-92).

A few studies have looked at possible evidence of autonomic dysfunction in insular epilepsies with some authors even suggesting a higher risk of SUDEP in such patients given that cardiac autonomic dysfunction is considered to play an essential role in the pathophysiology of SUDEP (35, 93). Seeck et al. reported a three-and-a-half-year-old epileptic child with a right insular dysplasia presenting ictal bradycardia (94). Surges et al. observed recurrent complete atrial-ventricular block episodes in a patient with focal epilepsy due to a left insular lesion (95). Tayah et al. reported ictal bradycardia-asystole in a 48-year-old man with refractory epilepsy related to a left insular lesion (90). Even more disturbing is the report by Lacuey et al., of two SUDEP patients with TLE and temporo-insular epilepsy; both had evidence of left insular damage on MRI after a previous unsuccessful temporal and temporo-insular resection, respectively (96). Analysis of their

HRV over time before their SUDEP revealed progressive HRV changes. The authors suggested that the presence of an intrinsic insular lesion or acquired insular damage in patients with refractory epilepsy could be an additional risk for SUDEP. These cases will be described in detail in the next chapter.

More recently, Allen et al. showed that patients at high SUDEP risk showed enhanced resting-state fMRI connectivity patterns from the frontal medial or orbitofrontal cortex to the insula (42). The same group also found retrospective evidence of progressive enlargement of the bilateral anterior insula in 25 SUDEP cases (97).

## 1.3. Heart rate variability

Heart rate variability (HRV), the R-R interval (RRI) variation between consecutive heartbeats, is widely used to evaluate cardiac autonomic function (98). The sinus rhythm is well-known to be irregular at resting state. Several different physiological systems integrate to modulate the sinoatrial node resulting in the fluctuation of heart rhythms: the efferent and afferent sympathetic and parasympathetic nerves, the heart's intrinsic nervous system, the afferent signals from mechanosensitive and chemosensory neurons, blood pressure, and the respiratory control system (99).

An optimal level of variability is essential for the adaptation to the life-changing and stressful situation in both emotional and physical problems of each healthy person. Too much instability is prejudicial to efficient physiological functioning and energy preservation. Conversely, too little variation indicates pathology (99). HRV indirectly reflects the heart's responses to various environmental factors that trigger autonomic activities. Stimuli such as stress, exercise, or heart disease activate sympathetic activities to increase heart rate. In contrast, parasympathetic activity decreases the firing rate of pacemaker cells and heart rate to regulate physiological autonomic function (99).

### 1.3.1. Overview of HRV metrics

HRV can be investigated from long-term (24 hours), short-term (2 - 5 minutes), or ultra-short-term (<2 minutes) recordings. In long-term HRV recordings, subjects are usually allowed to

go about their daily activities. In short-term HRV recordings, subjects are usually recorded under specific conditions (e.g., resting state, lying or sitting position, during vagal reflex etc.) (100). In any case, it is crucial to compare HRV indices obtained from recordings of the same duration and similar conditions (98).

The Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology recommend three classic quantitative methods to characterize HRV from continuous electrocardiogram (EKG) recordings: time-domain, frequency-domain, and non-linear methods (98). Table 1 summarizes the standard HRV measures and their interpretation in clinical HRV assessment.

#### 1.3.1.1. Time-domain method

The time-domain analysis calculates the variability in the intervals between consecutive QRS complexes originating from the sinus node (101). Low or reduced time-domain measures indicate a real autonomic dysfunction. High values may result from some cardiac abnormalities, such as conduction disorders and highly irregular heart rate patterns (e.g., atrial fibrillation) (102, 103). Simple time-domain variables include mean normal-to-normal (N-N) intervals (Figure 1), mean heart rate, the standard deviation of the NN (normal to normal) interval (SDNN), square root of the mean square differences of successive NN intervals (RMSSD), and proportion derived by dividing the number of interval differences of successive NN intervals greater than 50 ms by the total number of NN intervals (pNN50) (98).

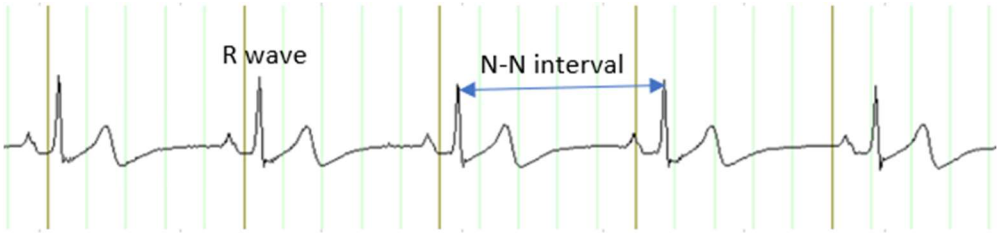



Figure 1. The normal-to-normal (N-N) intervals (Data from one patient)

##### *1.3.1.1.1. SDNN*

SDNN reflects all the cyclic components contributing to the HRV. Both sympathetic and parasympathetic activity contribute to SDNN, and it is strongly correlated with ultra-low

frequency (ULF), very low frequency (VLF), and low frequency (LF) band power (101). In 24-hour recordings, the SDNN is considered as the “gold standard” for medical stratification of cardiac risk (98).

The standard deviation of the average NN intervals for each of the 5-min segments (SDANN) measures long-term fluctuations. It is correlated with SDNN and does not provide additional useful information to SDNN (98, 101).

SDNN index (SDNNI) is the mean of the standard deviations of all the NN intervals for each 5-min segment of a 24-hour HRV recording. The SDNNI correlates with VLF power over a 24-hour period (99, 101).

*1.3.1.1.2. RMSSD*

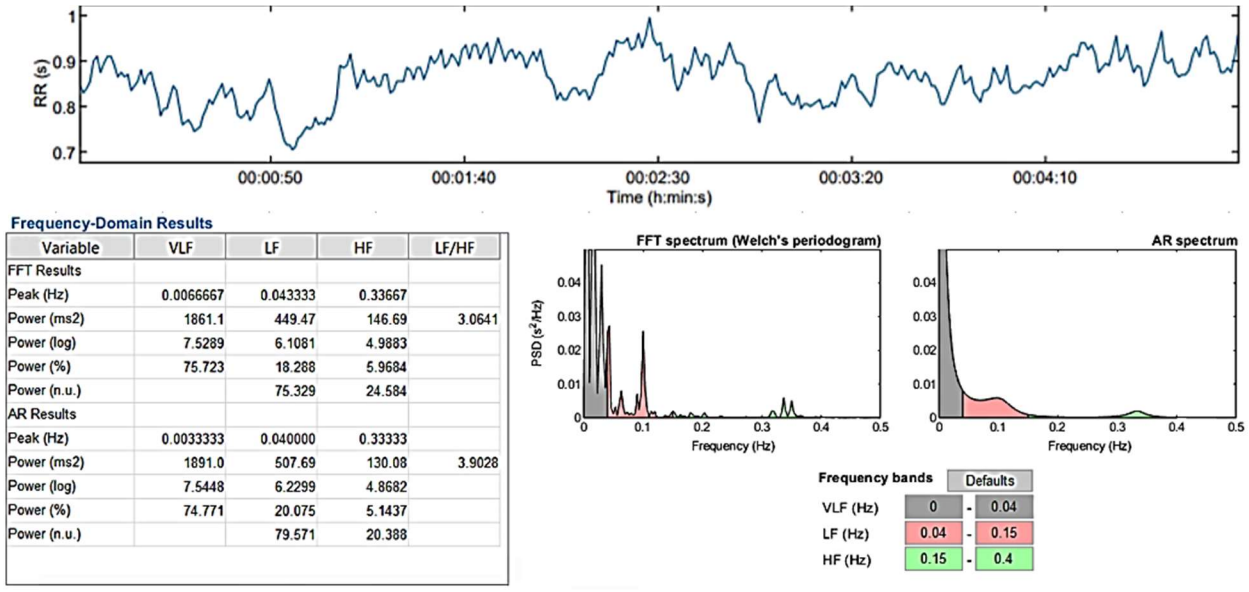
RMSSD is the root mean square of successive differences between normal heartbeats. RMSSD is the primary time-domain measure used to estimate vagally-mediated changes reflected in HRV because it is more sensitive and is not significantly affected by the breathing rate or recording duration (104). RMSSD is identical to the non-linear metric SD1, which reflects short-term HRV (101).

*1.3.1.1.3. pNN50*

pNN50 is also correlated with the RMSSD and HF power but RMSSD provides a better assessment of respiratory sinus arrhythmia (98, 99).

1.3.1.2. Frequency-domains

The frequency-domain or power spectral density analysis evaluates a given heart rhythm's cyclic fluctuations at various frequencies. Extremely low values are associated with a lack of heart rate autonomic modulation, but higher values cannot be assumed to reflect better HRV (102). According to the Task Force report (1996), three main spectral components are distinguished from short-term recordings: high frequency (HF), low frequency (LF), and very low frequency (VLF) bands. These components and an ultra-low frequency (ULF) component can be assessed in 24-hour recordings (98). Figure 2 show an example of spectral analysis of RR interval variability in a healthy individual at rest.




| Variable | VLF | LF | HF | LF/HF |
|---|---|---|---|---|
| FFT Results | | | | |
| Peak (Hz) | 0.0066667 | 0.043333 | 0.33667 | |
| Power (ms2) | 1861.1 | 449.47 | 146.69 | 3.0641 |
| Power (log) | 7.5289 | 6.1081 | 4.9883 | |
| Power (%) | 75.723 | 18.288 | 5.9684 | |
| Power (n.u.) | | 75.329 | 24.584 | |
| AR Results | | | | |
| Peak (Hz) | 0.0033333 | 0.040000 | 0.33333 | |
| Power (ms2) | 1891.0 | 507.69 | 130.08 | 3.9028 |
| Power (log) | 7.5448 | 6.2299 | 4.8682 | |
| Power (%) | 74.771 | 20.075 | 5.1437 | |
| Power (n.u.) | | 79.571 | 20.388 | |

Figure 2. Power spectrum density (PSD) obtained from the 5-min EKG recording during resting state in a healthy individual from our study. The upper trace shows the interval tachogram of consecutive RR values. The bottom trace shows the power spectra, calculated by a Fast Fourier Transform (FFT) (middle inset) and autoregressive (AR) modelling (right inset). VLF = very low frequency; LF = low frequency; HF = high frequency.

*1.3.1.2.1. High frequency band*

The HF spectrum is the power in the range from 0,15 to 0,4 Hz. This band reflects parasympathetic or vagal activity. HF power is frequently called the respiratory band because it is affected by respiratory sinus arrhythmia, defined as the heart rate variation related to the respiratory cycle (98, 101).

*1.3.1.2.2. Low frequency band*

The LF band ranges between 0,04 and 0,15 Hz. Both the parasympathetic and sympathetic activity may produce LF power. In resting conditions, the LF bands reflect baroreflex activity and not cardiac sympathetic innervation. In ambulatory 24-hour HRV recordings, it has been suggested that the LF band also reflects sympathetic activity (98, 101).

Physiological changes in HF and LF depend on different conditions. LF increases during physical activity, standing, moderate exercise in a healthy person, or emotional stress reaction, whereas an increase in HF is observed in controlled respiration, cold stimulation, and rotational stimuli (99).

*1.3.1.2.3. Autonomic balance and the LF/HF ratio*

The parasympathetic nervous system mainly contributes to the HF power while the LF power is affected by both parasympathetic and sympathetic nervous systems, it is assumed that the ratio between LF and HF reflects the autonomic balance (98). However, the interactions between these two branches in generating the LF component are complicated, non-linear, and depend on different contexts (e.g., lying or standing; resting or ambulatory conditions; normal breath or deep breath) and the interpretation of LF/HF is still controversial (99)

*1.3.1.2.4. Very low frequency and ultra-low frequency band*

Less than 10% of the total power of HRV in a 24-hour recording is in the VLF and ULF power spectrum (105).

VLF band ranges between 0,0033 – 0,04 Hz. Experimental evidence suggests that the heart's intrinsic nervous system and efferent sympathetic activity generate these oscillations. In addition, VLF power is affected by thermoregulation, vasomotor activity, and physical activity (99).

The ULF is the lowest frequency band in the 24-hour HRV power spectrum. It measures the variation in the heart rate with every period of 5 minutes once 24 hours (ULF < 0,003Hz), hence it can only be assessed with 24-hour and longer recordings (101, 105). ULF power is strongly associated with SDANN (105).

1.3.1.3. Non-linear methods

Non-linear measurements provide us the unpredictability of a time series and correlate with specific frequency- and time-domain measurements (101). Table 1 describes some common non-linear measurements. Figure 3 shows an example of a Poincaré plot which is graphed by plotting each R-R interval against the following interval, forming a scatter plot (101).

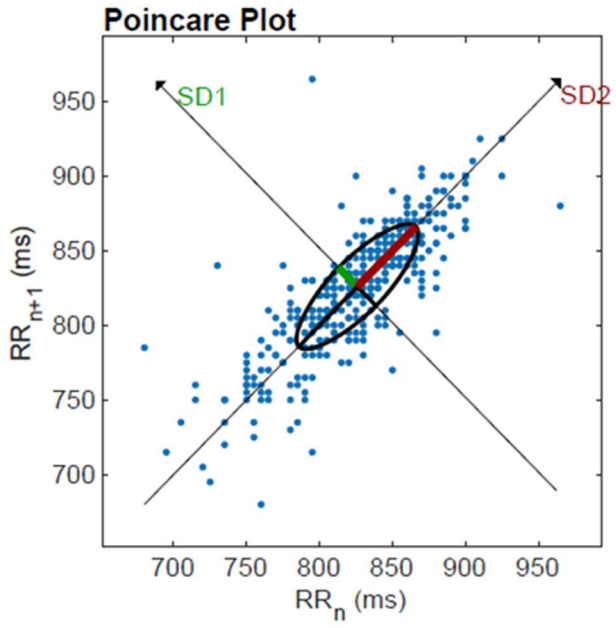



Figure 3. Poincaré plot obtained from the 5-min EKG recording during resting state in a healthy individual from our study. Poincaré plot is analyzed by fitting an ellipse to the plot. The area of the ellipse represents total HRV. The ellipse's width (SD1) is "the standard deviation of the distance of each point from y = x axis". The ellipse's length (SD2) is "the standard deviation of each point from the y = x + average R-R interval" (101).

### 1.3.2. Clinical application of HRV

During the past decades, HRV has increasingly been used to evaluate autonomic function in a variety of situations from physiological conditions (e.g., physical exercise, wakefulness and sleep, different body positions) to several pathological conditions (cardiovascular diseases, psychiatric disorders, neurological disorders, renal conditions etc.). In healthy humans, HRV decreases when people become older, but HRV seems to stabilize when people get above 55 years old (106). A higher HRV is found in men and athletes (107-109). Obesity, smoking and alcohol have adverse effects on HRV. Fortunately, weight loss, smoking cessation, and alcohol abstinence can improve HRV (110-112).

Table 1. Summary of the standard HRV measures used in clinical research

| **HRV time-domain measures** (98, 101) | | |
|---|---|---|
| **Parameter (unit)** | **Description** | **Meanings** |
| SDNN (ms) | Standard deviation of NN intervals | -Both SNS and PNS activity contribute to SDNN<br>-It is highly correlated with ULF, VLF and LF band power, and total power.<br>-It is the biomarker for medical stratification of cardiac risk when recorded over a 24-hour period. |
| SDANN (ms) | Standard deviation of the average NN intervals for each 5-min segment of a 24-hour HRV recording | -SDANN is not a surrogate for SDNN, and it does not provide additional useful information |
| SDNN index (ms) | Mean of the standard deviations of all the NN intervals for each 5-min segment of a 24-hour HRV recording | -It reflects autonomic influence on HRV and correlates with VLF power over a 24-hour period |
| pNN50 (%) | Percentage of successive RR intervals that differ by more than 50ms | -It reflects the parasympathetic activity; it is correlated with RMSSD and HF power. |
| HR max- HR min (bpm) | Average difference between the highest and lowest heart rates during each respiratory cycle | -It is sensitive to the effects of respiration rate, independent of vagus nerve traffic.<br>-It reflects the respiratory sinus arrhythmia |
| RMSSD (ms) | Root mean square of successive RR interval differences | -It is the primary time-domain measure<br>-It is used to estimate the vagally mediated changes reflected in HRV<br>-The RMSSD is identical to the non-linear metric SD1 reflecting short-term HRV.<br>-It is strongly correlated with pNN50 and HF power. |
| HTI | HRV triangular index | -It is a geometric measure based on 24-hour recordings<br>-HTI and RMSSD can jointly distinguish between normal heart rhythms and arrhythmias. |

| **HRV frequency-domain measures** (98, 101) | | |
|---|---|---|
| ULF power ($ms^2$) | Absolute power of the ultra-low-frequency band (≤0.0033Hz) | -It requires a recording period of at least 24 hours.<br>-It is correlated with the SDANN time-domain index. |
| VLF power ($ms^2$) | Absolute power of the very-low-frequency band (0.0033-0.04 Hz) | -Parasympathetic activity may contribute to VLF power.<br>-Sympathetic nervous system blockades do not affect VLF power. |
| LF power ($ms^2$) | Absolute power pf the low-frequency band (0.004-0.015Hz) | -LF power may be produced by both parasympathetic and sympathetic nervous systems. |
| HF power ($ms^2$) | Absolute power of the high-frequency band (0.015-0.4Hz) | -HF power reflects parasympathetic activity<br>-It is called the respiratory band<br>-It is highly correlated with the pNN50 and RMSSD |
| LF/HF | Ratio of LF to HF power | -It is used to measure autonomic balance<br>-Interpretation of 5-min resting LF/HF ratio depends on specific measurement conditions. |
| **HRV non-linear measures** (98, 101) | | |
| SD1 (ms) | Poincaré plot standard deviation perpendicular the line of identity | -SD1 is identical to RMSSD<br>-It reflects short-term HRV |
| SD2 (ms) | Poincaré plot standard deviation along the line of identity | -SD2 measures short- and long-term HRV and correlates with LF power and baroreflex sensitivity. |
| SD1/SD1 | Ratio of SD1 to SD2 | -It is correlated with the LF/HF ratio. |
| ApEn | Approximate entropy | -Measures the regularity and complexity of a time series.<br>-large ApEn values indicate low predictability of fluctuations in successive RR intervals.<br>-Small ApEn values mean that the signal is regular and predictable |
| DFA α1 | Detrended fluctuation analysis – slope α1 | -Describes short-term fluctuations<br>-Reflects the baroreceptor reflex. |
| DFA α2 | Detrended fluctuation analysis – slope α2 | -It reflects long-term fluctuations |

In summary, HRV provides indirect insight into the autonomic activity. Since the first report of its clinical relevance in 1965, studies on HRV in cardiovascular disorders have remarkably increased (98). A decrease in parasympathetic or increase in sympathetic components have been correlated with poor prognosis and an increased risk of sudden death in patients (113-118). Owing to its simplicity and reliability, HRV has also been increasingly used in the detection of autonomic impairment in a variety of neurological disorders such as diabetic neuropathy (119), stroke (120), multiple sclerosis (121), amyotrophic lateral sclerosis (122) and Parkinson's disease (123) or in neuropsychiatric conditions (58, 124, 125).

While several studies have looked at the effect of acute ischemic damage to the insula on HRV, little is known about the impact of chronic insular epilepsy on HRV. In this work, the primary objective is to investigate the interictal HRV changes in patients with insulo-opercular epilepsy (IOE) and the impact of insular epilepsy surgery on HRV.

# Chapter 2 – HRV in epilepsy: review of the literature

## 2.1. Interictal HRV in epilepsy

Given that chronic autonomic dysfunction or cardiovascular dysfunction may be involved in the mechanisms of SUDEP (35, 36), several studies since the late 1990s have measured HRV changes at different time points in different epileptic populations and assessed its potential use as a biomarker for SUDEP. Notably, interictal HRV studies have been conducted on patients with newly diagnosed epilepsy (126-128), generalized epilepsy (129), Lennox-Gastaut syndrome (130), Dravet syndrome (131), frontal lobe epilepsy (132), and temporal lobe epilepsy (TLE) (133, 134). Most studies suggest the presence of autonomic dysfunction in refractory epilepsy; whether these changes are associated with the risk of SUDEP is less clear with inconsistent results (131, 135-139).

### 2.1.1. Interictal HRV changes in children with epilepsy (CWE)

Few studies of HRV changes have been performed in children with epilepsy (CWE). Yang et al. analyzed the power spectrum of 5-min HRV during resting state for the first time in 30 CWE aged between 4 and 10. Thirty age- and sex-matched healthy controls were also enrolled (140). Each patient was tested in supine and then in the head-up tilt position, with 15 minutes between the two tests. The findings showed no significant difference in frequency indices or the LF/HF ratio between the study and control groups. In the CWE group, there was no HRV difference between supine and head-up tilt positions, whereas healthy subjects showed a significantly lower HF power and significantly higher LF power as well as LF/HF ratio in the head-up position than in the supine position. This finding may demonstrate a relative disturbance in the autonomic balance activity in CWE (140).

Subsequently, Ferri et al. evaluated interictal HRV changes during sleep in eleven children (mean age 11,5 years, SD: 3,65) with focal epilepsy (141). Patients slept in the laboratory for at least 8 hours and had EEG, EKG, electrooculogram, and electromyogram recordings. For each patient, nine 5-min epochs were chosen from three sleep stages (sleep stage 2, slow-wave sleep, and rapid eye movement sleep); three epochs for each stage. The standard time-domain and

frequency-domain measures were analyzed. They showed that epileptic patients tended to have lower HRV in both time-domain and frequency-domain (most evident for the HF power), mostly during rapid eye movement sleep. This finding may explain why SUDEP often occurs during sleep.

Harnod et al. revealed significant LF and HF reductions in children with refractory generalized tonic or generalized tonic-clonic epilepsy compared to aged, sex-matched healthy children (142). However, LF% or LF/(HF+LF), which are considered to mirror sympathetic regulation, did not significantly change. They concluded that the lower HRV in their patients resulted from parasympathetic or vagal reduction. These findings were in line with those from the study of Hirfanoglu et al. (143) but contradictory to a more recent study by Okanari et al. (2020) which showed no difference in interictal HRV among children with generalized convulsive seizures as compared to controls (144).

It seemed that most studies on patients with epileptic encephalopathies such as Dravet syndrome (131, 145), Lennox-Gastaut syndrome (130), and West syndrome (146) had revealed significant changes in HRV. These findings may implicate the specific genetic roles or the severity of seizures on HRV.

There was only one case report of HRV changes in Lennox-Gastaut syndrome (130). The authors described a girl with Lennox-Gastaut syndrome who had daily and multiple type seizures (atonic, tonic-clonic, and unconscious focal seizures). Her tonic-clonic seizures were well controlled with the vagus nerve stimulation (VNS). Analysis of HRV during VNS signal-off periods revealed an extreme suppression of frequency-domain components (VLF = 11,5 $ms^2/s$; LF = 13,87 $ms^2/s$ and HF = 20,8 $ms^2/s$) which was significantly improved with VNS signal-on (VLF = 641 $ms^2/s$; LF = 147,6 $ms^2/s$; and HF = 61,81 $ms^2/s$).

Hattori et al. examined HRV in 15 patients with West syndrome and nine age-matched controls during sleep stage 2 (147). Their results showed that only LF power was significantly higher than in the control group. This finding indicated a shift towards sympathetic dominance in West syndrome. In 2015, Moller et al. retrospectively analyzed 5-min interictal sleep HRV and 5-min interictal awake HRV in 23 patients with West syndrome at two different time points: at the time of diagnosis and the end of the study period when clinical spasms or the hypsarrythmia on

EEG disappeared (146). No patients had started treatment with glucocorticoids at the initial recording. At the time of diagnosis, only awake SDNN and total power were significantly lower in patients with West syndrome than controls. Both components significantly increased at the final examination, and they no longer differed from the controls.

Dravet syndrome (DS), a severe epileptic encephalopathy, is mainly caused by mutations in the sodium voltage-gated channel alpha-1 subunit (*SCN1A*) gene. Most studies agree on a decrease of HRV values in patients with DS (131, 145, 148, 149). For example, Delogu et al. (2011) reported a significant decrease in all 24-hour HRV parameter values in 20 patients with DS compared with the ASM-treated patients with other epilepsy syndromes, untreated patients with other epilepsy syndromes, and healthy controls (149). These findings were in line with those observed by Ergul et al. and Lyu et al. in which the 24-hour HRV values in patients with DS were lower than matched healthy controls (145, 148), and those by Myers et al. in which the 5-min-awake-RMSSD values of 40 patients with sodium channel mutations were significantly decreased compared to 40 age-matched patients with non-sodium channel drug-resistant epilepsy (131). Findings reflected a shift of the sympathovagal balance toward the sympathetic tone in DS. In addition to decreased HRV, Ergul et al. and Lyu et al. also found an increased QT dispersion (145, 148), a cardiac repolarization abnormality which may be linked with increased risk of ventricular dysrhythmias (150) in patients with DS. These findings suggested a potential risk of sudden cardiac death in DS patients resulting from a sympathetic overactivity (145, 148).

### 2.1.2. Interictal HRV changes in adults with epilepsy

#### 2.1.2.1. Temporal lobe epilepsy (TLE)

Since the temporal lobe is part of the central autonomic network and because TLE is the most common type of focal epilepsy, patients with TLE have been the most studied in terms of HRV research in adults with epilepsy. A summary of previous studies on HRV in TLE is presented in Table 2.

*2.1.2.1.1. Interictal HRV changes in TLE*

Some studies have found that patients with refractory TLE had a decrease in both parasympathetic and sympathetic HRV components (151-154) while other studies have revealed only a decrease in sympathetic tone (155) or a decrease in parasympathetic and increase in sympathetic HRV parameters (156).

Note however that other studies have failed to show differences with healthy controls (133, 157). The methodological differences may explain these discrepancies, as detailed below. For example, Suorsa et al. conducted a prospective study of 36 patients with TLE performing HRV analysis at the beginning (baseline) and at the end of the study (133). The mean duration of follow-up was 6.1 years. Overall, all HRV measures of TLE patients were not significantly different from those of controls at both study times. At the end of the study, 18 patients continued to have recurrent seizures, and 18 patients were well-controlled. They found that in well-controlled patients, HRV values had remained stable between baseline and after follow-up, while after follow-up, HRV values of refractory TLE patients had decreased compared with baseline. Although HRV measures had decreased after six years compared to baseline in uncontrolled patients, they were still in the normal range (compared with the control group).

Furthermore, there have also been inconsistent results with regards to the lateralization of the temporal focus. A lower LF value was reported with right TLE by Massetani et al. (151). Tomson et al. observed a decrease in the LF/HF ratio with right TLE, but no difference in LF or HF between right and left TLE (155). On the contrary, Dono et al. found that right TLE patients had significantly lower HF, higher LF, and LF/HF ratio than left TLE patients in a series of 52 TLE patients (26 left and 26 right) (158). Similar results were observed when comparing HRV measures between right and left TLE when looking only at seizure-free or non-seizure-free patients.

Discrepant findings observed in the literature may be explained by several factors: a) the small number of patients in most studies; b) the different methods of HRV analysis (long-term 24-hour HRV versus 2-min, 5-min, 30-min, 50-min or 1-hour short-term HRV; c) differences in study design (retrospective versus prospective study; with or without healthy control subjects, and also

level of certainty of focus localization as the diagnosis of temporal lobe epilepsy was sometimes only based on semiology or surface EEG).

*2.1.2.1.2. Effects of temporal lobe surgery on HRV*

In 2002, Hilz et al. compared LF and HF power of 2-min HRV between the preoperative period and 3-4 months after temporal lobe surgery in 18 patients with TLE (159). They found a significant LF decrease after the surgery. There was no differential effect between right and left surgeries. The authors did not however have a group of healthy controls to compare with.

In 2013, Dericioglu et al. also compared HRV parameters in TLE patients between the preoperative period and postoperative period (33-188 days after surgery) (154). Although all postoperative HRV parameters remained significantly lower than those of controls, there was a significant increase in sympathetic spectral values (LF power and LF/HF ratio) as compared to the preoperative values. The follow-up duration was too short to conclude on the long-term effect of surgery on HRV. Moreover, the authors did not analyze the effect of surgery in good outcome versus poor outcome patients separately.

Persson et al. (2005, 2006) showed that HRV values one year after surgery did not differ from those before the surgery (134, 160). When separating patients into two subgroups (good outcome and poor outcome patients), the authors found that the preoperative HRV values were significantly lower only in poor outcome patients. These values remained lower in the postoperative period when compared with the control group. On the other hand, preoperative HRV values of patients with good surgical outcomes were not different from those of controls. The authors proposed that a pronounced impairment of both parasympathetic and sympathetic HRV parameters could suggest an extension of a temporal epileptic focus, and predict a poor outcome after the temporal surgery. However, because of the small number of patients (n = 21), this observation needs to be confirmed with a larger patient cohort.

2.1.2.2. Frontal lobe epilepsy (FLE)

Only two studies have looked at interictal HRV in FLE. Harnod et al. analyzed frequency-domain of 5-min interictal HRV in 25 patients (11 females, age: 5-34 years, mean age = 16,16 years) with frontal lobe epilepsy identified by interictal EEG (132). All patients were MRI-negative.

The results showed that FLE patients had a decreased HF power as compared to matched healthy controls.

More recently, do Nascimento Vinholes et al. compared time-domain and frequency-domain parameters of 24-hour HRV between ten patients with FLE (range 18-51 years old) and 15 healthy controls (161). The authors reported a decrease in time-domain parameters (SDNN, SDNN index, RMSSD, and pNN50%) in FLE (but no difference in frequency-domain parameters). These parameters and LF power were inversely correlated with the SUDEP-7 score.

In summary, these studies describe an autonomic derangement in FLE although both are hardly comparable as they differed in patient populations (children and adults versus adult only) and duration of recordings (short-term versus 24-hour HRV analysis).

#### 2.1.2.3. Other epilepsy types

Many studies looking at interictal HRV changes in epilepsy have included various types of epilepsy (generalized and focal) or have not specified the type of focal epilepsies, rendering interpretation of findings more arduous. For example, Mativo et al. evaluated short-term resting HRV in 20 newly untreated epileptic patients (5 females, age: 17-55 years old) (127). Unfortunately, the type of epilepsy was not specified for each patient. Results showed decreased pNN50, RMSSD, and HF power but increased LF/HF ratio. Similarly, Goit et al. investigated 5-min HRV in 65 patients with untreated epilepsy, 44 with focal epilepsy and 21 with generalized epilepsy (128). Unfortunately, the authors did not look at HRV differences between patients with focal versus generalized epilepsy.

### 2.1.3. HRV and vagus nerve stimulation

Vagus nerve stimulation (VNS) is a type of neuromodulation in which the stimulation generator is implanted in the upper left chest area and the electrode wire is wrapped around the left vagus nerve (162). It delivers intermittent electrical stimulation to the brain via the vagus nerve. Currently, VNS is approved as an adjuvant treatment for patients with pharmacoresistant epilepsy who are not candidates for resective surgery. Approximately 45 - 65% of patients achieve a 50 - 100% seizure reduction with VNS (163). The most common adverse events of VNS treatment

are related to cyclic stimulation, such as stimulation-induced hoarseness, coughing, throat paresthesia (or sometimes pain), dyspnea, and headache (163).

Since several studies have found a depression of parasympathetic activity in patients with refractory epilepsy (the vagal-sympathetic balance is shifted towards the sympathetic tone), as mentioned above, researchers have looked at whether VNS could positively affect cardiac autonomic regulation. Such studies have generated mixed results until now.

By analyzing 24-hour HRV, Ronkainen et al. evaluated the effect of VNS on cardiac autonomic function in 14 patients with refractory epilepsy (mean age = 34,3±9,3 years) before and one year after the VNS implantation (164). All patients received different mono- or polytherapy treatment without achieving sufficient seizure control. A third of patients had had corpus callosotomy, two patients a lobectomy, and one patient radiation therapy. HRV parameters (RR intervals, SDNN, HF, and the Poincaré components SD1 and SD2) were significantly lower in the patient group versus the control group. One year after VNS implantation, nine patients had ≥50% seizure reduction, two patients had <50% seizure reduction, and no change in seizure frequency was seen in two patients. As was the case before VNS implantation, HRV parameters were lower in patients versus controls and no differences were found before and one year after VNS therapy. Seizure reduction was not associated with changes in the HRV. A year later, Barone et al. also reported no significant difference in 24-hour HRV variables before and three months after VNS implantation in eight refractory epileptic patients (mean age = 32 ± 24 years old) (165). Similar results were demonstrated by Garamendi et al. who found no major changes in cardiovascular autonomic control with VNS 15-30 days before VNS implantation, 6 months after VNS implantation, and when high stimulation parameters were achieved (mean = 12 months) in 15 refractory epileptic patients (166). More recently, Liu et al. also came to the same conclusion with a larger cohort (n=32 patients; range 6-38 years) with prospective analysis of 24h-hour HRV parameters before and one year after VNS implantation (167).

On the other hand, other studies have shown a partial benefit of VNS on HRV parameters. Jansen et al. extracted 50-min epochs of 24-h EEG-EKG recordings from stage 2 sleep and slow-wave sleep in the first sleep cycle in 17 patients aged from 3 to 16 years with various forms of

epilepsy (Lennox-Gastaut, myoclonic epilepsy, electrical status epilepticus during sleep, frontal lobe epilepsy, absence epilepsy, and temporal lobe epilepsy) (168). The authors found autonomic cardiac dysfunction during stage 2 and slow-wave sleep both before and after VNS therapy. VNS had a positive impact on autonomic modulation with a partial improvement of the LF component during stage 2 sleep but not during slow-wave sleep. Hirfanoglu et al. evaluated the evolution of long-term HRV before, six months, and 12 months after VNS therapy in 20 children (4-17 years old) with intractable epilepsy (143). 70% and 90% of patients achieved a 50% reduction in seizure frequency at six months and 12 months of VNS therapy. As compared to healthy controls, although a significant depression of all HRV parameters in the patient group was observed at all three study time points, there was a remarkable improvement after six months with VNS treatment. This improvement was no longer seen after 12 months when compared to HRV values after six months. Finally, Schomer et al. analyzed the effect of VNS on T-wave alternans (TWA) and HRV in nine refractory epilepsy patients (169). TWA level and frequency-domain HRV were measured in the 24-hour EKG recordings before VNS implantation and 7,6 weeks (range 1-14 weeks) after. The results showed an elevated TWA level in patients with refractory epilepsy which significantly decreased after VNS therapy. This finding was congruent with a decrease in LF power and LF/HF. A subsequent prospective, multicenter study of VNS with the AspireSR Model 106 Therapy System also demonstrated a significant decrease in TWA 2-4 weeks after implantation compared to pre-implantation in all 28 patients; however, no alteration in HRV after VNS treatment was noted (170). Since TWA is considered as a biomarker for sudden cardiac death in patients with cardiovascular disease (171), these findings suggest that VNS may have a cardioprotective role, reducing the risk for cardiac-mediated SUDEP, but may not necessarily modify HRV (169, 170).

### 2.1.4. HRV and antiseizure medications (ASM)

Several studies have examined the effect of antiseizure medications (ASM) on HRV. When comparing HRV between untreated epileptic patients and epileptic patients on ASM or before and after ASM therapy, some studies have showed that interictal HRV had improved when seizures became well controlled (133, 172). In contrast, other studies have failed to find a difference in HRV before and after ASM therapy or with specific ASM (173-175). A meta-analysis

until July 2011 was performed to evaluate the effects of ASM treatment on HRV (176). Six studies compared HRV between patients before and after ASM or between epileptic patients with and without ASM treatment. Their findings showed no significant differences between both groups for LF power but the LF/HF ratio tended to be lower, suggesting a possible shift of the sympathovagal balance toward the parasympathetic tone in treated patients.

With regards to ASM withdrawal, Kenneback et al. and Hennessy et al. suggested that a depression of parasympathetic activity and an increase in sympathetic activity may develop dramatically if ASMs are stopped abruptly (177, 178). Conversely, Stefani et al. investigated HRV changes in patients whose ASMs were withdrawn during their admission to the epilepsy monitoring unit for long-term video EEG monitoring and found no cardiac disturbances (179). A prospective, randomized and double-blinded study on the alteration of long-term HRV before and after the withdrawal of ASMs in seizure-free patients with epilepsy on monotherapy revealed that the slow withdrawal of ASMs increased both parasympathetic and sympathetic activity (180).

## 2.2. HRV – a biomarker of SUDEP?

Although several authors mention autonomic dysfunction as an important factor in SUDEP pathophysiology, evidence correlating HRV with SUDEP remains inconclusive.

DeGiorgio et al. correlated the SUDEP-7 inventory with time-domain measures of HRV in 18 refractory focal epilepsy patients (mean age = 35,8; SD = 9,4) (26). All patients underwent a 1-hour awake EKG. The SUDEP-7 inventory score was inversely correlated with RMSSD (Pearson r = -0,64; p=0,004). In 2015, the same group reported similar findings with a larger cohort of 25 severe refractory epilepsy patients (18 of which were included in their initial report). In another series of 10 patients with FLE and 15 healthy controls, do Nascimento Vinholes et al. found that 24-hour time-domain parameters and LF were inversely and significantly correlated with the SUDEP-7 inventory (161). On the other hand, Baysal-Kirac et al. failed to show such a correlation in their cohort of 47 patients with drug-resistant epilepsy (mean age: 34,6±11,3 years) (20 with TLE and 27 with extra-temporal or multifocal epilepsy) (138). Although, the authors found no relationship between all HRV parameters and the SUDEP-7 score, one patient with Dravet

syndrome and definite SUDEP had a SUDEP-7 score of 7 and a RMSSD 59,8% lower than healthy controls and 52,5% lower than other patients.

Myers et al. reported extreme HRV derangement in SUDEP patients as compared to non-SUDEP patients in those with epilepsy due to SCN mutations (131). All SUDEP patients had *SCN1A* mutations whereas non-SUDEP patients either had *SCN1A* mutations or *SCN2A* or *SCN8A* mutation. While 8 out of 10 SUDEP patients had very low awake RMSSD values, one 11-year-old patient had an unusually high value and a 38-year-old woman had an intermediate value. Then again, others have not found significant HRV differences in SUDEP versus non-SUDEP patients. Surges et al. found no significant difference in HRV parameters between seven patients who died of SUDEP and seven living refractory focal epileptic patients (135). Similarly, Odom and Batman found no difference in RMSSD between 16 cases of definite or probable SUDEP and 48 living epileptic patients. One possible reason underlying these contradictory results lies in the heterogeneity of seizure and epilepsies types (137).

The above small series of SUDEP cases are complemented by anecdotal case reports describing acute and subacute HRV changes in SUDEP cases. In 2014, Jeppesen et al. reported a 25-year-old man with drug-resistant epilepsy related to a focal cortical dysplasia who developed cardiac dysrhythmias following a cluster of GTCS during video-EEG monitoring and who eventually died despite cardiopulmonary resuscitation (181). The authors retrospectively analyzed the evolution of the 30 min-interictal HRV in the seven months and one day preceding the SUDEP. They also calculated the HRV in the 30-min preictal period of the first of cluster of GTCS leading SUDEP and compared it with the mean preictal HRV values from 11 GTCS from 10 patients. The findings revealed an increase in parasympathetic activity in the hours preceding the fatal seizures as compared to the seven months before. Furthermore, his preictal HRV values were also higher than those of other patients with GTCS without SUDEP. In 2017, Myers et al. described a child with a chromosomal disorder and febrile seizures who had SUDEP during continuous EEG monitoring (182). In that particular case, HRV was extremely low in the hours prior to death; however, eight minutes prior to death, there was a dramatic spike in HRV indicating a sudden parasympathetic shift. Rauscher et al. reported HRV changes over a period of seven months in a 33-year-old man with intractable post-traumatic epilepsy who underwent regular EKG as part of

an n-3 fatty acids clinical trial (139). From baseline to the last visit prior to his SUDEP, the RMSSD, pNN50, LF and HF values progressively decreased by 35,9%; 72,9%; 62,4% and 71,1% respectively. The last RMSSD and HF values were 56,2% and 50,4% lower than those of healthy controls reported by Evrengül et al. (129) which included 43 healthy men (mean age 20,02±1,2 years). Finally, Lacuey et al. retrospectively analyzed the series of HRV in two patients prior their SUDEP (96). HRV measurements were calculated over 5-min periods during 30-min interictal awake periods in different video-EEG admissions. The first patient was a 28-year-old man with left refractory temporal epilepsy due to anoxic brain damage in early childhood. His seizures were not controlled by a left temporal resection and he died (definite SUDEP) 11 years after the surgery (and 21 months after the last evaluation). HRV analysis from the most recent recording in 2012 showed increased SDNN (352,84%), RMSSD (196,03%), and HF (271,64%) and decreased LF (18,63%) and LF/HF ratio (80,26%) as compared to values calculated from recordings performed in 2009. Both recordings were performed postoperatively. The second patient was a 33-year-old man with refractory left hemispheric epilepsy who underwent an unsuccessful left posterior insular and opercular resection. This patient also died of SUDEP. His interictal HRV during postoperative monitoring in 2011 showed a decrease of SDNN (21,14%), RMSSD (48,96%), and HF (21,35%) and an increase in LF (63,65%) and LF/HF ratio (307,95%), as compared to values calculated from a preoperative monitoring recording in 2006.

## 2.3. HRV changes in insulo-opercular epilepsy

Very little is known regarding HRV changes in insular epilepsy. As alluded to above, Lacuey et al. reported progressive changes in HRV prior to SUDEP in two patients with left insular damage from previous failed temporal/temporo-insular epilepsy surgeries. In the first patient, a brain MRI after left temporal lobe surgery showed post-operative anterior inferior insular gliosis, which was not present on the presurgical brain MRI. The second patient had a left posterior insular and opercular resection following an invasive evaluation. Pathological findings showed focal cortical dysplasia type IIA. Comparing interictal HRV parameters between the most recent and the earliest available video-EEG monitoring performed postoperatively, the authors showed a significant increase in parasympathetic and decrease in sympathetic HRV measures in the first patient, and a significant decrease in parasympathetic and an increase in sympathetic HRV measures in the

second patient. The authors suggested that the acquired insular damage (for patient one) or the intrinsic insular lesion (for patient two) could have contributed to their SUDEP. Given that the first HRV analysis was performed after surgery, it is impossible to know whether the new insular damage resulted in any autonomic changes in the first patient. Moreover, because both patients continued to have seizures after surgery, it is impossible to assess if autonomic deteriorations are the consequence of insular damage or due to the persistence of frequent seizures. The same group then examined HRV changes in 21 epileptic patients operated in the temporal lobe and a variable portion of the insula (183). The authors found a significant decrease in RMSSD and coefficient of variation (CV) in patients who had <25% (n=7) or ≥ 25% (n=1) of those whose insula was resected compared to those whose insula was not (n=4) or minimally/marginally (n=9) resected. Because most patients had temporal or temporal plus epilepsy, the effect of insular epilepsy per se could not be disentangled and remains unknown. Furthermore, because close to half of patients continued to have seizures post-operatively, it is unclear if HRV changes were due to insular surgery or persistent seizures. Finally, there was no control group to help account for the physiological decrease of HRV that occurs with aging.

In another study, Mishra et al. looked at 5-min HRV in 50 patients with newly diagnosed insular glioma. As compared to controls, patients with insular glioma had a decrease in all time-domain parameters and HF power as well as an increase in LF power and LF/HF ratio. Patients with seizures have greater suppression as compared to the patients without seizures (86). Hard conclusions are however difficult since most patients had tumors extending beyond the insula and presented with acute seizures (rather than chronic epilepsy) and evidence of increased intracranial pressure.

To our knowledge, no studies have looked at the effect of chronic insular epilepsy on HRV and the effect of insulo-opercular surgery on autonomic function.

Table 2. Summary of the HRV studies in TLE

| Study Author/ Year | Sub-group | Patient group | | Healthy control group | | EKG recording | | HRV variables | Main findings | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean age (y) | N | Mean age (y) | N | Duration | Recording state | | | |
| Massetani et al. 1997 (151) | | 40,9 (SD:13, 3) | 30 (19F) | NA | 20 | 50 mins | Rest in supine position after 20 mins of rest in a sitting position. | TP, LF, HF, and LF/HF ratio | -LF and HF values were significantly lower in patients than in controls<br>-LF/HF ratio was not different between two groups<br>-LF values was lower in right focus than in left focus. | There was a significant decrease in both LF and HF components in TLE with a more severity in right focus. |
| | | | | | | 40 mins | Passive-tilt position at 60º 50 mins after the first recording. | | - LF and HF values were also lower in patients than in controls but statistical significance was only seen in LF.<br>- LF/HF ratio was reduced in patients compared with controls ($p<0.01$)<br>- no difference in LF and HF values between right and left focus | |
| Tomson et al. 1998 (155) | | 37 (SD:9) | 21 | 37 (SD:9) | 21 | 24 hours | Free to perform normal daily activities and slept at home | mean RR, SDNN, TP, VLF, LF, HF, and LF/HF ratio | -SDNN, LF and LF/HF ratio were lower than those of healthy controls. | Patients with TLE may have a decrease in sympathetic tone. |

| Study Author/ Year | Sub-group | Patient group | | Healthy control group | | EKG recording | | HRV variables | Main findings | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean age (y) | N | Mean age (y) | N | Duration | Recording state | | | |
| Ansakorpi et al. 2002 (152) | Refractory TLE | 32.8 (SD:7) | 19 (14F) | 33.3 (SD: 9,7) | 34 (25F) | 24 hours | Free to perform normal daily activities | mean RRI, SDNN, VLF, LF, HF and ApEn, α1, α2 | In patients with TLE:<br>-SDNN, VLF, LF and HF were lower than those of healthy controls.<br>-ApEn was also diminished<br>Compared refractory TLE and well-controlled TLE, there were no differences in most of HRV parameters except for ApEn and α2<br>-ApEn value of the refractory TLE was lower than that of well-controlled patients (p=0.021)<br>- Long term fractal correlation value α2 was smaller in patients with well-controlled TLE (p=0.037) | In TLE, overall HRV was reduced |
| | Well-controlled TLE | 33.3 (SD:6,2) | 25 (14F) | | | | | | | |
| Hilz et al. 2002 (159) | TLE patients were treated with temporal lobectomy | 37.3 (SD:6,4) | 18 (10F) | None | None | 2 mins | -Resting state, after at least 35 mins of rest in supine position<br>-Before and 3-4 months after surgery. | LF and HF | -After surgery, LF value was significantly lower than before surgery<br>-Post-surgical HF value did not differ from that before surgery.<br>- No different effects between right and left side of the surgery. | TLE surgery seems to stabilized the cardiovascular control by reducing the risk of sympathetically mediated tachyarrhythmias |

| Study Author/ Year | Sub-group | Patient group | | Healthy control group | | EKG recording | | HRV variables | Main findings | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean age (y) | N | Mean age (y) | N | Duration | Recording state | | | |
| Ansakorpi et al. 2004 (153) | Patients with HS | 36 (SD:5) | 8 (6F) | 33.6 (SD: 8,2) | 72 (55F) | 24 hours | | mean RRI, SDNN, VLF, LF, HF and ApEn, α1, α2 | -All HRV values of TLE patients were significantly lower than those of controls.<br>-In patients with HS, the mean values of most of HRV parameters tended to be lower than those of patients without HS, but no statistical significance. | Functional rather than structural changes related to TLE are involved mainly as a mechanism of altered cardiac regulatory function |
| | Patients without HS | 31 (SD:7) | 31 (20F) | | | | | | | |
| Persson et al. 2005 (134) | | NA | 21 | NA | 21 | 24 hours | Before and one year after surgery | SDNN, TP, VLF, LF, HF and LF/HF ratio | Before surgery,<br>- TLE patients had significantly lower SDNN, TP, VLF and LF values than those of control. HF values and LF/HF ratio were not different.<br>- In poor outcome patients, all analyzed parameters except LF/HF ratio were significantly lower than those in good outcome patients and also lower than those of controls.<br>- All HRV parameters of patients with good outcome did not differ from those of controls. | TLE patients with a poor outcome of surgery have a more pronounced impairment of both sympathetic and parasympathetic activities than those with good outcome. Good surgery candidates may have a less impaired autonomic cardiac control before surgery than those with poor outcome. |
| | Good outcome (Engel I) | NA | 11 | | | | | | | |
| | Poor outcome (Engel II-IV) | NA | 10 | | | | | | | |

| Study Author/ Year | Sub-group | Patient group | | Healthy control group | | EKG recording | | HRV variables | Main findings | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean age (y) | N | Mean age (y) | N | Duration | Recording state | | | |
| Persson et al. 2006 (160) | The same patients and HRV analysis method as the Persson et al. 2005 | | | | | | | | After surgery,<br>- TLE patients also had significantly lower SDNN, TP, VLF and LF values than those of control. HF and LF/HF values were not different between two groups<br><br>- Poor outcome patients had significantly lower SDNN, TP, VLF and LF than those of controls while good outcome patients did not differ from the controls.<br><br>- There were no differences in any HRV measures between preoperative and postoperative in the entire TLE group, or in the good outcome group, or in the poor outcome group. | There was no effect on HRV of temporal lobe epilepsy surgery |
| Dutsch et al. 2006 (184) | | median = 36,5 | 22 (12F) | median = 34,8 | 20 | 10 mins | -free of seizures 48 hours prior to the recording.<br>-Resting state, after at least 35 mins of rest in supine position | -LF, HF, LF/HF ratio | Compared with controls:<br>- LF values of TLE patients were higher<br><br>- HF values of TLE patients were lower<br><br>- LF/HF ratio values of TLE patients were higher | In TLE, the parasympathetic tone was decreased and the sympathetic tone was increased |

| Study Author/ Year | Sub-group | Patient group | | Healthy control group | | EKG recording | | HRV variables | Main findings | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean age (y) | N | Mean age (y) | N | Duration | Recording state | | | |
| Suorsa et al. 2011 (133) | Refractory TLE | 32,4 (SD:7,1) | 18 (14F) | None | None | 24 hours | At the baseline and after follow-up (mean FU duration: 6,1 years) | RRI, SDNN, LF, HF, VLF, SD1, SD2 and α | -All mean values of HRV measures tended to be lower in patients with refractory TLE compared to those with well-controlled TLE at the baseline and after the follow-up but no statistical difference<br><br>-Compared baseline HRV and after FU in two groups:<br>+After FU, SD1 and SD2 were significantly decreased in patients with refractory TLE<br>+In well-controlled group, there was no difference between baseline HRV and FU HRV. | The reduction in HRV seems to be progressive in patients with chronic refractory TLE. |
| | Well-controlled TLE | 32,2 (SD:6,6) | 18 (9F) | | | | | | | |
| Varon et al. 2015 (157) | | 10.3 (SD:2,7) | 10 | 10.8 (SD:4,3) | 10 | 30 seg of 5 mins | -No epileptic discharges in EEG<br><br>-The onset of any seizures is at least 30mins away | RRI, LF, HF | No difference between TLE and controls | |

| Study Author/ Year | Sub-group | Patient group | | Healthy control group | | EKG recording | | HRV variables | Main findings | Conclusion |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean age (y) | N | Mean age (y) | N | Duration | Recording state | | | |
| Dericioglu et al. 2013 (154) | | 27.5 (range: 15-48) | 24 (13F) | 27 (range 15-50) | 23 (13F) | 1 hour | -Resting state -Before surgery -3-8 days after surgery (early period) -35-188 days after surgery (late period) | RRI, SDNN, RMSSD, TP, LF, HF, LF/HF ratio | -Preoperatively, compared to the controls, all-time and frequency domain measures were significantly lower in the patient group, except for LF/HF ratio. -Similar results were obtained in the early period -In the late period, results remained similar, except for the LF/HF ratio which was significantly increased. - Within the patient group, compared to preoperative period, HF was significantly increased in the early period while in the late period, LF and LF/HF ratio were significantly increased and HF tended to be decreased. | -Patients with refractory TLE, HRV is decreased in both sympathetic and parasympathetic parameters. -After surgery, the sympathovagal balance shifts toward the parasympathetic side in the early postoperative period and toward the sympathetic side after the first postoperative month. |
| Dono et al. 2020 (158) | All patients | 42.9 (SD:16, 4) | 52 (28F) | None | None | 5 mins | -Resting state. | RRI, SDNN, RMSSD, LF, HF, VLF, SD1, and SD2 | Right-TLE vs Left-TLE: -No difference in all time-domain parameters -Left TLE had significantly higher HF, lower LF and lower LF/HF ratio than those of Right TLE. -Significantly higher SD1 and lower SD2 were observed in Left TLE. | Patients with TLE exhibit a lateralized cardiac autonomic control. Left TLE is associated with increased cardiac vagal tone compared to Right TLE. |
| | Left TLE | NA | 26 | | | | | | | |
| | Right TLE | NA | 26 | | | | | | | |

**Legends:** N = number; SD = standard deviation; TLE = temporal lobe epilepsy; TP = total power; VLF = very low frequency; LF = low frequency; HF = high frequency; RRI = RR interval; SDNN = Standard deviation of NN intervals; RMSSD = Root mean square of successive RR interval differences; ApEn = Approximate entropy, which measures the regularity and complexity of a time series; α1 = Detrended fluctuation analysis, which describes long-term fluctuations; α2 = Detrended fluctuation analysis, which describes long-term fluctuations; SD1 = Poincaré plot standard deviation perpendicular the line of identity; SD2 = Poincaré plot standard deviation along the line of identity; MRI = magnetic resonance imaging; EEG = Electroencephalography; EKG = Electrocardiogram; F = female; NA = not available; mins = minutes; seg = segments.

# Chapter 3 – Research objectives and hypotheses

Given the important role of the insula in autonomic function, this work aimed to determine if an insulo-opercular epileptogenic focus is associated with abnormalities in HRV, a marker of cardiac autonomic function.

**Objective #1**: To investigate the interictal HRV changes in patients with refractory IOE.

**Hypothesis #1**: Previous studies have shown a decrease of interictal HRV in patients with refractory epilepsy (151, 152, 156). Animal and human studies have shown that the insula plays a role in both parasympathetic and sympathetic controls (51, 53). We hypothesized that in refractory IOE patients, interictal HRV is impaired with the autonomic balance shifted towards either the parasympathetic or sympathetic side.

**Hypothesis #2:** Since cardiac autonomic dysfunction may be involved in the pathology of SUDEP, we also hypothesized that the severity of interictal HRV impairment in IOE is correlated with the SUDEP-7 score.

**Objective #2:** To determine the long-term effect of insulo-opercular resection on HRV

**Hypothesis #3**: Previous studies have shown that operculo-insular cortectomy in experienced hands is relatively safe and efficacious for refractory IOE (88, 185). Furthermore, studies have shown that the improvement in quality of life, physical activity, and reduction in anxiety and depressive disorders as a result of post-operative seizure control may have a positive impact on HRV (186, 187). Hence, we hypothesized that successful insular surgery for refractory IOE will have a positive impact on HRV.

# Chapter 4 – Methodology

Our study is part retrospective and part prospective. Clinical and HRV data were analyzed before and at least six months after the surgery. This study was approved by our institutional ethics committee (Project 16.146). All participants signed a written informed consent.

## 4.1. Study population

We retrospectively reviewed all patients with IOE investigated and treated at our institution between 2000 and 2018 (Figure 4). We identified 19 patients who met our criteria from a group of 43 IOE patients evaluated at our epilepsy center. Five of them declined to pass a postoperative EKG recording. Thus, 14 patients were included in the study.

Fourteen age- and sex-matched patients with TLE who had a good outcome after anterior temporal lobectomy were also recruited as an active control group. Similar inclusion and exclusion criteria were used except for the location of the seizure focus and surgery.

Each IOE and TLE patient was matched for age and sex with two healthy controls. Since the IOE and TLE patient HRV analyses were carried out twice (pre- and post-operatively), two healthy control groups matched for age and sex with patients in two studied periods.

## 4.2. Recording procedures and HRV analysis

The presurgical video-EEG monitoring of each IOE and TLE patient was analyzed to select 12 5-min segments in the awake, resting state for HRV analysis. At least six months after the surgery, all patients underwent a one-hour three-lead EKG awake recording at resting state in a lying position. Healthy individuals also underwent a single one-hour three lead EKG recording at our laboratory.

HRV variables including time-domain (RMSSD, pNN50) and frequency parameters (LF and HF) were calculated from 5-min selected preoperative and postoperative segments for each participant. The detail of the period selection and HRV analysis can be found in chapter 5.

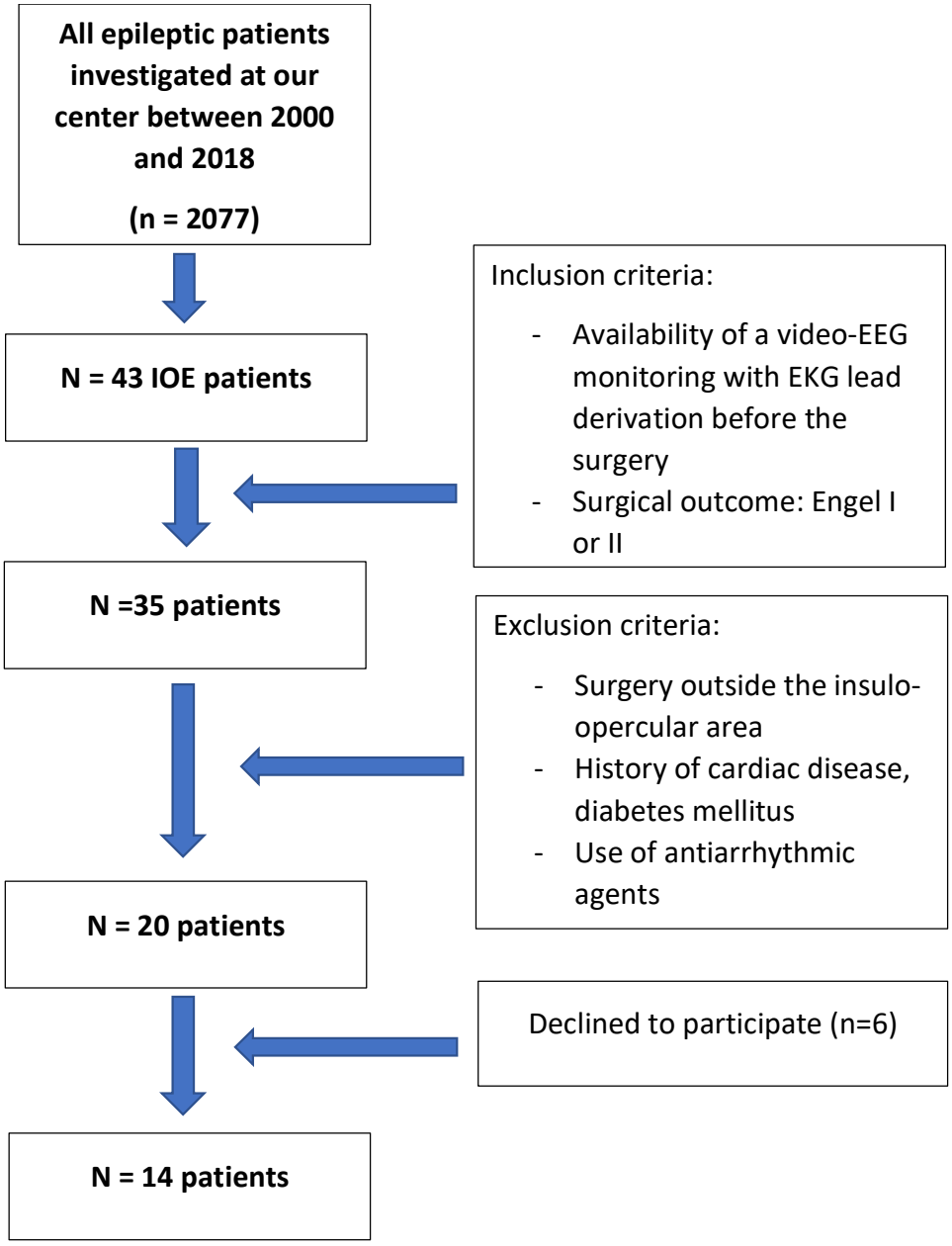



Figure 4. Flowchart of the study.

(IOE = insulo-opercular epilepsy; EKG = electrocardiogram; EEG = electroencephalography)

## 4.3. Statistical analysis

The statistical analysis was performed with Statistical package for the social sciences (SPSS) version 25.

The Kolmogorov-Smirnov test was used to test the normality of each HRV variable. Since the data were not normally distributed, a natural logarithmic transformation was applied to RMSSD, LF, and HF.

The mean and standard deviation (SD) were calculated for each HRV variable and values were expressed as means (SD). The one-way analysis of variance (ANOVA) was used for comparing the HRV values of three groups: IOE, TLE, and control. Statistical comparisons between two groups (IOE group versus healthy control group or TLE group versus healthy control group) were made using an unpaired t-test. Within a group, a paired t-test was used to compare the pre-operative with the post-operative HRV values. Spearman's correlation coefficients were calculated to evaluate the relationship between HRV values and the SUDEP-7 score. The results were considered significant at $p<0.05$.

## 4.4. Exploratory study

The IOE group was then divided into two subgroups based on their LnRMSSD below (Group 1a) or above (Group 1b) the mean value of the healthy control group. Similar statistical tests were performed to compare the HRV parameter values of each subgroup with their matched healthy control and between preoperative and postoperative periods.

The same methodology was applied to the TLE group.

## 4.5. Age correction method

Previous studies have shown age dependency in HRV. Since there were different age groups among the participants as well as variable time intervals between surgery and postoperative EKG acquisition, it was necessary to control for age-related HRV changes. The statistical analyses were performed on the original values first, then on the age-corrected values.

In this work, to control for the effects of age, we applied a regression model based on age-related changes assessed by Voss et al. (106) who calculated reference values for five-minute resting HRV indices from a population of 1906 healthy subjects aged 25 to 74 years.

For all four parameters, the data from Voss et al. (106) were fitted to quadratic regressions with a correlation coefficient (r) of more than 0.99, and $p<0.01$.

- For RMSSD: $y = 75.78 – 1.618x + 0.011x^2$, $r = 0.9982$
- For pNN50: $y = 60 – 1.89x + 0.015x^2$, $r = 0.9989$
- For LF: $y = 310.114 – 6.6771x + 0.03857x^2$, $r = 0.9964$
- For HF: $y = 271.214 – 7.4701x + 0.05407x^2$, $r = 0.9983$,

  With y = HRV value, x = age

The value determined from this quadratic regression was considered as the "theoretical value." By subtracting the theoretical value from the participant's actual value, the individual's changes related to the disease itself were obtained. To remove age-related effects, participants at any studied period were considered at the same age (reference age). The adjusted HRV value was calculated by adding the reference value (the theoretical value at the reference age) to the difference between the actual value and the theoretical value at the participant's age.

# Chapter 5 – Article

## Heart rate variability in insulo-opercular epilepsy

Authors: Yen Thi Phuoc Tran[1], Philippe Pouliot[3,4,5], Pierre Rainville[6], Kenneth A. Myers[7,8,9], Manon Robert[1]; Alain Bouthillier[10], Mark R. Keezer[1,2], Dang Khoa Nguyen[1,2]

**Affiliations**

[1] Division of Neurosciences, CHUM Research Center, University of Montreal, Canada

[2] Division of Neurology, CHUM, University of Montreal, Canada

[3] Safe Engineering Services and Technologies, Laval, Canada

[4] Labeo Technologies, Montreal, Canada

[5] On leave from Department of Electrical Engineering, Ecole Polytechnique, Montreal, Canada

[6] Department of Somatology, University of Montreal, Canada, and Research Centre of Institut universitaire de gériatrie de Montréal, Canada

[7] Research Institute of the McGill University Medical Centre, Montreal, Quebec, Canada

[8] Division of Neurology, Department of Pediatrics, Montreal Children's Hospital, McGill University Health Centre, Montreal, Quebec, Canada

[9] Department of Neurology and Neurosurgery, Montreal Children's Hospital, McGill University Health Centre, Montreal, Quebec, Canada

[10] Division of Neurosurgery, CHUM, University of Montreal, Canada

**Corresponding author**: Yen Thi Phuoc Tran, email address: yen.tran@umontreal.ca. Address: Centre de recherche du CHUM, Pavillon R, porte R04.298, 900 rue Saint-Denis, Montréal (Québec) H2X O9A.

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* This manuscript describes the work accomplished during my MSc training in neurosciences. I substantially contributed to the design of the study, data collection (including clinical, paraclinical data, EKG selection from video-EEG monitoring, postoperative EKG recordings, and HRV analysis),

statistical analysis, and result interpretation. I also drafted and edited the manuscript with the inputs from the other authors.

* The co-authorship agreement in which the co-authors stating their acceptance of the inclusion of this manuscript in my memoir was obtained.

## 5.1. Abstract

**Purpose**: Accumulating evidence suggests that the insula plays a key role in the control of the cardiac autonomic system whose dysfunction may be involved in the pathophysiology of sudden unexpected death in epilepsy (SUDEP). The aim of this study was to evaluate the changes of interictal heart rate variability (HRV), a reliable index of autonomic activity, in insulo-opercular epilepsy (IOE). We further investigated the effect of insulo-opercular surgery on these HRV changes.

**Method**: Short-term interictal HRV parameters were retrospectively evaluated in 14 IOE patients who had a good outcome (Engel I-II) after an insulo-opercular surgery at our epilepsy center between 2000 and 2018. HRV measurements including time domain (root mean square of successive RR interval differences (RMSSD) and percentage of successive RR intervals that differ by more than 50ms (pNN50)) and frequency domain (low-frequency (LF) and high-frequency (HF)) were carried out in pre- and post-operative periods (6-204 months). Patients' pre-operative data were collected to calculate the SUDEP-7 risk inventory score. Results were compared with age- and sex-matched patients with temporal lobe epilepsy (TLE) and healthy individuals.

**Results**: Before surgery, there were no statistically significant differences in all HRV measurements between groups of IOE patients, TLE patients and healthy controls. In IOE patients, the SUDEP-7 score ranged from 1 to 6 (mean 2,9; SD:1,6) and was positively correlated with pNN50 (r=0,671; p=0,009) and LnRMSSD (natural logarithm of RMSSD) (r=0,591; p=0,0026). Post-operatively, HRV measurements were not statistically different from either pre-operative values or from those of controls. We conducted exploratory analyses where we stratified IOE patients into those whose preoperative LnRMSSD values were below (Group 1a) versus above (Group 1b) a cut-off threshold of 3,52 set to the mean value of our healthy sample. Preoperatively, in the

Group 1a, all time and frequency domain values (LnRMSSD, pNN50, LnLF (natural logarithm of LF) and LnHF (natural logarithm of HF)) were significantly lower than those of control ($p<0,01$) while in the Group 1b, only time domain values (LnRMSSD and pNN50) were significantly higher than those of control subjects ($p<0,01$). In both groups, HRV values tended to normalize postoperatively. In contrast, anterior temporal lobectomy for TLE patients did not alter HRV values.

**Conclusion**: Our preliminary results suggest that in refractory IOE, HRV may be either decreased globally in both sympathetic and parasympathetic tones or increased in parasympathetic tone. Such changes may reverse after successful insulo-opercular surgery. The increase in parasympathetic tone observed pre-operatively may be of clinical concern as it was positively correlated with the SUDEP-7 score. Confirmation with a larger sample size is necessary.

**Keywords:** Heart rate variability, insulo-opercular epilepsy, temporal lobe epilepsy, sudden unexpected death in epilepsy.

## 5.2. Introduction

Heart rate variability (HRV) is considered an index of autonomic activity that reflects heart-brain interactions and the balance between sympathetic and parasympathetic activity (99). It has been shown to be a reliable biomarker of cardiovascular dysfunction in cardiovascular diseases (113-117) and is increasingly used for the evaluation of autonomic changes in some neurological disorders such as diabetic neuropathy (119), stroke (120), multiple sclerosis (121) and Parkinson's disease (123).

Given that chronic autonomic dysfunction or cardiovascular dysfunction may be involved in the mechanisms of Sudden Unexpected Death in Epilepsy (SUDEP) (35, 36), several studies since the late 1990s have measured HRV changes at different time points in different epileptic populations and assessed its potential use as a biomarker for SUDEP. Notably, interictal HRV studies have been conducted on patients with newly diagnosed epilepsy (126-128), generalized epilepsy (129), Lennox-Gastaut syndrome (130), Dravet syndrome (131), frontal lobe epilepsy (132), and temporal lobe epilepsy (TLE) (133, 152, 154, 159, 188). Most studies suggest the

presence of an autonomic dysfunction in refractory epilepsy; whether these changes are associated with the risk of SUDEP is less clear given inconsistent results (131, 135-139).

Recently, the role of the insula in drug-resistant epilepsy has been increasingly recognized with the report of several cases of insular, insulo-opercular or temporo-insular epilepsy successfully treated by resection, radio-frequency thermocoagulation or laser ablation (87-92). While several studies have looked at the effect of acute ischemic damage to the insula on HRV (81, 189), the impact of chronic insular epilepsy or insular epilepsy surgery on HRV has not been studied thoroughly. In 2016, Lacuey et al. reported progressive changes in HRV prior to SUDEP in two patients with left insular damage from previous failed temporal/temporo-insular epilepsy surgeries (96). The same group then examined HRV changes in 21 epileptic patients operated in the temporal lobe and a variable portion of the insula (183). The authors found a significant decrease in root mean square difference of successive RR intervals (RMSSD) and coefficient of variation (CV) in patients who had <25% (n=7) or ≥ 25% (n=1) of their insula resected compared to those whose insula was not (n=4) or minimally/marginally (n=9) resected. Because most patients had temporal or temporal plus epilepsy, the effect of insular epilepsy per se remains unknown. Furthermore, because close to half of patients continued to have seizures post-operatively, it is unclear if HRV changes were due to insular surgery or persistent seizures. Finally, there was no control group to help account for the physiological decrease of HRV that occurs with aging.

In this study, we assessed pre- and post-operative HRV in patients with insulo-opercular epilepsy (IOE). Considering the role of the insula in autonomic and cardiovascular control, we hypothesized that HRV could be altered by long-standing insulo-opercular seizures as well as insulo-opercular resections. We also sought to look at the relationship between HRV changes and SUDEP risk factors.

## 5.3. Material and methods

### 5.3.1. Study population

We retrospectively reviewed all patients with IOE investigated and treated at our institution between 2000 and 2018. Inclusion criteria were the following: (1) at least one pre-operative video-electroencephalography (VEEG) monitoring study with electrocardiographic (EKG) lead derivations; (2) free or nearly free of disabling seizures (Engel I or II) after an insulo-opercular resection at the time of postsurgical EKG recording. Exclusion criteria were: (1) surgery outside the insulo-opercular area (in the same or previous surgery); (2) history of cardiac disease (e.g., heart failure, cardiac ischemia, arrhythmias) or any disease that may affect autonomic function (e.g., diabetes mellitus); (3) use of antiarrhythmic agents (e.g., beta blockers).

The active control group consisted in age- and sex-matched patients with TLE who had a good outcome after anterior temporal lobectomy (ratio 1:1). Similar inclusion and exclusion criteria were used except for the location of the seizure focus and surgery.

Each IOE and TLE patient was matched for age and sex with two healthy controls. Since the IOE and TLE patient HRV analyses were carried out twice (pre- and post-operatively), there were two healthy control groups that matched for age and sex with patients in two studied periods.

Patients' clinical charts were also carefully reviewed to retrieve demographic characteristics and clinical information, neuroimaging findings, scalp and intracranial VEEG results, details of the surgical treatment, and outcome. The revised SUDEP-7 risk inventory scores (SUDEP-7 score) were calculated from seven elements: (1) more than three tonic-clonic seizures in the last year; (2) one or more tonic-clonic seizures in the last year (if risk factor 1 selected, score as 0); (3) one or more seizures of any type over the last 12 months (if risk factor 4 selected, score as 0); (4) more than 50 seizures of any type per month over the last 12 months; (5) duration of epilepsy ≥ 30 years; (6) current use of three or more antiseizure medications; and (7) mental retardation, IQ <70, or too impaired to test (136).

### 5.3.2. Recording procedures

For all patients, short-term five-minute HRV analyses were carried out twice: in the pre-operative period and in the post-operative period. In the pre-operative period, for each patient, 12 5-min segments were selected from VEEG recordings performed during their presurgical assessment. EEG and three-lead EKG were recorded and digitized at a sampling frequency of 200 Hz for scalp EEG recordings and 2000 Hz for intracranial EEG recordings. Periods were selected based on the following criteria, inspired by Myers et al. (190): (1) awake state during daytime (from 8am to 4pm), (2) fewer movement artifacts, (3) not during eating, (4) not during the hyperventilation procedure, (5) at least eight hours after the last tonic-clonic seizure; (6) at least one hour after the last known clinical seizure (excluding tonic-clonic seizure); (7) at least one hour before the next clinical seizure. Since video recording was not available during interictal periods (since they were not archived), in order to best select the segment closest to resting state, the segment out of the 12 5-min segments with the lowest mean heart rate was chosen for HRV analysis. In the post-operative period, patients underwent a one-hour three-lead EKG awake recording at resting state in a lying position. Five-minute segments were selected for HRV analysis using the same criteria as in the pre-operative period. Healthy individuals also underwent a single one-hour three-lead EKG awake recording (while resting in a lying position) from which a five-minute segment was chosen for HRV analysis.

### 5.3.3. HRV analysis

Consecutive R-R intervals were measured using Brain Vision Analyser 2.1 software. R peaks were initially automatically detected from EKG data then subsequently visually inspected to detect and manually correct artifacts, missed beats or ectopic beats. Ectopic beats were removed from the recordings and replaced by an interpolated R-R interval (101).

Kubios HRV 3.2.0 software was used to calculate the standard HRV parameters. Because the standard deviation of RR intervals (SDNN) is more accurate when calculated over 24 hours than during short periods, only two time-domain parameters were therefore analyzed: the root mean square of successive RR interval differences (RMSSD) and the percentage of successive RR intervals that differ by more than 50 ms (pNN50). Frequency-domain parameters, including low-

frequency (LF) power (0,04-0,15 Hz) and high-frequency (HF) power (0,15-0,4 Hz), were obtained by fast Fourier transform (FFT) of the RR interval tachogram (RR durations versus number of progressive beats). We did not analyze the LF/HF ratio, as its role in reflecting the sympathovagal balances is controversial (101).

Out of the time-domain parameters, RMSSD is considered as the primary measure to assess the vagal tone. Both RMSSD and pNN50 are influenced mainly by the parasympathetic nervous system. Out of the frequency-domain parameters, the HF power represents the parasympathetic influences on the heart rate while the LF power reflects predominantly sympathetic activity (101).

### 5.3.4. Statistical analysis

The statistical analysis was performed with Statistical package for the social sciences (SPSS) version 25.

Since the data were not normally distributed, a natural logarithmic transformation was applied to RMSSD, LF and HF.

The mean and standard deviation (SD) were calculated for each HRV variable and values were expressed as means (SD). The one-way analysis of variance (ANOVA) was used for comparing the HRV values of three groups: IOE, TLE and control. An unpaired t-test was performed to compare these values between two groups: IOE and control or TLE and control. Within a group, to compare the pre-operative HRV values with those after surgery, a paired t-test was used. Spearman's correlation coefficients were calculated to evaluate the relationship between HRV values and the SUDEP-7 score. The results were considered significant at $p<0,05$.

### 5.3.5. Age correction method

Previous studies showed the age dependency of both time- and frequency HRV domains so the main statistical analyses were also performed on age-corrected values. In order to remove age-related effects, we used a regression model based on age-related changes assessed by Voss et al. who calculated reference values for five-minute resting HRV indices from a population of 1906 healthy subjects aged 25 to 74 years (106) .

For all four parameters, the data from Voss et al. were fitted to quadratic regressions with a correlation coefficient (r) of more than 0,99:

- For RMSSD: $y = 75,78 – 1,618x + 0,011x^2$, $r = 0,9982$, $p = 0.004$
- For pNN50: $y = 60 – 1,89x + 0,015x^2$, $r = 0,9989$, $p = 0.002$
- For LF: $y = 310,114 – 6,6771x + 0,03857x^2$, $r = 0,9964$, $p = 0.007$
- For HF: $y = 271,214 – 7,4701x + 0,05407x^2$, $r = 0,9983$, $p = 0.003$

With y = HRV value, x = age

The value determined from this quadratic regression was considered as the "theoretical value." By subtracting the theoretical value from the participant's actual value, the individual's changes related to the disease itself were obtained. To remove age-related effects, participants at any studied period were considered at the same age (reference age). The adjusted HRV value was calculated by adding the reference value (the theoretical value at the reference age) to the difference between the actual value and the theoretical value at the participant's age.

## 5.4. Results

### 5.4.1. Patients and demographics

Fourteen IOE epileptic patients (nine females; mean age 35 years (SD: 8,5; range 19 – 49) at the time of presurgical evaluation) met our inclusion criteria and were recruited in our study. Findings from the presurgical evaluation, presurgical SUDEP-7 score, and surgical data for all patients are summarized in Table 3. Their SUDEP-7 score ranged from 1 to 6 (mean 2,9, SD: 1,6) (details of the score components for each patient can be found in Supplementary Table A). The epileptic focus was lateralized to the right side in 7 patients and the left in 7 patients. Mean epilepsy duration until the pre-operative evaluation was 19.1 years (SD: 11,3; range 8 - 46). Duration from surgery to post-operative EKG recording ranged from 6 to 204 months (mean = 58,2; SD: 56,7). Mean age at this post-operative period was 40,4 (SD: 10,6; range 19 to 54). Seizure outcome was Engel I for 13 patients and Engel IID for one patient.

Fourteen TLE patients (nine females; mean age 34,4 years (SD: 7,8; range 21 – 46) at the time of presurgical EEG-video monitoring) were also enrolled (Table 4). Mean SUDEP-7 score for

TLE patients was 2,5 (SD: 1,5; range 1-5). The seizure focus was lateralized to the right in 4 patients and to the left in 10. Epilepsy duration for TLE patients was 15,6 years (SD: 12,4). Duration from surgery to post-operative EKG recording ranged from 13 to 120 months (mean 53,9; SD: 34,2). Mean age at the post-operative period was 39,4 years (SD: 8,3; range 22 - 52). Seizure outcome was Engel IA for all TLE patients.

Two aged- and sex-matched healthy control groups were formed from a database of 33 volunteers. Each group consisted of 28 subjects (18 F, 10M) with a mean age of 34,6 years (SD: 8,6) and 37,5 years (SD: 8,8) for the pre- and post-operative control group, respectively. There were no significant differences in age and sex between the IOE, TLE and healthy control groups at pre- and post-operative periods (Supplementary table B).

HRV parameters for each participant can be found in Supplementary table C.

## 5.4.2. HRV differences in IOE and TLE

5.4.2.1. Comparison of HRV parameters between epileptic patients and age-sex matched healthy controls in pre-operative period

Table 5 summarizes the results of pre-operative HRV values of IOE and TLE patients as well as of healthy controls. No statistically significant differences were found in all HRV parameters ($p>0,05$) among the three groups. We did not find any difference in HRV parameters between the right and left side for both IOE and TLE groups. There was no difference when comparing HRV parameters of each epileptic group according to their laterality with those of the matched control group.

5.4.2.2. Correlation between HRV parameters and SUDEP-7 score

In IOE patients, the SUDEP-7 score was positively correlated with pNN50 ($r = 0,671$; $p=0,009$; figure 5a) and with RMSSD ($r = 0,591$; $p=0,026$, figure 5b). We were unable to detect any relationship between the HRV variables and SUDEP-7 scores in TLE patients (Supplementary table D).

### 5.4.3. Effect of epilepsy surgery on HRV

Post-operative HRV parameters were not significantly different between IOE and TLE operated patients as well as healthy controls. Within the two patient groups, there were no statistically significant differences between pre-operative and post-operative HRV measures (Table 5). We did not find any statistical difference between right and left foci for both epileptic groups after the surgery.

These conclusions were unchanged after correction for age (Supplementary table E).

### 5.4.4. Exploratory analyses

Since RMSSD was considered as the primary HRV measure in previous studies (101, 190), we subsequently examined this parameter in further detail for each patient. When individually examining the behavior of LnRMSSD pre- and post-operatively, two different trends were observed: patients with a low pre-operative LnRMSSD value appeared to have an increase in LnRMSSD post-operatively while patients with a high pre-operative LnRMSSD value appeared to present a decrease in LnRMSSD post-operatively (Figure 6A). Similar behaviors were observed for pNN50 but not for LnLF and LnHF (Figure 6B-D).

To quantify these observations, we divided the IOE group into two subgroups (Group 1a versus Group 1b) based on whether their LnRMSSD were, respectively below or above the mean value of the control group which was 3,52 (Table 6). RMSSD, pNN50, LF and HF values were significantly lower for Group 1a compared to the control group ($p<0.01$) pre-operatively. On the other hand, LnRMSSD and pNN50 were significantly higher for Group 1b (n=8) compared to the control group ($p<0,01$ and $p<0,05$ respectively) while LnHF tended to be higher ($p=0,059$). After surgery, LnRMSSD, pNN50 and LnLF values in Group 1a significantly increased even though post-operative mean values were not statistically different from those of matched controls. In Group 1b, LnRMSSD and pNN50 had a tendency to decrease post-operatively ($p=0,058$ and 0,053 respectively). As for Group 1a, all post-operative HRV values of group 1b were not statistically different from those of control subjects. There was no significant difference between pre-operative and post-operative LnLF for Group 1b (Table 6).

With correction for age, similar results were obtained except that LnLF in Group 1a did not change after the surgery and post-operative values were still significantly lower than those of controls (Appendix table E).

Using the same methodology, we did not find any statistically significant changes in the TLE group pre- versus post-operatively in patients with either low or high pre-operative LnRMSSD values (Figure 5E-F).

## 5.5. Discussion

To our knowledge, this is the first study to look at interictal HRV changes in IOE versus TLE patients and healthy controls. We also looked at the effect of epilepsy surgery on these HRV parameters.

### 5.5.1. HRV in IOE

We did not find significant differences in mean HRV values between the group of patients with IOE versus TLE patients and healthy controls. This was somewhat unexpected since the insula is known to be part of the central autonomic network (57, 191). While mean group values did not differ from healthy controls, examination of individual results within the IOE group showed that some patients appeared to have a decrease in both parasympathetic and sympathetic tones (low in all parameters) while others appeared to have an increase in parasympathetic tone (high LnRMSSD and pNN50) compared to healthy controls. These changes can potentially be explained by the fact that the insular cortex plays a role in both sympathetic and parasympathetic control (51, 57, 191, 192) and that resulting HRV changes could vary from patient to patient according to the side and site of subinsular epileptogenicity.

### 5.5.2. Effect of insulo-opercular epilepsy surgery on HRV

In our study, mean pre-operative HRV values did not significantly change after insulo-opercular surgery. In exploratory analyses, we observed a normalization of low or high pre-operative parasympathetic HRV values in individuals successfully operated in the insulo-opercular region (Figure 3). The low sympathetic measure (LnLF) was normalized post-operatively but was

still significantly lower compared to those of controls after correction for age. At first glance, these results appear to contradict findings from Lacuey et al. (2016, 2019) (96, 183). Lacuey et al. (2016) reported a 33-year-old patient who presented a significant decrease of mean of normal-to-normal heart beats (NNM) (13,75%), SDNN (21,14%), RMSSD (48.96%), and HF (21,35%), and an increase in LF (63,65%) and LF/HF ratio (307,95%) when comparing HRV parameters obtained the year before an unsuccessful left posterior insular and opercular resection versus those acquired 4 years after the surgery. Because the patient eventually died (SUDEP), the authors suggested that autonomic dysfunction from the failed insular surgery might have put him at additional risk for SUDEP. One could argue that HRV changes after his insulo-opercular surgery were due to persistent seizures from an unresected focus and not the resection of the insula itself. In 2019, the same group looked at HRV changes in patients undergoing temporal epilepsy surgery with variable resection/damage to the insula (183). The authors found a significant decrease in RMSSD and CV when the temporal lobe surgery was combined with partial (<25%; n=7) or extensive (≥25%; n=1) insular removal compared to temporal surgeries with no (n=4) or marginal (n=9) insular damage. Once again, because almost half of patients had persistent seizures (12 ILAE outcome class 1-2/21 patients), one cannot exclude the possibility that HRV changes were related to ongoing seizures rather than surgical damage to the insula. Moreover, both populations are hardly comparable as in our series, surgery was limited to the insulo-opercular areas while in their series, surgery included the temporal lobe and variable portions of the insula.

It would have been interesting to look at HRV changes in patients from our institution with failed insular surgeries. However, in this study, only patients with good surgical outcome after insulo-opercular seizures were included to guarantee accurate seizure focus identification. Potential explanations for such a recovery include a) arrest of seizure activity in the epileptogenic insula and areas of propagation involved in the central autonomic network; b) gradual compensation by the intact contralateral insula; c) reduction or withdrawal of antiepileptic drug after successful epilepsy surgery; d) reduction in co-morbid anxiety and depressive disorders, which may impact HRV as well; e) improvement in quality of life and a favorable psychosocial outcome (180, 186, 187).

### 5.5.3. HRV in TLE

In our study, mean pre-operative HRV values in the TLE group did not significantly differ from those with IOE as well as healthy controls. Less than a dozen studies have previously looked at interictal HRV in TLE, generating conflicting results. Similar to us, Varon et al. failed to demonstrate significant changes in HRV (157). Some have observed a decrease in parasympathetic tone while others have shown a decrease in both parasympathetic and sympathetic tones (151, 153-155). Suorsa et al. suggested that HRV is progressively reduced in refractory TLE but remains stable in well-controlled TLE patients (133).

### 5.5.4. Effect of TLE surgery on HRV

In our study, no significant changes were noted between mean pre-operative and post-operative HRV parameters. This is in line with findings from Persson et al. who found that TLE surgery did not affect HRV. In the latter study, patients with a good surgical outcome had similar HRV values than control subjects while patients with poor outcome had lower values; one year after TLE surgery, post-operative HRV was not different from pre-operative HRV (160). Although the poor outcome group had lower HRV post-operatively versus matched controls, this was already the case before the surgery. In contrast, Dericioglu et al. found a reduction in total HRV after surgery with the sympathovagal balance altered towards the sympathetic side (154) and Hilz et al. found a reduction of sympathetic cardiovascular modulation after surgery (159). Possible reasons for such contradictory results include inconsistent protocol for measurement and reporting of HRV, confounding factors (e.g., age, sex, medications, comorbid medical conditions), and the small numbers of patients, as discussed in a recent review by Myers et al. (2018) (190).

### 5.5.5. Correlation between parasympathetic parameters (RMSDD and pNN50) and SUDEP-7 score.

We found a positive correlation between pNN50 and RMSSD with the SUDEP-7 score. This positive correlation between these parasympathetic parameters and the SUDEP-7 score may suggest that, in IOE, the increase in parasympathetic tone could be more worrisome than its

decrease. This is partly supported by case reports of ictal bradycardia, atrioventricular block or asystole in patients with insular epilepsy (90, 94, 95). However, because all of our patients were operated and had a good seizure outcome (as per our inclusion criteria), it is unlikely that we will be able to assess in the long run if patients with either an increase or decrease in parasympathetic parameters have a higher risk of SUDEP. Further prospective work will be required to validate this finding.

### 5.5.6. Limitation

We acknowledge that our study has several limitations mainly related to its retrospective design: the need to resort to EKG recordings obtained during pre-operative VEEG sessions (rather than dedicated EKG recording sessions), variable time intervals between surgery and post-operative EKG acquisitions, and variable antiepileptic drug regimens between patients. As SUDEP occurs frequently at night or during sleep (36, 193), it would have been interesting to analyze the sleep HRV. Unfortunately, albeit applying the minimum protocol for interictal HRV analysis of Myers et al., we did not obtain 5-min sleep recording for patients at post-operative periods as well as for matched control participants. We also recognize that trend towards normalization observed in IOE subgroups could reflect regression to the mean rather than a true effect from successful epilepsy surgery. However, this effect was only noted in the IOE group and not the TLE group, which could support a real contribution from insulo-opercular resection. Even if the regression to the mean is genuinely responsible for these observed findings, the good news is that the insular resection does not appear to induce further HRV impairments in patients with IOE. Finally, we are also well aware of the implications of conducting an exploratory study with a modest number of patients in which several pairwise comparisons were conducted and for which an accurate correction to control for Type I errors could not be estimated. Nevertheless, we deemed that some of the observations found with low p-values could be interesting enough to be worth reporting to encourage further work. On the other side, one must contend with the fact that IOE is relatively rare; to reach adequate power will require multicenter collaboration. We did try to enhance the power of our findings by including twice the number of age- and sex matched control subjects for each patient at both studied periods as well as correct for age-related HRV changes using a quadratic regression model.

## 5.6. Conclusion

In refractory IOE, our findings suggest that HRV may be either lower globally in both sympathetic and parasympathetic tones or higher in parasympathetic tone. Higher parasympathetic tone was also positively correlated with the SUDEP-7 inventory score. After successful IOE surgery, HRV changes appear to normalize. A multicenter prospective study with a larger number of patients is warranted to confirm these observations.

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## Conflict of interest

Authors report no conflict of interest pertaining to this study.

Table 3. Summary of presurgical, surgical and postsurgical features of IOE patients

| Pt | S | Lat | AO (yo) | Presurgical period | | Epilepsy duration (years) | Postsurgical period | | SUDEP-7 score (over past 12 months) at A1 | Presurgical evaluation | | | | | Surgery | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | A1 (yo) | ASMs | | Elapsed time from surgery to postsurgical EKG (months) | ASMs | | Operculo-INS lesion on MRI | PET | iSPECT | MEG | icEEG | Resected areas | Engel |
| I1 | F | R | 13 | 46 | TPM, CBZ, LEV, LCM | 33 | 64 | LEV, LCM | **6** | yes | Normal | R inf FG, INS | no spike | N/A | superior INS + F-P opercule | Ia |
| I2 | F | L | 10 | 19 | OXC, PHT, PER | 9 | 6 | PHT, OXC | **2** | no | L perisylvian region | L ant-INS | L operculo-INS | ant-INS, F-opercule | ant-INS | IId |
| I3 | M | L | 29 | 37 | PHT, CLB | 8 | 142 | None | **2** | no | Normal | L post-INS, Post-F lobe | L post-INS, L mesial T | post-INS | post-INS | Ib |
| I4 | F | L | 4 | 31 | LEV, CBZ, CLB | 27 | 29 | CBZ, CLB | **3** | yes | L INS | non-localizing | no spike | N/A | INS + F-P opercule | Ia |
| I5 | F | L | 5 | 36 | LCM, CLB, CBZ, TPM | 31 | 25 | CBZ | **6** | no | L T pole | L IFG, ant-INS | L F-T opercula-INS | ant-INS, IFG, STG | 2/3 ant-INS + partial F-T opercule | Ia |
| I6 | F | L | 3 | 49 | CBZ, LTG, CLB | 46 | 12 | CBZ, LTG, CLB | **5** | yes | Normal | multiple | L post-INS, P opercule | post-INS | INS | Ia |
| I7 | M | R | 26 | 35 | PHT, LTG | 9 | 60 | OXC | **2** | no | T lobes (L>R) | L post-orbitoF | R IFG | IFG | ant-INS + F opercule | Ia |
| I8 | F | R | 18 | 37 | CBZ, LTG | 19 | 204 | CBZ, CLB | **2** | yes | NA | NA | NA | NA | INS + F-P-R opercule | Ia |

| Pt | S | Lat | AO (yo) | Presurgical period | | Epilepsy duration (years) | Postsurgical period | | SUDEP-7 score (over past 12 months) at A1 | Presurgical evaluation | | | | | Surgery | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | A1 (yo) | ASMs | | Elapsed time from surgery to postsurgical EKG (months) | ASMs | | Operculo-INS lesion on MRI | PET | iSPECT | MEG | icEEG | Resected areas | Engel |
| I9 | F | R | 9 | 27 | TPM, LCM | 12 | 58 | None | **2** | no | R mesial F | multiple | Junction between R ant-INS and orbitoF | Junction between R ant-INS and orbitoF | ant-INS + F opercule | Ia |
| I10 | M | L | 16 | 30 | CBZ, CLB | 14 | 28 | CBZ | **2** | no | L INS, orbitoF, ant-T | N/A | L orbito-F | N/A | ant-INS + orbitoF | Ia |
| I11 | M | L | 24 | 33 | LTG, CBZ | 9 | 6 | LTG, CBZ | **2** | yes (L ant-INS cavernoma) | L INS, T lobe | L INS, orbitoF, T lobe | N/A | N/A | INS | Ia |
| I12 | F | R | 5 | 24 | TPM, CLB, LCM | 19 | 70 | OXC, TPM, CLB | **3** | yes (TSC) | R INS, MFG | R INS, peri-INS | R operculo-INS | N/A | INS + F-P-T opercule | Ib |
| I13 | F | R | 21 | 35 | CBZ, TPM | 14 | 18 | CBZ, TPM | **1** | no | R cerebellum | R post-INS | R superior ant-INS, F opercule, STG | ant-INS, IFG | ant-INS + F opercule | Ia |
| I14 | M | R | 35 | 46 | PGB, OXC | 11 | 93 | PGB, LCM, CNZ | **2** | no | R F pole | R IFG, F pole | R mesial F, both orbitoF, L IFG, L SFG | R mesial F, R F pole, IFG, pCG | ant-INS + F opercule + orbitoF | Ia |

Pt = patient, S = sex, Lat = lateralization of the epileptic focus, AO = age of onset, A1 = age at preoperative period, yo = years old, TCS = tonic-clonic seizures, ASMs = antiseizure medications; Epilepsy duration = from onset to preoperative EEG; MRI = magnetic resonance imaging, PET = positron emission tomography, iSPECT = ictal single photon emission computed tomography, MEG = magnetoencephalography, icEEG = intracranial electroencephalography, M = male; F = female; R = right, L = left, INS = insula, F = frontal, P = parietal, T = temporal, CG = central gyrus, STG = superior temporal gyrus, IFG = inferior frontal gyrus, MFG = middle frontal gyrus, ant = anterior; post = posterior; inf = inferior; LEV = Levetiracetam, LTG = Lamotrigine, TPM = Topiramate, CBZ = Carbamazepine, OXC = Oxcabazepine, LCM = Lacosamide, PER = Perampanel, PHT = Phenytoin, CLB = Clobazam, CNZ = Clonazepam, PGB = Pregabalin, NA = not available

Table 4. Summary of presurgical and postsurgical features of TLE patients

| Pt | S | Lat | AO (yo) | Epilepsy duration (years) | Presurgical period | | Postsurgical period | | SUDEP-7 score (over past 12 months) at A1 |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | A1 (yo) | ASMs | elapsed time from surgery to postsurgical EKG (months) | ASMs | |
| T1 | F | L | 0.7 | 39 | 40 | VPA, CBZ | 120 | None | **4** |
| T2 | M | L | 19 | 15 | 34 | LEV, LTG, CLB | 41 | LEV, LTG | **3** |
| T3 | F | L | 2 | 19 | 21 | CBZ, LEV | 14 | CBZ, LEV | **1** |
| T4 | F | L | 4 | 24 | 28 | TPM, LTG | 79 | LEV | **1** |
| T5 | M | L | 30 | 16 | 46 | OXC, BRV, Pb | 71 | OXC, Pb, GPN, LCM | **4** |
| T6 | F | L | 27 | 4 | 31 | Pb, PGB, PHT | 104 | PGB, LCM | **2** |
| T7 | M | L | 11 | 12 | 33 | TPM, LTG | 22 | LTG, CLB | **1** |
| T8 | F | L | 33 | 3 | 36 | CLB, TPM, OXC, LTG | 48 | OXC, TPM, CLB | **4** |
| T9 | F | R | 33 | 11 | 44 | PER, CNZ, LCM | 17 | TPM | **4** |
| T10 | M | R | 25 | 2 | 27 | LTG, LCM, LEV | 64 | LCM, CLB | **2** |
| T11 | F | R | 2 | 41 | 43 | PHT, TPM, LTG | 77 | PHT, LCM | **5** |
| T12 | F | L | 23 | 1 | 24 | LTG, LEV, CLB | 60 | LTG | **2** |
| T13 | M | R | 17 | 15 | 32 | CBZ | 24 | CZB | **1** |
| T14 | F | L | 13 | 16 | 39 | CBZ | 13 | CBZ | **1** |

Pt = patient, S = sex, Lat = lateralization of the epileptic focus, AO = age of onset, A1 = age at preoperative period, yo = years old, TCS = tonic-clonic seizures, ASMs = antiseizure medications, VPA = Valproic acid, LEV = Levetiracetam, LTG = Lamotrigine, TPM = Topiramate, CBZ = Carbamazepine, OXC = Oxcabazepine, LCM = Lacosamide, PER = Perampanel, PHT = Phenytoin, CLB = Clobazam, CNZ = Clonazepam; GPN = Gabapentin, PGB = Pregabalin; Pb = Phenobarbital; SUDEP = sudden unexpected death in epilepsy.

Table 5. Comparing HRV values of IOE, TLE and control groups in preoperative and postoperative

| HRV parameters | | IOE group (n=14) | | | TLE group (n=14) | | | Control group (n=28) | | IOE vs TLE vs Control group | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Preop | Postop | P | Preop | Postop | P | Preop | Postop | Preop p value | Postop p value |
| **LnRMSSD** | Mean | 3,56 | 3,51 | 0,76 | 3,61 | 3,54 | 0,54 | 3,52 | 3,50 | 0,87 | 0,96 |
| | SD | 0,45 | 0,44 | | 0,45 | 0,55 | | 0,60 | 0,57 | | |
| **pNN50 (%)** | Mean | 16,09 | 13,61 | 0,59 | 17,92 | 13,37 | 0,31 | 16,37 | 14,83 | 0,95 | 0,95 |
| | SD | 15,12 | 11,03 | | 16,94 | 17,79 | | 16,39 | 16,13 | | |
| **LnLF** | Mean | 6,16 | 6,55 | 0,20 | 6,73 | 6,52 | 0,37 | 6,43 | 6,43 | 0,27 | 0,92 |
| | SD | 0,89 | 0,97 | | 0,72 | 1,12 | | 1,00 | 0,95 | | |
| **LnHF** | Mean | 5,90 | 5,73 | 0,61 | 5,98 | 5,88 | 0,59 | 5,90 | 5,89 | 0,97 | 0,92 |
| | SD | 0,92 | 1,14 | | 1,00 | 1,05 | | 1,25 | 1,20 | | |
| **IOE GROUP** | | | | | | | | | | | |
| **HRV parameters** | | **L-IOE (n=7)** | | | **R-IOE (n=7)** | | | **L-IOE vs control (p)** | | **R-IOE vs control (p)** | |
| | | **Preop** | **postop** | **P** | **Preop** | **Postop** | **p** | **Preop** | **Postop** | **Preop** | **Postop** |
| **LnRMSSD** | Mean | 3,65 | 3,78 | 0,52 | 3,46 | 3,54 | 0,69 | 0,57 | 0,70 | 0,90 | 0,64 |
| | SD | 0,43 | 0,54 | | 0,49 | 0,37 | | | | | |
| **pNN50 (%)** | Mean | 19,94 | 14,84 | 0,56 | 12,24 | 12,37 | 0,98 | 0,50 | 0,65 | 0,27 | 0,15 |
| | SD | 18,62 | 12,93 | | 10,67 | 9,62 | | | | | |
| **LnLF** | Mean | 6,24 | 6,64 | 0,43 | 6,09 | 6,45 | 0,36 | 0,82 | 0,67 | 0,38 | 0,052 |
| | SD | 0,63 | 1,11 | | 1,14 | 0,89 | | | | | |
| **LnHF** | Mean | 6,17 | 5,52 | 0,43 | 5,64 | 5,95 | 0,22 | 0,55 | 0,34 | 0,71 | 0,55 |
| | SD | 0,86 | 1,29 | | 0,96 | 1,03 | | | | | |

| TLE group | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **HRV parameters** | | **L-TLE (n=10)** | | | **R-TLE (n=4)** | | | **L-TLE vs control** | | **R-TLE vs control** | |
| | | **Preop** | **postop** | **P** | **Preop** | **Postop** | **p** | **Preop** | **Postop** | **Preop** | **Postop** |
| **LnRMSSD** | Mean | 3,66 | 3,62 | 0,74 | 3,47 | 3,36 | 0,52 | 0,90 | 0,66 | 0,26 | 0,54 |
| | SD | 0,53 | 0,60 | | 0,06 | 0,36 | | | | | |
| **pNN50 (%)** | Mean | 21,74 | 15,09 | 0,26 | 8,38 | 9,08 | 0,91 | 0,78 | 0,95 | 0,90 | 0,74 |
| | SD | 18,89 | 20,02 | | 1,78 | 22,59 | | | | | |
| **LnLF** | Mean | 6,77 | 6,67 | 0,75 | 6,62 | 6,12 | 0,21 | 0,66 | 0,68 | 0,32 | 0,99 |
| | SD | 0,77 | 1,24 | | 0,69 | 0,68 | | | | | |
| **LnHF** | Mean | 6,02 | 5,92 | 0,70 | 5,89 | 5,77 | 0,71 | 0,75 | 0,99 | 0,82 | 0,55 |
| | SD | 1,20 | 1,18 | | 0,25 | 0,75 | | | | | |
| | | | | | | | | | | | |

IOE = Insulo-opercular epilepsy; TLE = Temporal lobe epilepsy; pNN50 = percentage of successive RR intervals that differ by more than 50ms; RMSSD = root mean square of successive RR interval differences; Ln = natural logarithm
Preop = preoperative; Postop = postoperative
The results were not changed after age correction.

Table 6. Preoperative and postoperative HRV values of IOE subgroups compared to those of matched controls.

| HRV parameters | | Control for group 1a | | Group 1a (n=6) | | Group 1a vs control | | | Control for group 1b | | Group 1b (n=8) | | | Group 1b vs control | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Preop (n=12) | Postop (n=12) | Preop | Postop | Preop vs Postop | Preop p value | Postop p value | Preop (n=16) | Postop (n=16) | Preop | Postop | Preop vs postop | Preop p value | Postop p value |
| **LnRMSSD** | Mean | 3,80 | 3,49 | 3,17 | 3,59 | **0,007** | **0,008** | 0,694 | 3,31 | 3,50 | 3,84 | 3,45 | 0,058 | **0,006** | 0,836 |
| | SD | 0,48 | 0,58 | 0,36 | 0,39 | | | | 0,60 | 0,58 | 0,26 | 0,49 | | | |
| **pNN50 (%)** | Mean | 22,31 | 13,85 | 3,48 | 13,85 | **0,035** | **0,003** | 0,917 | 11,91 | 15,05 | 25,55 | 13,43 | 0,053 | **0,037** | 0,785 |
| | SD | 17,33 | 11,34 | 3,25 | 11,34 | | | | 14,63 | 16,83 | 13,36 | 11,57 | | | |
| **LnLF** | Mean | 6,85 | 6,31 | 5,48 | 6,34 | **0,006** (*) | **0,005** | 0,944 (**) | 6,11 | 6,51 | 6,68 | 6,70 | 0,964 | 0,103 | 0,687 |
| | SD | 0,88 | 1,07 | 0,74 | 0,66 | | | | 0,99 | 0,89 | 0,61 | 1,17 | | | |
| **LnHF** | Mean | 6,47 | 5,57 | 5,28 | 5,73 | 0,123 | **0,004** | 0,756 | 5,48 | 5,89 | 5,62 | 5,73 | 0,235 | 0,059 | 0,793 |
| | SD | 0,92 | 1,19 | 0,57 | 0,67 | | | | 1,32 | 1,23 | 1,47 | 1,45 | | | |

IOE = Insulo-opercular epilepsy; pNN50 = percentage of successive RR intervals that differ by more than 50ms; RMSSD = root mean square of successive RR interval differences; Ln = natural logarithm

Group 1a and group 1b consisted of patients whose preoperative LnRMSSD values were below or above 3,53 respectively.

Preop = preoperative; Postop = postoperative

(*) After correction for age, the results were nearly unchanged except for (LnLF of group 1a): * p value = 0,71; ** p value = 0,01.

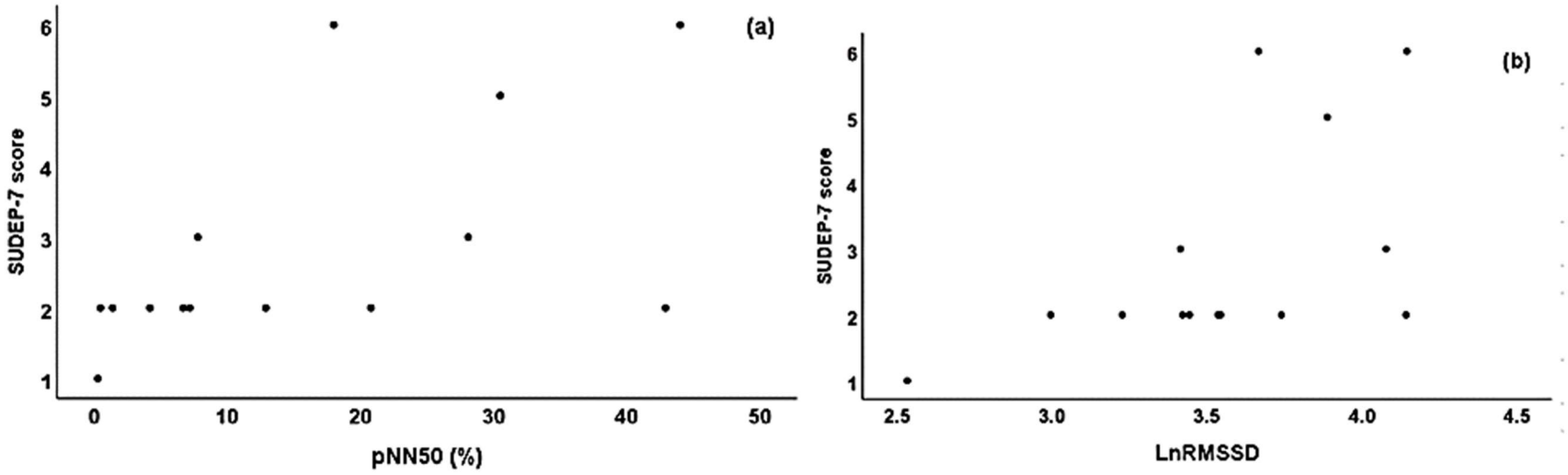


Figure 5. The correlation between LnRMSSD, pNN50 and the SUDEP-7 score in IOE group.

(a) Plot of pNN50 (%) versus SUDEP-7 score. (b) Plot of LnRMSSD versus SUDEP-7 score. In IOE group, the SUDEP-7 score was positively correlated with pNN50 (r=0,671; p<0,01) and with LnRMSSD) (r=0,591; p<0,05). IOE = Insulo-opercular epilepsy; pNN50 = percentage of successive RR intervals that differ by more than 50ms; RMSSD = root mean square of successive RR interval differences; Ln = natural logarithm; SUDEP = sudden unexpected death in epilepsy.

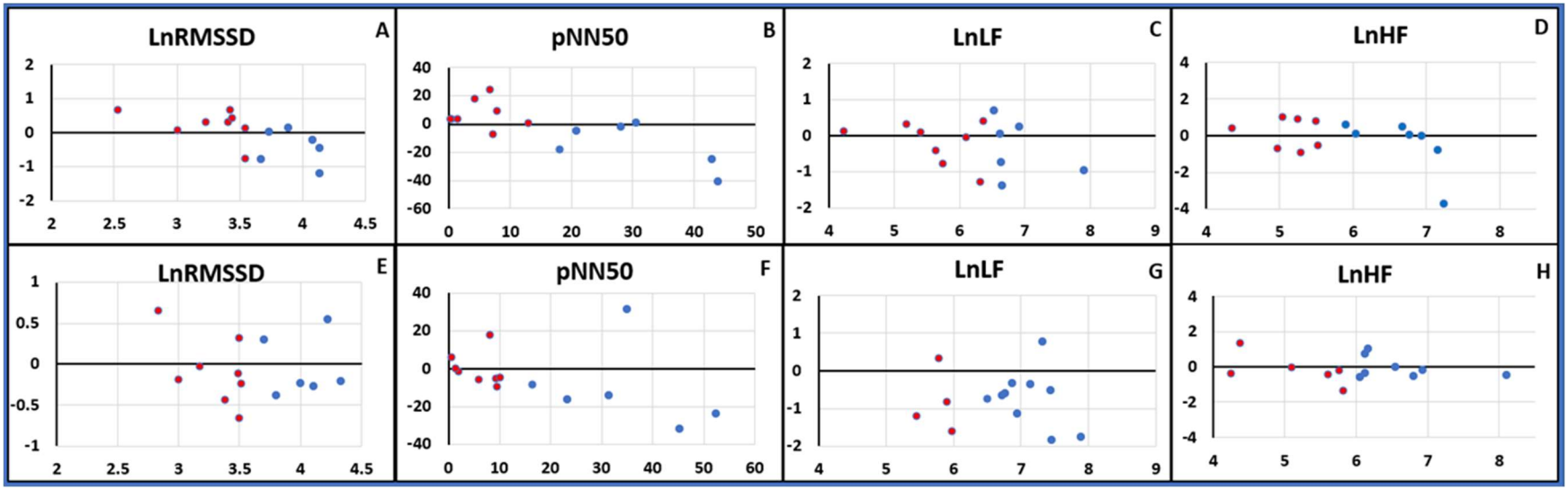


Figure 6. Comparing pre-operative and postoperative HRV parameters of each patient in the IOE group (A-D) and TLE group (E-H)

For each parameter, patients in the IOE or TLE group were divided into two subgroups based on whether their pre-operative values were below (red dots) or above (blue dots) the mean LnRMSSD value of control group. In the IOE group, low LnRMSSD and pNN50 tended to increase post-operatively, whereas high values tended to decrease after surgery. This pattern of HRV changes was not seen in the TLE group. (X-axis represents the pre-operative HRV measures; y-axis represents the difference between pre-operative and post-operative HRV. Each dot represents a patient). IOE = Insulo-opercular epilepsy; TLE = Temporal lobe epilepsy; pNN50 = percentage of successive RR intervals that differ by more than 50ms; RMSSD = root mean square of successive RR interval differences; Ln = natural logarithm.

# Chapter 6 – General discussion

## 6.1. Summary

The insular cortex is part of the central autonomic network; hence insular damage could result in cardiac autonomic dysregulation. While several studies investigated the effects of insular infarction on autonomic activity by assessing HRV, none have until now assessed HRV changes in chronic insular epilepsy. The purpose of the present study was to determine the cardio-autonomic function in IOE by evaluating the interictal HRV in IOE patients and how the resection of this epileptogenic focus affected interictal HRV. We also looked at the relationship between HRV parameters and the risk of SUDEP in IOE patients, as measured by the SUDEP-7 inventory. Our preliminary findings were that:

1. In IOE, the whole group's HRV mean values were not statistically different from those of healthy controls, but the individual's HRV values may be either increased or decreased. There was no significant difference between right and left insular epileptic foci;

2. The increased parasympathetic HRV parameters may be more worrisome as they were correlated to the SUDEP risk scale;

3. Insulo-opercular resection may have a positive impact on HRV impairment in IOE patients;

4. There were no differences in HRV parameters between the TLE group and the healthy control group.

5. Temporal lobectomies did not impact HRV.

## 6.2. HRV changes in IOE

In our study, mean HRV measures in patients with IOE did not differ from age- and sex-matched control subjects. However, when examining on a patient-by-patient basis, we observed that 43% of patients had significantly lower HRV parameter values (RMSSD, pNN50, LF, and HF values) and 57% of patients had significantly higher parasympathetic HRV values (RMSSD and

pNN50) than those of the control group. Since this is the first study looking at the HRV changes in IOE, we could not compare our results with other studies. Although HRV changes are well described in acute ischemic stroke involving the insula, the underlying pathophysiology is different from epilepsy. Further, as previously mentioned, most stroke series were composed of MCA ischemic damage extending well beyond the insula. The same limitations apply to series evaluating HRV with insular tumors.

Our findings are in line with electrical stimulation studies, which have provided evidence that the insula is involved in autonomic control. Indeed, cortical stimulations of the human insula induced bradycardia in 26% of stimulations and tachycardia in 21% (51). The authors reported a posterior predominance of sympathetic control, whereas parasympathetic control seemed to be more anteriorly positioned within the insula (without left/right asymmetry). While there is still some debate in the antero-posterior distribution or lateralization of the insular cortex in terms of cardiac autonomic function, functional MRI studies have also supported the role of the insula in both parasympathetic and sympathetic regulation (57, 191).

## 6.3. Relationship between HRV and SUDEP risk

In our study, we found a positive correlation between the SUDEP-7 score and the parasympathetic parameters (RMSSD and pNN50) in IOE patients. This is in line with a few case reports indicating that insular seizures may be associated with parasympathetic overactivity-induced life-threatening arrhythmias, such as bradycardia/asystole or complete atrioventricular block (90, 94, 95). In the MORTEMUS study (Mortality in Epilepsy Monitoring Unit Study), the authors also reported two near-SUDEP patients with an epileptic focus in the insular cortex, one presenting postictal cardiorespiratory arrest after the partial seizure and the other ictal asystole (93). These observations could possibly mean that IOE with interictal parasympathetic HRV should be monitored more closely although more evidence is required to confirm this. With other forms of epilepsy, others have found an association between decreased RMSSD (a parasympathetic parameter) and an increase in the SUDEP-7 score (26, 131, 136, 161).

## 6.4. Effects of insulo-opercular resection on HRV

When comparing the mean HRV value of the whole group, we did not find any differences between preoperative and postoperative values. However, we found that postoperative HRV values significantly increased for patients whose preoperative HRV values were low and tended to decrease for patients whose preoperative HRV values were high. Notably, HRV values after surgery in both groups were not different from those of matched controls.

To our knowledge, only two articles have reported the effect of insular resection on autonomic function. De Morree et al. (2016) described HRV changes during the resection of a right or left insular glioma in two patients (85). An increased parasympathetic activity reflected via temporary bradycardia/asystole was observed during removal of the right insular glioma while no effect was observed during the removal of the left insular tumor. Short- and long-term evolution of their autonomic activity after the surgery was not reported however. Lacuey et al. (2019) explored in patients with refractory epilepsy the effect of insular resection of variable extent, with temporal lobectomies on HRV changes (183). They found that patients with significant insular resection had decreased RMSSD and coefficient of variation after surgery. Also, right-sided surgeries were associated with an increased LF/HF ratio independent of the extent of insular involvement. The authors suggested that insular resections can alter autonomic function, particularly on the right side. This cohort of patients is hardly comparable to our series of patients. For one, patients had much larger resections with the removal of the temporal lobe in addition to variable amounts of insular cortex; hence, disentangling the contribution of the insula alone to HRV changes is difficult. Also, only 12/21 patients achieved a good seizure outcome after surgery (ILAE outcome classification 1-2); thus, it is unclear if these postoperative HRV changes are due to the resection of a portion of the insula or to recurring seizures. We noted that only two of eight patients with significant insular resection had poor surgical outcome (ILAE outcome classification 4 and 5). It would be important to know whether these two patients' values were outliers and how the results would have changed if these two patients had been removed from the analysis. Moreover, since there was no control group, it remains unclear how preoperative and postoperative values stand compared to healthy individuals. Therefore, from this study, it remains uncertain if insular resection directly induces HRV deterioration.

At this moment, based on available evidence from the literature and our findings, it may be possible that removal of the insular cortex leads to acute HRV changes (as noted by de Morree et al. 2016); however, if such resections lead to seizure-freedom, it might have a positive impact on autonomic function in the long run (as our findings would suggest). However, if such resections do not lead to seizure freedom, then it may possibly worsen autonomic function (as reported by Lacuey et al. (2019). Several factors could contribute to long-term improvement of autonomic function after successful insular surgery for refractory epilepsy: a) arrest of seizure activity within the insular epileptogenic focus, as well as within areas of seizure propagation in the central autonomic network; b) compensation by the intact contralateral insula; c) reduction or withdrawal of ASM after successful epilepsy surgery; d) reduction in co-morbid anxiety and depressive disorders; and (e) favorable psychosocial outcome and improvement in quality of life. Indeed, studies in adults and children have shown cognitive improvement after successful partial or complete insulectomy (194, 195).

## 6.5. HRV in TLE

The present study did not find any differences in preoperative and postoperative HRV between the TLE and the IOE groups. There were no significant differences between preoperative and postoperative HRV values. Our findings are in line with other studies of Persson et al. (2005, 2006), who showed that preoperative HRV values did not differ from those of healthy controls in TLE patients who had good surgical outcomes and that temporal lobe epilepsy surgery had no impact on HRV (134, 160). On the contrary, for TLE patients who had poor surgical outcomes, preoperative and postoperative HRV values were significantly lower than those of controls. The authors suggested that the pronounced abnormalities in HRV were possibly because they had temporal plus epilepsy, explaining both HRV changes and poor surgical outcome after temporal lobectomy. In addition, they hypothesized that the increased risk of SUDEP in patients who failed epilepsy surgery may result from the existing abnormalities rather than the influence of surgical intervention since postoperative HRV values (although low) were not different from preoperative values.

While in line with the work of Persson et al. (2005a, 2006), our findings are in contradiction with other studies showing HRV changes in TLE patients. These discrepant findings are potentially explained by methodological differences: diagnosis of TLE solely based on clinical semiology and interictal surface EEG findings (133, 152, 157), short-term versus long-term HRV measures and number of patients. Regarding HRV changes after temporal lobectomies, our findings are different from Hilz et al. (2002) who showed a decrease in LF after surgery (159) and Dericioglu et al. (2013) who found an increase in LF and LF/HF ratio (154). However, in both studies, postoperative follow-up was only 1-3 months, and not all patients were seizure-free; their results are hence difficult to compare to our findings.

## 6.6. Clinical significance of the current study

Results presented in this memoir remain preliminary and no hard conclusions can be made. Since we found a positive correlation between parasympathetic tone and the SUDEP-7 score in IOE patients, suggesting a potentially higher risk of SUDEP, these patients may require more attentive and long-term monitoring. This has little clinical bearing since physicians have already striven to reach seizure control to reduce the risk of SUDEP. Nevertheless, when epilepsy becomes drug-resistant, many general physicians or neurologists have been reluctant to refer patients to the epilepsy center for epilepsy surgery (196-198). One of the several reasons for non-referral is the assumed postoperative deficits (196). Even the epileptologists also show vigilance in IOE surgery regarding the risk of postoperative autonomic dysfunction. More importantly, we found no evidence that insulo-opercular surgeries lead to major autonomic dysfunction when a good seizure outcome is reached. Hence, when one is convinced that the seizure focus lies in the insula and believes that a good outcome can be reasonably attained with surgery rather than medical therapy, epilepsy surgery in this region is justified. The same goes for refractory temporal lobe epilepsy as temporal lobectomies leading to a good outcome does not have a negative impact on HRV.

# Chapter 7 – Future perspectives

Since our study contained several limitations, more work is necessary to confirm the observations made in our study. Future endeavors should ideally be prospective rather than retrospective. Considering the low prevalence of insular epilepsy, a multicenter study will be necessary to reach a sufficient number of patients to confirm our findings. If we consider the prevalence of IOE in our center representative of its prevalence in Canada, with a confidence level of 90%, and a 5% of margin of error, we estimate that ~250 patients are required. A standardized protocol among sites for EKG recording and HRV analyses both pre- and post-operatively should be set in place to ensure that autonomic activity is assessed at resting state, during sleep stages, and during autonomic activation tests (Ewing tests, including standing test, handgrip test, deep breathing test, and Valsalva maneuver). To better understand the evolution of HRV over time, HRV assessment should be obtained at scheduled follow-up visits, pre- and post-operative timepoints (e.g., <one month, <6 months, 6-12 months, 1-3 years, 3-5 years, …) for both patients with good and poor surgical outcomes. Adding other biomarkers for sudden cardiac death, such as QTc dispersion and T-wave alternans (TWA) in refractory epilepsy, may improve the ability to identify the patients at high-risk. Such better designed studies should obviously not be limited to IOE but also include other types of epilepsy.

The increasing availability of non-obtrusive smart wearables (e.g., smart watch, smart shirt) or more invasive devices (e.g., Neuropace) that allow the clinician to record the cardiac rhythm at home over a long period of time also provides a unique opportunity to understand how epilepsy (and epilepsy surgery) impacts autonomic function over years.

Our findings pave the way for larger multicentric studies that could ultimately impact patient care in favor of earlier surgical interventions for eligible candidates and better long-term monitoring of cardiac markers of SUDEP risk.

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# Appendix

**Table A: The revised SUDEP-7 inventory scores of IOE and TLE patients**

| | SUDEP-7 risk factor inventory (over past 12 months) at presurgical period | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **IOE patient** | **More than 3TCS** | **1 or more TCS** | **1 or more sz of any type** | **>50sz of any type/month** | **Duration>30yrs** | **Current use of 3 or more ASMs** | **Mental retardation, IQ<70, or too impaired to test** | **Score** |
| I1 | 0 | 0 | 0 | 2 | 3 | 1 | 0 | **6** |
| I2 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | **2** |
| I3 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | **2** |
| I4 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | **3** |
| I5 | 0 | 1 | 1 | 0 | 3 | 1 | 0 | **6** |
| I6 | 0 | 0 | 1 | 0 | 3 | 1 | 0 | **5** |
| I7 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | **2** |
| I8 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | **2** |
| I9 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | **2** |
| I10 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | **2** |
| I11 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | **2** |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| I12 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | **3** |
| I13 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | **1** |
| I14 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | **2** |
| **TLE patient** | **More than 3TCS** | **1 or more TCS** | **1 or more sz of any type** | **>50sz of any type/month** | **Duration>30yrs** | **Current use of 3 or more ASMs** | **Mental retardation, IQ<70, or too impaired to test** | **Score** |
| T1 | 0 | 0 | 1 | 0 | 3 | 0 | 0 | **4** |
| T2 | 0 | 0 | 0 | 2 | 0 | 1 | 0 | **3** |
| T3 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | **1** |
| T4 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | **1** |
| T5 | 2 | 0 | 1 | 0 | 0 | 1 | 0 | **4** |
| T6 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | **2** |
| T7 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | **1** |
| T8 | 2 | 0 | 1 | 0 | 0 | 1 | 0 | **4** |
| T9 | 2 | 0 | 1 | 0 | 0 | 1 | 0 | **4** |
| T10 | 2 | 0 | 1 | 0 | 0 | 1 | 0 | **2** |
| T11 | 0 | 0 | 1 | 0 | 3 | 1 | 0 | **5** |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| T12 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | **2** |
| T13 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | **1** |
| T14 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | **1** |

SUDEP = sudden unexpected death of epilepsy; IOE = insulo-opercular epilepsy; TLE = temporal lobe epilepsy; TCS = tonic-clonic seizure; sz = seizure; yrs = years; ASM = antiepileptic drugs; IQ = intelligence quotient

**Table B: Age-, sex matched controls for patients' groups at preoperative and postoperative periods.**

| IOE group | S | Matched TLE | Preop age | Preop matched Control | Postop age | Postop matched control |
|---|---|---|---|---|---|---|
| I1 | F | T11 | 47 | C5, 32 | 54 | C1, 12 |
| I2 | F | T3 | 19 | C3, 26 | 19 | C3, 26 |
| I3 | M | T7 | 38 | C29, 30 | 49 | C24, 30 |
| I4 | F | T6 | 32 | C14, 31 | 36 | C21, 22 |
| I5 | F | T8 | 36 | C9, 21 | 40 | C2, 33 |
| I6 | F | T9 | 49 | C7, 25 | 51 | C5, 25 |
| I7 | M | T2 | 35 | C4, 23 | 42 | C8, 16 |
| I8 | F | T1 | 37 | C12, 22 | 54 | C7, 32 |
| I9 | F | T4 | 28 | C18, 28 | 33 | C14, 31 |
| I10 | M | T10 | 31 | C11, 17 | 35 | C4, 23 |
| I11 | M | T13 | 34 | C20, 27 | 34 | C20, 27 |
| I12 | F | T12 | 23 | C6, 19 | 30 | C18, 28 |
| I13 | F | T14 | 35 | C2, C33 | 36 | C9, 15 |
| I14 | M | T5 | 46 | C13, 24 | 53 | C10, 29 |
| | | | | | | |

| TLE group | S | Preop age | Postop age | CONTROL | S | Age |
|---|---|---|---|---|---|---|
| T1 | F | 40 | 50 | C1 | F | 53 |
| T2 | M | 35 | 41 | C2 | F | 39 |
| T3 | F | 21 | 22 | C3 | F | 20 |
| T4 | F | 28 | 36 | C4 | M | 33 |
| T5 | M | 48 | 52 | C5 | F | 52 |
| T6 | F | 31 | 39 | C6 | F | 24 |
| T7 | M | 33 | 35 | C7 | F | 52 |
| T8 | F | 36 | 41 | C8 | M | 40 |
| T9 | F | 44 | 46 | C9 | F | 36 |
| T10 | M | 28 | 35 | C10 | M | 53 |
| T11 | F | 43 | 50 | C11 | M | 27 |
| T12 | F | 24 | 29 | C12 | F | 41 |
| T13 | M | 32 | 36 | C13 | M | 40 |
| T14 | F | 39 | 40 | C14 | F | 31 |
| **Legends:** IOE = insulo-opercular epilepsy; TLE = temporal lobe epilepsy; S = sex; F = female; M = male; preop = preoperative; postop = postoperative | | | | C15 | F | 38 |
| | | | | C16 | M | 40 |
| | | | | C17 | M | 29 |
| | | | | C18 | F | 28 |
| | | | | C19 | F | 23 |
| | | | | C20 | M | 33 |
| | | | | C21 | F | 36 |
| | | | | C22 | F | 36 |
| | | | | C23 | M | 35 |
| | | | | C24 | M | 43 |
| | | | | C25 | F | 49 |
| | | | | C26 | F | 19 |
| | | | | C27 | M | 33 |

| | | | |
|---|---|---|---|
| | C28 | F | 27 |
| | C29 | M | 38 |
| | C30 | M | 37 |
| | C31 | F | 31 |
| | C32 | F | 42 |
| | C33 | F | 36 |

**Table C: HRV measures of each participant**

| Control Group | HRV parameters | | | | Preoperative/Postoperative control group |
|---|---|---|---|---|---|
| Subject | LnRMSSD | pNN50 | LnLF | LnHF | |
| **1** | 3,37 | 5,1 | 5,67 | 5,30 | **Postoperative** |
| **2** | 3,10 | 0,0 | 6,27 | 5,23 | **Both** |
| **3** | 4,28 | 49,8 | 6,72 | 7,44 | **Both** |
| **4** | 4,27 | 50,7 | 6,71 | 7,97 | **Both** |
| **5** | 2,52 | 0,0 | 5,55 | 3,87 | **Both** |
| **6** | 4,06 | 34,9 | 7,50 | 7,10 | **Preoperative** |
| **7** | 2,54 | 0,0 | 4,33 | 3,29 | **Both** |
| **8** | 3,36 | 4,1 | 6,40 | 5,92 | **Postoperative** |
| **9** | 3,59 | 13,8 | 7,36 | 5,71 | **Both** |
| **10** | 2,63 | 0,5 | 6,30 | 4,01 | **Postoperative** |
| **11** | 3,39 | 5,2 | 5,93 | 5,50 | **Preoperative** |
| **12** | 3,85 | 29,2 | 7,08 | 6,57 | **Both** |
| **13** | 2,37 | 0,0 | 4,99 | 3,56 | **Preoperative** |
| **14** | 3,61 | 11,3 | 7,02 | 6,39 | **Both** |
| **15** | 3,58 | 10,7 | 5,94 | 6,23 | **Postoperative** |
| **16** | 3,65 | 11,7 | 7,50 | 6,09 | **Postoperative** |
| **17** | 3,79 | 19,6 | 6,40 | 6,51 | **Preoperative** |
| **18** | 4,62 | 48,5 | 8,12 | 8,13 | **Both** |
| **19** | 3,58 | 15,4 | 7,13 | 5,20 | **Preoperative** |
| **20** | 3,40 | 1,0 | 6,21 | 5,18 | **Both** |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **21** | 3,54 | 10,8 | 5,49 | 6,52 | **Both** | | | |
| **22** | 3,84 | 16,9 | 7,62 | 6,64 | **Both** | | | |
| **23** | 3,56 | 14,4 | 5,90 | 5,48 | **Both** | | | |
| **24** | 3,05 | 1,2 | 5,38 | 4,97 | **Both** | | | |
| **25** | 2,60 | 0,2 | 5,28 | 4,84 | **Both** | | | |
| **26** | 4,45 | 41,6 | 7,79 | 7,48 | **Both** | | | |
| **27** | 3,91 | 23,0 | 7,88 | 7,05 | **Both** | | | |
| **28** | 3,73 | 21,1 | 5,73 | 6,35 | **Both** | | | |
| **29** | 3,48 | 4,6 | 5,89 | 5,51 | **Both** | | | |
| **30** | 3,73 | 16,2 | 5,38 | 5,93 | **Both** | | | |
| **31** | 3,02 | 1,8 | 6,86 | 5,16 | **Both** | | | |
| **32** | 2,71 | 0,4 | 5,88 | 4,73 | **Both** | | | |
| **33** | 3,88 | 26,7 | 7,70 | 6,79 | **Both** | | | |
| **IOE group** | **Preoperative HRV Measures** | | | | **Postoperative HRV Measures** | | | |
| **Subject** | **LnRMSSD** | **pNN50** | **LnLF** | **LnHF** | **LnRMSSD** | **pNN50** | **LnLF** | **LnHF** |
| **I1** | 3,67 | 18,0 | 5,75 | 4,97 | 2,88 | 0,3 | 6,05 | 4,26 |
| **I2** | 3,00 | 0,5 | 5,19 | 5,52 | 3,07 | 3,9 | 6,73 | 4,97 |
| **I3** | 3,42 | 6,7 | 6,10 | 6,05 | 4,10 | 30,9 | 6,79 | 6,13 |
| **I4** | 3,41 | 7,8 | 5,64 | 5,24 | 3,73 | 17,0 | 6,81 | 6,14 |
| **I5** | 4,14 | 44,0 | 6,54 | 7,24 | 2,94 | 3,0 | 4,56 | 3,48 |
| **I6** | 3,89 | 30,5 | 6,63 | 6,68 | 4,02 | 31,0 | 6,69 | 7,16 |
| **I7** | 3,54 | 12,9 | 6,36 | 6,78 | 3,66 | 13,6 | 7,58 | 6,82 |
| **I8** | 3,23 | 1,4 | 5,40 | 5,49 | 3,54 | 5,0 | 6,12 | 6,32 |
| **I9** | 3,44 | 4,2 | 6,31 | 5,04 | 3,87 | 22,2 | 6,50 | 6,06 |
| **I10** | 4,14 | 42,9 | 6,92 | 7,16 | 3,69 | 17,8 | 8,38 | 6,38 |
| **I11** | 3,54 | 7,2 | 6,66 | 5,28 | 2,79 | 0,3 | 6,51 | 4,38 |
| **I12** | 4,08 | 28,1 | 7,92 | 6,94 | 3,85 | 25,7 | 6,22 | 6,90 |
| **I13** | 2,53 | 0,3 | 4,23 | 4,35 | 3,21 | 4,1 | 5,10 | 4,78 |
| **I14** | 3,74 | 20,8 | 6,64 | 5,90 | 3,76 | 15,7 | 7,61 | 6,48 |
| **TLE group** | **Preoperative HRV Measures** | | | | **Postoperative HRV Measures** | | | |
| **Subject** | **LnRMSSD** | **pNN50** | **LnLF** | **LnHF** | **LnRMSSD** | **pNN50** | **LnLF** | **LnHF** |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **T1** | 3,80 | 23,4 | 7,89 | 6,12 | 3,42 | 7,1 | 7,07 | 5,77 |
| **T2** | 2,83 | 0,5 | 5,98 | 4,37 | 3,48 | 6,8 | 7,17 | 5,73 |
| **T3** | 4,33 | 52,4 | 7,32 | 8,10 | 4,12 | 28,5 | 8,79 | 7,60 |
| **T4** | 4,11 | 45,4 | 7,15 | 6,80 | 3,84 | 13,7 | 7,11 | 6,27 |
| **T5** | 3,00 | 1,9 | 5,45 | 4,24 | 2,82 | 0,5 | 3,95 | 3,86 |
| **T6** | 3,17 | 1,4 | 5,90 | 5,09 | 3,15 | 1,9 | 5,86 | 5,07 |
| **T7** | 3,70 | 16,4 | 6,88 | 6,55 | 4,00 | 8,2 | 6,46 | 6,54 |
| **T8** | 4,00 | 31,5 | 7,45 | 6,93 | 3,76 | 17,5 | 6,67 | 6,72 |
| **T9** | 3,50 | 8,1 | 5,78 | 6,13 | 3,82 | 26,2 | 6,08 | 6,86 |
| **T10** | 3,49 | 9,3 | 6,50 | 5,76 | 3,38 | 4,2 | 5,71 | 5,58 |
| **T11** | 3,38 | 6,0 | 6,73 | 6,06 | 2,95 | 0,6 | 5,60 | 5,45 |
| **T12** | 4,23 | 35,0 | 6,77 | 6,17 | 4,77 | 66,4 | 7,41 | 7,16 |
| **T13** | 3,51 | 10,1 | 7,46 | 5,61 | 3,27 | 5,3 | 7,10 | 5,19 |
| **T14** | 3,50 | 9,5 | 6,95 | 5,81 | 2,85 | 0,3 | 6,25 | 4,49 |

**Table D: Correlation between HRV parameters and SUDEP-7 scores**

| IOE group | | | SUDEP-7 | TLE group | | | SUDEP-7 |
|---|---|---|---|---|---|---|---|
| **Spearman's rho** | **lnRMSSD** | Correlation Coefficient | ,591* | **Spearman's rho** | **lnRMSSD** | Correlation Coefficient | -0,401 |
| | | Sig, (2-tailed) | **0,026** | | | Sig, (2-tailed) | 0,155 |
| | | N | 14 | | | N | 14 |
| | **pNN50** | Correlation Coefficient | ,671** | | **pNN50** | Correlation Coefficient | -0,439 |
| | | Sig, (2-tailed) | **0,009** | | | Sig, (2-tailed) | 0,117 |
| | | N | 14 | | | N | 14 |
| | **lnLF** | Correlation Coefficient | 0,256 | | **lnLF** | Correlation Coefficient | -0,347 |
| | | Sig, (2-tailed) | 0,377 | | | Sig, (2-tailed) | 0,224 |
| | | N | 14 | | | N | 14 |
| | **lnHF** | Correlation Coefficient | 0,286 | | **lnHF** | Correlation Coefficient | -0,219 |
| | | Sig, (2-tailed) | 0,322 | | | Sig, (2-tailed) | 0,451 |
| | | N | 14 | | | N | 14 |
| | **SUDEP-7** | Correlation Coefficient | 1,000 | | **SUDEP-7** | Correlation Coefficient | 1,000 |
| | | Sig, (2-tailed) | | | | Sig, (2-tailed) | |
| | | N | 14 | | | N | 14 |

** Correlation is significant at the 0,01 level (2-tailed)
* Correlation is significant at the 0,05 level (2-tailed)

**Table E: Results after correction for age**

**Table E.1: HRV values of IOE, TLE and matched controls after correction for age.**

| HRV parameters | | Preoperative period | | | | Postoperative period | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | IOE group N=14 | TLE group N=14 | Control group N=28 | P value | IOE group N=14 | TLE group N=14 | Control group N=28 | P value |
| **LnRMSSD** | Mean | 3,50 | 3,64 | 3,60 | 0,72 | 3,57 | 3,71 | 3,64 | 0,74 |
| | SD | 0,68 | 0,39 | 0,43 | | 0,62 | 0,42 | 0,38 | |
| **pNN50 (%)** | Mean | 17,27 | 17,66 | 16,28 | 0,95 | 18,25 | 17,03 | 16,94 | 0,95 |
| | SD | 16,26 | 13,48 | 12,92 | | 12,76 | 15,21 | 12,14 | |
| **LnLF** | Mean | 6,15 | 6,74 | 6,48 | 0,21 | 5,96 | 6,10 | 6,49 | 0,15 |
| | SD | 0,92 | 0,69 | 0,92 | | 0,90 | 0,87 | 0,88 | |
| **LnHF** | Mean | 5,90 | 6,01 | 5,99 | 0,95 | 5,80 | 5,99 | 6,02 | 0,79 |
| | SD | 0,93 | 0,95 | 1,08 | | 1,08 | 0,91 | 1,00 | |

**Table E.2: Preoperative and postoperative HRV values of IOE subgroups compared to those of matched controls after correction for age with a threshold of 3.52.**

| HRV parameters | | **Group 1a (n=6)** | | | Control for Group 1a (n=12) | | Control vs Group 1a | | **Group 1b (n=8)** | | | Control for Group 1b (n=16) | | Control vs Group 1b | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Preop | Postop | p | Preop | Postop | Preop P | Postop P | Preop | Postop | p | Preop | Postop | Preop P | Postop P |
| LnRMSSD | Mean | 2,96 | 3,50 | **0,000** | 3,80 | 3,59 | **0,032** | 0,80 | 3,90 | 3,63 | 0,12 | 3,46 | 3,67 | **0,002** | 0,791 |
| | SD | 0,71 | 0,84 | | 0,41 | 0,39 | | | 0,24 | 0,45 | | 0,37 | 0,37 | | |
| pNN50 | Mean | 3,45 | 17,41 | 0,030 | 20,67 | 14,66 | **0,001** | 0,69 | 27,65 | 18,88 | 0,14 | 13,41 | 18,64 | **0,029** | 0,968 |
| | SD | 3,71 | 14,17 | | 12,78 | 9,33 | | | 13,94 | 12,56 | | 12,04 | 13,93 | | |
| LnLF | Mean | 5,41 | 5,31 | 0,708 | 6,77 | 6,39 | **0,006** | **0,01** | 6,71 | 6,45 | 0,36 | 6,20 | 6,59 | 0,106 | 0,753 |
| | SD | 0,77 | 0,59 | | 0,91 | 0,95 | | | 0,57 | 0,79 | | 0,87 | 0,82 | | |
| LnHF | Mean | 5,21 | 5,31 | 0,192 | 6,46 | 6,00 | **0,003** | 0,48 | 6,41 | 5,89 | 0,26 | 5,63 | 6,06 | 0,064 | 0,753 |
| | SD | 0,57 | 0,59 | | 0,92 | 0,96 | | | 0,82 | 1,27 | | 1,09 | 1,02 | | |

** In order to correction for age, the reference value of RMSSD was set to the threshold of 33,8 (LnRMSSD = 3,52) which corresponded to the age of 33,6 ($y= 75,78 – 1,618x + 0,011x^2$). At this age, the reference values for pNN50, LF and HF were 13,43; 129,34 (LnLF=4,86) and 81,26 (LnHF=4,40) respectively.

**Table E.3. HRV values of IOE subgroups using the LnRMSSD threshold of 3.43**

| HRV parameters | | Group 1a (n=5) | | | Control for Group 1a (n=10) | | Control vs Group 1a | | Group 1b (n=9) | | | Control for Group 1b (n=18) | | Control vs Group 1a | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Preop | Postop | p | Preop | Postop | Preop P | Postop P | Preop | Postop | p | Preop | Postop | Preop P | Postop P |
| LnRMSSD | Mean | 3,12 | 3,53 | 0,02 | 3,72 | 3,53 | 0,020 | 1,00 | 3,80 | 3,49 | 0,13 | 3,40 | 3,48 | 0,035 | 0,93 |
| | SD | 0,37 | 0,41 | | 0,45 | 0,62 | | | 0,27 | 0,48 | | 0,65 | 0,56 | | |
| pNN50 | Mean | 3,34 | 12,18 | 0,09 | 19,81 | 16,14 | 0,014 | 0,61 | 23,18 | 14,40 | 0,16 | 14,46 | 14,11 | 0,173 | 0,96 |
| | SD | 3,61 | 11,82 | | 16,84 | 16,92 | | | 14,38 | 11,21 | | 16,30 | 16,13 | | |
| LnLF | Mean | 5,31 | 6,31 | **0,003** | 6,83 | 6,19 | **0,004** | 0,81 | 6,64 | 6,68 | 0,92 | 6,21 | 6,56 | 0,186 | 0,78 |
| | SD | 0,69 | 0,73 | | 0,80 | 1,14 | | | 0,58 | 1,10 | | 1,05 | 0,85 | | |
| LnHF | Mean | 5,33 | 5,67 | 0,27 | 6,32 | 5,90 | 0,027 | 0,67 | 6,22 | 5,77 | 0,36 | 5,66 | 5,88 | 0,442 | 0,84 |
| | SD | 0,62 | 0,73 | | 0,84 | 1,29 | | | 0,93 | 1,36 | | 1,40 | 1,17 | | |