Doctoral thesis

Curriculum Vitae

Stefanie Dedeurwaerdere was born in 1979 in the medieval city of Bruges, Belgium. She moved to Ghent to study biology (M.Sc.) at the University of Ghent. Intrigued by the human brain and more specifically epilepsy research, she then started working at the Department of Neurology (Ghent University Hospital) in the experimental laboratory directed by Professor Paul Boon (Laboratory for Clinical and Experimental Neurophysiology-LCEN). During this period, she obtained a Master in Animal Science and achieved her PhD title on a thesis on "Neuromodulation in experimental animal models of epilepsy" in October 2005. Dr. Dedeurwaerdere has contributed to 13 original articles in international peer-reviewed journals. Several grants and bursaries have been awarded to her research work during her PhD. This allowed Stefanie to contribute to international meetings and to be trained in experimental laboratories in the Netherlands and Canada. Since January 2006, Stefanie is working at the Department of Medicine of the University of Melbourne as a post-doctoral researcher in the group of Professor Terrence O'Brien. Currently, her main topics of interest are imaging (FDG and flumazenil PET, and MRI) as a tool to investigate epilepsy and epilepsy treatments like vagus nerve stimulation in small animal models. Besides research, she enjoys exploring Melbourne and the Australian outdoors.

Acta neurol. belg., 2006, **106**, 91-97

Neuromodulation with levetiracetam and vagus nerve stimulation in experimental animal models of epilepsy

Stefanie Dedeurwaerdere[1,2], Kristl Vonck[1], Veerle De Herdt[1], Liesbeth Waterschoot[1], Tim De Smedt[1], Robrecht Raedt[1], Tine Wyckhuys[1], Benjamin Legros[1], Peter Van Hese[1,3], Koen Van Laere[4], Jean Delbeke[5], Wytse Wadman[1,6] and Paul Boon[1]

[1]Laboratory for Clinical and Experimental Neurophysiology, and Reference Centre for Refractory Epilepsy, Department of Neurology, Ghent University Hospital, Ghent, Belgium ; [2]Department of Medicine, The University of Melbourne, Royal Melbourne Hospital, Parkville, VIC, Australia ; [3]MEDISIP, Department of Electronics and Information Systems, Ghent University, Ghent, Belgium ; [4]Division of Nuclear Medicine, UZ Gasthuisberg, KU Leuven, Belgium ; [5]Neural Rehabilitation Engineering Laboratory, Department of Physiology and Pharmacology, Université Catholique de Louvain, Brussels, Belgium ; [6]Institute of Neurobiology, University of Amsterdam, Amsterdam, The Netherlands

Abstract

Epilepsy is a neurological disorder consisting of recurrent seizures, resulting from excessive, uncontrolled electrical activity in the brain. Epilepsy treatment is successful in the majority of the cases ; however, still one third of the epilepsy patients are refractory to treatment. Besides the ongoing research on the efficacy of anti-epileptic treatments in suppressing seizures (anti-seizure effect), we want to seek for therapies that can lead to plastic, neuromodulatory changes in the epileptic network. Neuropharmacological therapy with levetiracetam (LEV) and vagus nerve stimulation (VNS) are two novel treatments for refractory epilepsy. LEV acts rapidly on seizures in both animal models and humans. In addition, preclinical studies suggest that LEV may have anti-epileptogenic and neuroprotective effects, with the potential to slow or arrest disease progression. VNS as well can have an immediate effect on seizures in epilepsy models and patients with, in addition, a cumulative effect after prolonged treatment. Studies in man are hampered by the heterogeneity of patient populations and the difficulty to study therapy-related effects in a systematic way. Therefore, investigation was performed utilizing two rodent models mimicking epilepsy in humans. Genetic absence epilepsy rats from Strasbourg (GAERS) have inborn absence epilepsy and Fast rats have a genetically determined sensitivity for electrical amygdala kindling, which is an excellent model of temporal lobe epilepsy. Our findings support the hypothesis that treatment with LEV and VNS can be considered as neuromodulatory : changes are induced in central nervous system function or organization as a result of influencing and initiating neurophysiological signals.

Key words : Vagus nerve stimulation ; levetiracetam ; GAERS ; kindling ; positron emission tomography.

Introduction

Epilepsy is the most common serious brain disorder affecting 0.5-1% of the general population (1). Nevertheless there is still a lot of prejudice and misunderstanding about the disease. The word 'epilepsy' derives from the Greek verb *έπιλαμβανειν* (epilambanein), meaning 'to be seized, to be overwhelmed by surprise'. Epileptic seizures are characterized by a paroxysmal manifestation of highly synchronized abnormal neuronal activity of a part of the brain (partial epilepsy) or the whole brain (generalized epilepsy) resulting in various clinical symptoms, which can manifest as motor, sensory, emotional or mixed phenomena possibly with alteration or loss of consciousness. Although 9% of the population experiences a seizure once in a lifetime, epilepsy is only diagnosed when seizures are recurring. The conversion from a normal neuronal network into a hyperexcitable epileptic network is called epileptogenesis, which consists of complex and dynamic processes. Several epilepsy syndromes exist, but they are all characterized by repetitive seizures. There are multiple causes for epilepsy, which can be classified according to its etiology into three categories : idiopathic (primary, without a known cause or with a suggested genetic origin), symptomatic (secondary, resulting from known origins e.g. tumors, lesions, infections, vascular causes) or cryptogenic (presumably symptomatic but currently of unknown specific etiology) (2).

Epilepsy treatment is indicated following two or more unprovoked epileptic seizures and is successful in the majority of the cases. Despite the pharmacological development of new treatments, still one third of the epilepsy patients does not respond sufficiently to anti-epileptic drugs (AED) and are called refractory patients (3). Hence, there is a constant impetus to search for other treatment strategies like epilepsy surgery, gamma knife surgery, ketogenic diet, deep brain stimulation, vagus nerve stimulation and transcranial magnetic stimulation

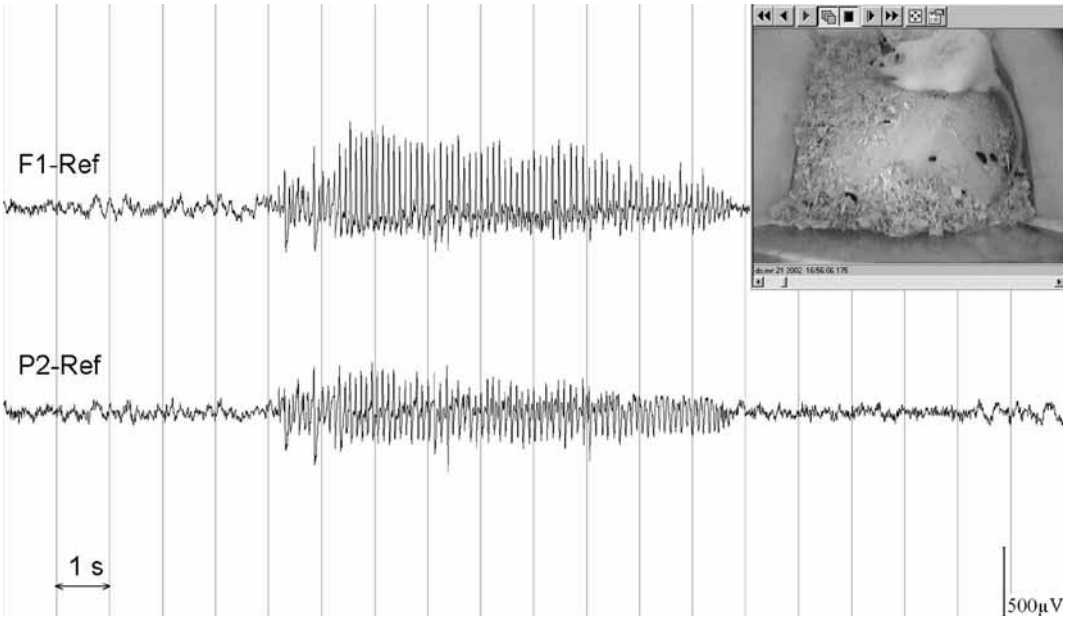

FIG. 1. — Video-EEG monitorig output with bilateral generalized SWDs (7-12 Hz) on the EEG of a GAERS rat. Abbreviations : F1, frontal left ; P2, parietal right ; Ref, reference.

but also the development of new AEDs with novel mechanisms of action. Besides the traditional research on the efficacy of anti-epileptic treatments in suppressing seizures (anti-seizure effect), we want to seek for therapies that can lead to plastic changes in the epileptic network and in this way have a modulating effect.

Neuromodulation as a treatment strategy for epilepsy

Neuromodulation is the science of how electrical, chemical and mechanical interventions can modulate or change central and peripheral nervous system functioning by initiating and influencing neurophysiological signals. Modulatory synapses in the central nervous system transmit information that will have long-lasting effects on the postsynaptic neuron's metabolic activity and on its response to subsequent input. These effects are fundamental to the development and adaptation of the nervous system and are believed to underlie higher functions such as learning and memory. Lately, neuromodulation has been applied to achieve therapeutic effects in several research fields, e.g. heart rate disorders, breathing disorders, movement disorders, pain, incontinence, spasticity, paralysis, depression and epilepsy often by means of implanted devices. The potential of such modulating and maybe even anti-epileptogenic therapies is of great significance. They could slow down or alter processes underlying epilepsy or they might prevent and even cure epilepsy. The current paper describes the modulation capability of levetiracetam and vagus nerve stimulation.

Animal models

Studies in man are hampered by the heterogeneity of patient populations (age, course of the epilepsy, type of epilepsy, AED regime and genetic background) and the difficulty to study therapy-related effects in a systematic way. Therefore, investigation was performed utilizing two rodent models mimicking epilepsy in humans. They are both chronic models with seizures evolving from true, genetically-driven epileptogenesis.

Genetic absence epilepsy rats from Strasbourg (GAERS) have inborn absence epilepsy (idiopathic epilepsy). It has become increasingly obvious during recent decades that genetic factors play a main role in the idiopathic generalized epilepsies, including absence epilepsy. Absence seizures are characterized by paroxysmal unresponsiveness to environmental stimuli and cessation of ongoing activity. In GAERS, absence seizures are associated with the appearance of bilateral synchronous 7-12 Hz spike and wave discharges (SWDs) on the EEG (Fig. 1). They occur mainly during quiet wakefulness, inattention and in the transition between sleep and waking (4, 5). Absence epilepsy has a genetic predisposition without evidence of any structural lesion as its substrate (6). A thalamocortical dysfunction is assumed to play a major role in the underlying pathophysiology (7). The frequent spontaneous seizures, the similarity with human absence epilepsy and the gradual development of epilepsy make this an attractive model to study epileptogenesis and treatments that can interfere with epilepsy. Another strong point is that SWDs appearing on the cortical EEG strictly

correlate with the occurrence of the numerous clinical absences. Therefore, EEG recordings can be used to quantify the appearance of SWDs and absences.

Of the various epilepsies and epilepsy syndromes, the symptomatic epilepsies (acquired) account for approximately 30-49% of the new cases (8). Temporal lobe epilepsy is the most common form of epilepsy in humans (9). An excellent model of temporal lobe epilepsy is the kindling model. Different brain structures (e.g. amygdala, hippocampus, piriform cortex) can be targeted electrically or chemically (e.g. glutamate, kainate). During the kindling process seizure severity and duration gradually progress. Its relatively slow onset due to daily or more frequent brief stimulation, allows detailed study of the events associated with the epileptogenic process. Because of the growing need for an animal model of complex partial seizures based on a genetic predisposition, two new lines of rats have been developed that are kindling-prone (Fast rats) or kindling-resistant (Slow rats) (10). These Fast-kindling and Slow-kindling rats are a parent mixture of two outbred strains that showed strong genetic control in the rate of amygdala kindling, with faster kindling rates in the Fast rats (10). Besides differences in excitability and epileptogenesis, the Fast strain also shows other natural differences with the Slow strain such as behavioral comorbidities including impulsivity, learning impairment, an attention deficit disorder and increased body weight during development (11). Therefore, these Fast rats are of great interest to determine the therapeutic effects of VNS on memory and body weight next to kindling development and seizures.

Levetiracetam

Levetiracetam (LEV) is a new well tolerated AED approved as an adjunctive therapy for epilepsy patients with refractory partial seizures with or without secondary generalization. It is believed to belong to a novel class of AEDs having anti-epileptogenic properties and it was discovered by unconventional drug screening. LEV has a favorable pharmacokinetic profile with rapid absorption following oral administration, excellent bioavailability, quick achievement of steady-state concentrations, linear kinetics and a minimal plasma binding (12). The mechanism of action (MOA) of LEV differs from other AEDs and is as yet not fully elucidated.

Anti-epileptogenic effects of LEV in addition to anti-epileptic effects have been reported in the rat amygdala kindling model for temporal lobe epilepsy (13-15) and the spontaneously epileptic rat (SER), a model of primary generalized epilepsy characterized by spontaneous tonic convulsions and absence seizures (16). LEV suppresses kindling development at doses devoid of adverse effects with persistent reduction in afterdischarge duration after termination of treatment (13). In the SER model study (published around the same period as our study), LEV was administered before the appearance of spontaneous seizures and was terminated at the expected age for seizure expression, which resulted in a lower seizure number in pretreated animals (17).

Administration of LEV strongly suppresses the occurrence of absence seizures in GAERS. In a pilot study in GAERS, the robust anti-seizure effect of LEV was confirmed and a trend towards an anti-epileptogenic effect was found. This encouraged us to further investigate the neuromodulatory properties of LEV in GAERS ; it was felt that investigating the effect of chronic LEV treatment in young GAERS could provide new insights and strategies for the treatment of epilepsy. We investigated the effect of LEV on the age-related development of spike and wave discharges (SWDs) in GAERS by chronic administration of LEV (postnatal day (PN) 23-PN60) starting before the age of occurrence of SWDs. We found that chronic LEV administration induced a reduction in epileptiform events in young GAERS (PN57-PN60) (Fig. 2). This effect persisted to some extent after treatment cessation (PN61-PN64), which might indicate a slowing down of epileptogenic processes (18). Such a long lasting effect has also been confirmed in the SER model (19).

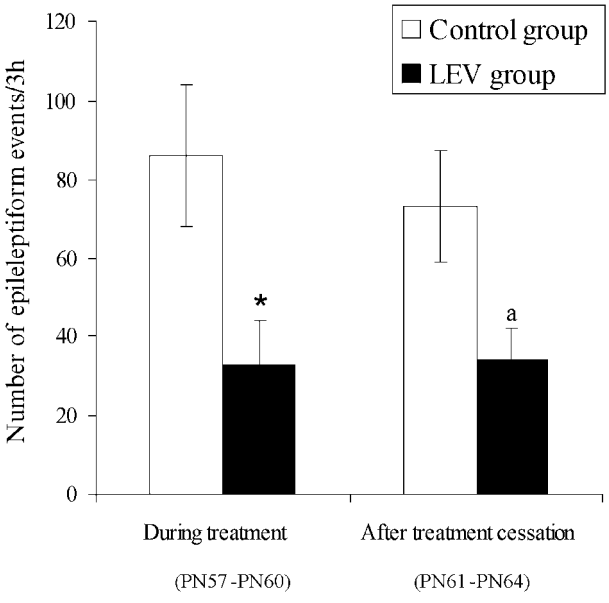

FIG. 2. — Effect of chronic LEV treatment early in life (PN23-PN60) on the development of epileptiform events in young GAERS. EEG was recorded during 3 h from PN57 untill PN64. During PN57-PN60, saline or LEV is administered in respectively the control (white bars) and LEV (black bars) group ; from PN61 treatment was discontinued. Data are presented as mean ± SEM and significance is set at $P < 0.05$. * indicates $P < 0.05$ and ^{a} indicates $P = 0.064$.

However, in our study this effect was only temporary. At the age of four months all animals revealed a similar expression of epileptiform discharges (18). Further studies should determine the optimal time window to interfere more permanently with epileptogenesis in GAERS.

Vagus nerve stimulation

Vagus nerve stimulation (VNS) has been used since 1988 and at present over 35 000 patients are being treated with VNS worldwide. The left vagus nerve is stimulated intermittently by means of a pulse generator to reduce frequency and severity of epileptic seizures. Experience and knowledge about VNS is rapidly increasing, however several questions remain unresolved. VNS is used in generalized and partial epilepsy (20), although responder groups are not clearly identified. Controlled randomized studies showed a 50% decrease in overall seizure frequency in approximately one third of the patients, between 30-50% decrease in seizure frequency in another third of patients and, finally, one third of the patients has less than 30% decrease in seizure frequency and are considered to be non-responders (21). VNS is believed to induce its effect by affecting a large number of intracerebral structures through stimulation of the vagal fibers in the neck (22). The precise MOA by which VNS exerts its anti-epileptic effect has not been elucidated yet. Understanding VNS could improve seizure outcome by identifying specific epilepsy syndromes or types of epilepsy that respond well to VNS or by optimizing stimulation parameters.

Initial animal studies with VNS showed promising results in reducing both ictal and interictal EEG abnormalities (23-28). These findings laid the foundation for further development of VNS as a treatment for human epilepsy. However, VNS efficacy in animals has primarily been assessed in acute models (3-mercaptopropionate, pentylenetetrazole, maximal electroshock, penicillin or strychnine application) utilizing application protocols immediately before and/or after seizure provocation. Only a few studies have assessed the effect of VNS in chronic animal models of epilepsy (23, 29), which is probably related to practical issues of chronic VNS in animals. It is clear that additional research is needed using chronic animal models and using both acute and chronic VNS protocols. Therefore, in our laboratory we have established an electrode-to-vagus nerve interface, implantable in rats, which is suitable for chronic experiments. Compound action potentials of the vagus nerve evoked by this electrode-to-nerve interface have been successfully measured (Fig. 3).

Several functional imaging studies have been conducted to investigate the activation or inhibition of brain structures by VNS. These studies found changes on both sides of the brain by unilateral left

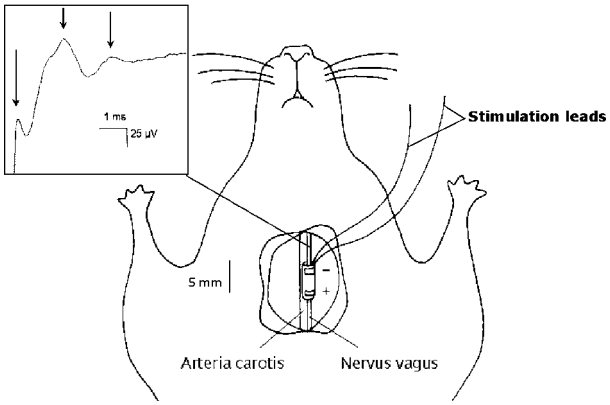

FIG. 3. — Schematic overview of the implanted VNS cuff-electrode and evoked compound action potentials (CAPs) of the nerve. The cuff-electrode is implanted in the cervical region around the left vagus nerve, which is located next to the arteria carotis. The cathode and anode are indicated on the picture as '?' and '+', respectively. The electrode wires are later on subcutaneously tunneled towards the head of the rat. CAPs of A, B and C fibers of the vagus nerve evoked at output current 1000 µA and pulse duration 50 µs using a silicone spiral cuff-electrode. The response of the A fibers appeared first and was partially enclosed by the stimulation artifact. Subsequently, the CAPs of the slower B and C fibers could be measured. Distance between cathode and registration electrode was 3.6 mm.

VNS and pointed out a key role for the thalamus and medial temporal lobe structures in the MOA of VNS (30-32). However, there is no consensus on the type of changes (inhibition or excitation) neither on other potentially activated structures. This discrepancy can be attributed to a number of confounding factors such as the differences between the imaging techniques used (PET, SPECT, fMRI), the contrast agents, scanning protocols, stimulation parameters, medication regimes, course of the illness and treatment response. Heterogeneity of the patient samples is difficult to avoid. In addition, data gathering from healthy subjects is impossible for ethical reasons as VNS is a relatively invasive procedure. Therefore, we explored whether it is feasible to investigate the effect of acute and chronic VNS on brain glucose metabolism in rats using small animal PET (Fig. 4). We showed that acute and chronic VNS induced changes in glucose metabolism in regions important for seizure control such as hippocampus and striatum, respectively (33). Our pilot study demonstrated that small animal PET is a useful and promising technique for imaging cerebral activation in long-term studies in rats.

Fundamental data on the effect of VNS in animal models of idiopathic epilepsy was completely lacking. Information about the potential efficacy of VNS in GAERS, a validated animal model of absence epilepsy, could help to clarify the general principles that underlie VNS, although extensive therapeutic use of VNS in absence epilepsy is

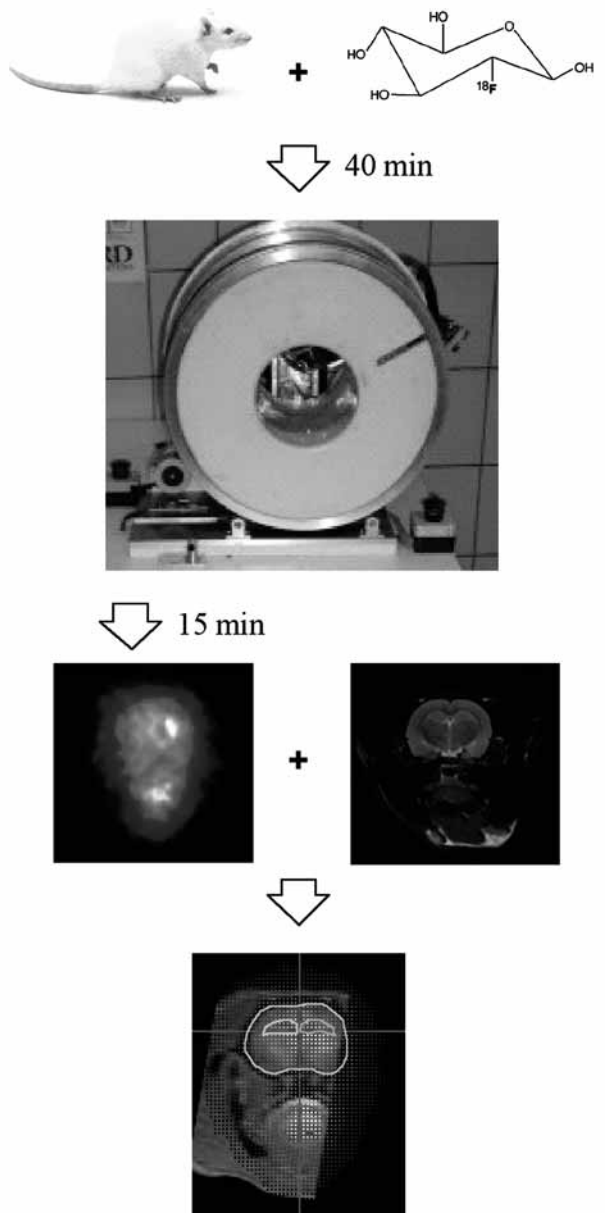

FIG. 4. — Small animal positron emission tomography in the rat. Firstly, the rats were injected intraveneously with 2-deoxy-2[^{18}F]fluoro-D-glucose (FDG), which reflects regional glucose metabolism correlating with the degree of neuronal activity. Thereafter the animals were placed fully awake in a dark, quiet room for 40 min. Subsequently, animals were brought under light anesthesia (medetomidine : 0.1 mg/kg and ketamine : 5 mg/kg, i.p.) to immobilizeated on the PET/MRI co-registration in blue : brain, in yellow : left hippocampus, in green : right hippocampus and in red : tongue.

unlikely because of the high success rate of conventional antiepileptic drug treatment. In this chronic model of spontaneous absence epilepsy, we observed a transient increase in seizure duration following acute VNS, which disappeared in a subacute setting (34). However, when VNS was applied at higher intensities perceptible for the animals, the typical SWDs were shut down immediately (35). When chronic VNS was applied during one week in GAERS, the decrease in SWDs did not significantly differ from the control group (35). It can be hypothesized that a longer period of VNS or earlier intervention during life might be required to affect an already established and genetically driven epilepsy syndrome.

In the genetic seizure-prone kindling-strain (Fast rats), VNS stimulation appeared to be devoid of significant cognitive side effects in the Morris water maze test. However, epileptogenesis was not prevented by two hours of daily VNS (36). Again both excitatory and inhibitory effects of VNS were observed. Seizure profiles in these fully kindled Fast rats were worsened when VNS was applied before the kindling pulse, whereas VNS applied immediately after the kindling pulse could completely prevent stage-5 seizures in a subset of animals. Our findings also demonstrate VNS benefit in only a subpopulation of our Fast rats while other rats appeared relatively unaffected. This shows that this animal model gives an accurate representation of the current human condition wherein VNS is efficacious in only a subpopulation of epilepsy patients as well. In addition.

Finally, VNS reduced body weight after two weeks of treatment in GAERS. In Fast rats, VNS prevented weight gain associated with the kindling process presumably via the observed reduction in food intake (36).

Clearly, the complexities of VNS treatment should be further investigated in order to optimize treatment in patients with refractory epilepsy. Further research directed towards identification of critical criteria that leads to success for VNS application is warranted. GAERS will be treated with VNS over prolonged periods to determine whether a cumulative effect of VNS resulting in increased efficacy can be found. This is a phenomenon well described by several studies in humans (37). Future experimentation will also include an investigation into the effects of repeated daily VNS stimulation in fully kindled rats using various stimulation parameters. Ultimately, this should identify optimal conditions for VNS delivery and should also allow for the selection of VNS-resistant vs. -sensitive rats. Such a categorization may provide information pertaining to the MOA of VNS and thereby lead to the identification of patient responder groups.

Acknowledgements

Stefanie Dedeurwaerdere, Liesbeth Waterschoot, Tim De Smet, Peter Van Hese and Tine Wyckhuys are supported by a grant of Ghent University Research Fund. Kristl Vonck,Veerle De Herdt and Annelies Van Dycke are supported by junior researcher (“Aspirant”) grants from the Fund for Scientific Research-Flanders. Robrecht Raedt is supported by a grant from the Institute for the Promotion of Innovation by Science and Technology (IWT) in Flanders. Paul Boon is a Senior

Clinical Investigator of the Fund for Scientific Research-Flanders and is supported by grants from the Fund for Scientific Research-Flanders (FWO) ; grants from Ghent University Research Fund and by the Clinical Epilepsy Grant from Ghent University Hospital 2000-2008.

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S. DEDEURWAERDERE, M.Sc., M.L.A.S., Ph.D.,
The University of Melbourne,
Department of Medicine,
Royal Melbourne Hospital,
Parkville, VIC 3050,
Australia.
E-mail : Stefanie.Dedeurwaerdere@Hotmail.be