INITIAL DEVELOPMENT OF AN
IMPLANTABLE DRUG DELIVERY DEVICE
FOR THE TREATMENT OF EPILEPSY

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Abstract

Brief abstract
Epilepsy is a chronic neurological condition, characterized by recurrent seizures. Treatment with conventional anti-epileptic drugs (AED) results in only 33% of patients having no seizure recurrence after a prolonged period. Some evidence suggests that the lack of effectiveness of AED penetration into the brain parenchyma could be one of the mechanisms for resistance to treatment. Furthermore, AED side effects often prevent large increases in their dose. It was for these reasons that a new alternative approach to therapy, intracranial implantation of polymer-based drug delivery systems aimed at improving the bioavailability of AED, was investigated in this PhD research project.

Genetic Absent Epilepsy Rats from Strasbourg (GAERS) is a strain of rat that has 100% of the animals with recurrent generalised non-convulsive seizures. This animal model has become the gold standard to study the mechanisms underlying absence epilepsy. After validating an automated spike and wave discharges (SWDs) detection algorithm applied to GAERS’ electroencephalograms (EEG) and demonstrating some effects of enrofloxacin on the GAERS’ epileptic activity, in vitro characterisation and in vivo testing of the antiepileptic efficacy of Poly(D,L-lactide-co-glycolide) (PLGA) polymers loaded with anticonvulsants, either phenytoin or lacosamide, were performed. PLGA is a biodegradable copolymer that it is very well tolerated by the brain and can be used as a passive release substrate.

In prospective randomized masked experiments, GAERS underwent surgery for implantation of skull electrodes, skull electrodes and blank polymers, or skull electrodes and AED loaded polymers. The polymers were implanted bilaterally and subdurally on the surface of the cortex. Electroencephalogram recordings were started at day 7 post-surgery and continued for eight weeks. The number of SWDs and mean duration of one SWD were compared week-by-week between the groups. Although temporary changes were seen, phenytoin loaded PLGA polymer sheets did not decrease seizure activity in GAERS. However lacosamide loaded PLGA polymer sheets affected seizure activity in GAERS by decreasing the mean duration of SWDs for a sustained period of up to 7
weeks. This provided proof of concept that intracranial implantation of polymer-based drug delivery systems could be used in the treatment of epilepsy.

The last chapter of this thesis evaluated the in vivo biocompatibility of Polypyrrole (PPy) implanted subdurally and unilaterally on the surface of the motor cortex in GAERS. Polypyrrole offers the advantage of being electrically conductive which could allow a controlled release of the anti-epileptic medications. Due to the absence of evidence of toxic injury or immune mediated inflammation, it was concluded that PPy offers good histocompatibility with central nervous system cells. Furthermore, the comparison of immunohistochemical scores reveals that the amount of neuronal death and gliosis was significantly less in the PPy side than in the sham surgery side of the cortices (p values of 0.005 and 0.002 respectively) implying that the application of PPy could protect the CNS tissue after surgery.

With improvements in polymer technologies and episodic release offering potentially much longer lasting release durations, intracranial polymer-based drug delivery systems may provide an effective therapeutic strategy for chronic epilepsy. While the development of such an antiepileptic device is worthwhile but still years away, the development of an implantable device combining the potential neuroprotective effect of PPy and an anti-inflammatory drug like dexamethasone would certainly be quicker to develop.
Declaration

I, Sébastien Hyacinthe Bauquier, hereby certified that:

✓ The thesis comprises only my original work towards the PhD except where indicated in the Preface,
✓ Due acknowledgement has been made in the text to all other material used,
✓ The thesis is fewer than 100 000 words in length, exclusive of tables, maps, bibliographies and appendices.

Date: 13th of December 2017

Signature:

[Signature]

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First and foremost I would like to thank Professor Mark Cook for welcoming me into his team of exceptional scientists and engineers and for giving me the opportunity to complete this PhD under his trusting supervision. Mark is a brilliant and supportive supervisor who gave me the freedom to perform various experiments without objections. Mark is a professional role model for me and I am extremely grateful to have had the opportunity to work with him.

I would like to thank my other supervisors, Dr Sam Long for introducing me to Professor Cook and for being at the origin this project, and Professor Ted Whittem for his mentoring. Ted has and continues to provide me with insightful guidance and support throughout my time at the University of Melbourne. Ted’s office and home doors have always been open and Ted has always been very generous with his time.

I would like to thank Professor Andrew Fisher for his role as advisory committee chair. Andrew’s calm and confident attitude and his valuable contributions were very much valued.

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I would like to thank my parents Aline and Gérard for their unconditional love, support and encouragement, and for their sacrifices that allowed me to get where I am today. To my late father who always believed in me when all others were giving up: I miss you!

I would like to thank my wife Jennifer and our two children Julien and Miles; I love you so infinity much! Jenni, you remind me every day what generosity looks like. Petit Bonhomme and Milo, you are so different and still best friends; the world would be a much better place if we could all be as non-judgemental and tolerant of each other’s differences as you are. Please don’t change!
Preface

The format of the thesis is “Thesis with Publications”. The published manuscripts include multiple co-authors. Below are their affiliations and a chapter by chapter short description of the authors’ contributions.

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EVALUATION OF AN AUTOMATED SPIKE AND WAVE COMPLEXES DETECTION ALGORITHM APPLIED TO GAERS EEGS.
All the work described in chapter 2 was performed at Centre for Clinical Neurosciences and Neurological Research (St. Vincent’s Hospital Melbourne, P.O. Box 2900, Fitzroy, Victoria 3065, Australia). The first author contributed 70% of the work. The establishment of the algorithm was mainly the work of the first two authors, and the manual marking of the EEGs was done by both Sebastien Bauquier and Jonathan Jiang.

**List of co-authors for Chapter 3: Chapter with publication**

CLONIC SEIZURES INDUCED BY ORAL ADMINISTRATION OF ENROFLOXACIN IN GAERS

Sebastien H. Bauquier, Jonathan L. Jiang, Alan Lai and Mark J. Cook


All the work described in chapter 3 was performed at Centre for Clinical Neurosciences and Neurological Research (St. Vincent’s Hospital Melbourne, P.O. Box 2900, Fitzroy, Victoria 3065, Australia). The first author contributed 80% of the work.

**Contributions for Chapter 4: Unpublished material not submitted for publication**

ANTI-EPILEPTIC EFFECTS OF PHENYTOIN LOADED POLYMERS IMPLANTED SUB-DURALLY IN GAERS RATS

The work described in chapter 4 was performed at Intelligent Polymer Research Institute and ARC Centre of Excellence for Electromaterials Science (AIIM Facility, Innovation Campus, University of Wollongong, Wollongong, New South Wales 2522, Australia) and at Centre for Clinical Neurosciences and Neurological Research, (St. Vincent’s Hospital Melbourne, P.O. Box 2900, Fitzroy, Victoria 3065, Australia). Sebastien Bauquier contributed 70% of the work. The in vitro experiments were mainly
performed at the University of Wollongong in collaboration with Sebastien Bauquier and the in vivo experiments were mainly performed by Sebastien Bauquier.

This work also required the use of the facilities and the assistance of Mr. Tony Romeo at the University of Wollongong Electron Microscopy Centre.

List of co-authors for Chapter 5: Chapter with publication
ANTI-EPILEPTIC EFFECTS OF LACOSAMIDE LOADED POLYMERS IMPLANTED SUB-DURALLY IN GAERS RATS


The work described in chapter 5 was performed at Intelligent Polymer Research Institute and ARC Centre of Excellence for Electromaterials Science (AIIM Facility, Innovation Campus, University of Wollongong, Wollongong, New South Wales 2522, Australia) and at Centre for Clinical Neurosciences and Neurological Research, (St. Vincent’s Hospital Melbourne, P.O. Box 2900, Fitzroy, Victoria 3065, Australia). The first author contributed 70% of the work. The in vitro experiments were mainly performed by the authors from the University of Wollongong in collaboration with the other authors including the first author and the in vivo experiments were mainly performed by the first author.

The authors also acknowledge the use of the facilities and the assistance of Mr. Tony Romeo at the University of Wollongong Electron Microscopy Centre.

List of co-authors for Chapter 6: Chapter with publication
EVALUATION OF THE BIOCOMPATIBILITY OF PPY ACTIVE RELEASE POLYMERS IMPLANTED SUB-DURALLY IN GAERS
The work described in chapter 6 was performed at Centre for Clinical Neurosciences and Neurological Research, (St. Vincent’s Hospital Melbourne, P.O. Box 2900, Fitzroy, Victoria 3065, Australia) in collaboration with the Intelligent Polymer Research Institute and ARC Centre of Excellence for Electromaterials Science (AIIM Facility, Innovation Campus, University of Wollongong, Wollongong, New South Wales 2522, Australia). The first author contributed 70% of the work. The immuno-histologic preparations were performed in collaboration with Karen McLean and the grading of the immuno-histologic changes was performed by both the first author and Jonathan Jiang.
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Presentations arising from work undertaken as part of this thesis


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<th>Description</th>
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<tbody>
<tr>
<td>α-MSH</td>
<td>Neuropeptide α-melanocyte stimulating hormone</td>
</tr>
<tr>
<td>aCSF</td>
<td>Artificial cerebrospinal fluid</td>
</tr>
<tr>
<td>AED</td>
<td>Antiepileptic drug</td>
</tr>
<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid</td>
</tr>
<tr>
<td>ANG</td>
<td>Angiopep</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood brain barrier</td>
</tr>
<tr>
<td>BCSFB</td>
<td>Blood-CSF barrier</td>
</tr>
<tr>
<td>BID</td>
<td>“bis in die” - Twice a day</td>
</tr>
<tr>
<td>CEC</td>
<td>Triblock copolymers synthesized by non-catalyzed ring opening polymerization of caprolactone in the presence of PEG</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>D</td>
<td>Day</td>
</tr>
<tr>
<td>DAPI</td>
<td>4',6-diamidino-2-phenylindole</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>DPX</td>
<td>Mountant for histology made of distyrene, a plasticizer, and xylene</td>
</tr>
<tr>
<td>DS</td>
<td>Dextran sulfate</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalogram</td>
</tr>
<tr>
<td>EOE</td>
<td>End of experiment</td>
</tr>
<tr>
<td>ERHNPs</td>
<td>Electro-responsive hydrogel nanoparticles</td>
</tr>
<tr>
<td>EVA</td>
<td>Ethylene vinylacetate</td>
</tr>
<tr>
<td>FAD</td>
<td>Fatty acid dimer</td>
</tr>
<tr>
<td>FESEM</td>
<td>Field emission scanning electron microscope</td>
</tr>
<tr>
<td>GoM</td>
<td>Goat anti mouse</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>GAERS</td>
<td>Genetic absence epilepsy rats from Strasbourg</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma-aminobutyric acid</td>
</tr>
<tr>
<td>GFAP</td>
<td>Glial fibrillary acidic protein</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Hematoxylin and Eosin</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HPMC</td>
<td>Hydroxypropyl methylcellulose</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IHC</td>
<td>Immunohistochemical</td>
</tr>
<tr>
<td>IP</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>LPSPs</td>
<td>Lipid protein sugar particles</td>
</tr>
<tr>
<td>MK-801</td>
<td>Methyl-10,11-dihydro-SH-dibenzo[a,d]cyclohepten-5,10-imine maleate</td>
</tr>
<tr>
<td>NA</td>
<td>Not available</td>
</tr>
<tr>
<td>NBF</td>
<td>Neutral buffered formalin</td>
</tr>
<tr>
<td>NeuN</td>
<td>Neuron-specific nuclear protein</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>NPs</td>
<td>Nanoparticles</td>
</tr>
<tr>
<td>NPV</td>
<td>Negative predictive value (NPV)</td>
</tr>
<tr>
<td>PBLG</td>
<td>Polybenzyl L-glutamate</td>
</tr>
<tr>
<td>PCL</td>
<td>Polycaprolactone</td>
</tr>
<tr>
<td>PDLLA</td>
<td>Poly(D,L-Lactide)</td>
</tr>
<tr>
<td>PECA</td>
<td>Polyethylcyanoacrylate</td>
</tr>
<tr>
<td>PEO</td>
<td>Poly(ethylene oxide)</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>PHBV</td>
<td>Poly(3-hydroxybutyrate-co-hydroxyvalerate)</td>
</tr>
<tr>
<td>PHT</td>
<td>Phenytoin</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>PLA</td>
<td>Poly(D,L-lactide)</td>
</tr>
<tr>
<td>PLGA</td>
<td>Poly(D,L-lactide-co-glycolide)</td>
</tr>
<tr>
<td>PNP</td>
<td>N,N-dimethylacrylamide-based pegylated polymeric nanoparticles</td>
</tr>
<tr>
<td>PPV</td>
<td>Positive predictive value</td>
</tr>
<tr>
<td>PPy</td>
<td>Polypyrrole</td>
</tr>
<tr>
<td>PTZ</td>
<td>Pentylenetetrazole</td>
</tr>
<tr>
<td>PVA</td>
<td>Polyvinyl acetate</td>
</tr>
<tr>
<td>PVP</td>
<td>Polyvinyl pyrrolidone</td>
</tr>
<tr>
<td>Py</td>
<td>Pyrrole</td>
</tr>
<tr>
<td>RNS</td>
<td>Responsive neurostimulation</td>
</tr>
<tr>
<td>RT</td>
<td>Room temperature</td>
</tr>
<tr>
<td>SA</td>
<td>Sebacic acid</td>
</tr>
<tr>
<td>SQ</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>STTP</td>
<td>Sodium tripolyphosphate</td>
</tr>
<tr>
<td>SWD</td>
<td>Spike-and-wave discharges</td>
</tr>
<tr>
<td>TRH</td>
<td>Thyrotropin-releasing hormone</td>
</tr>
<tr>
<td>WE</td>
<td>Working electrode</td>
</tr>
<tr>
<td>WK</td>
<td>Week</td>
</tr>
</tbody>
</table>
Chapter 1 – A new approach to the treatment of epilepsy.

1.1. Epilepsy

1.1.1. What is epilepsy?
The international league against epilepsy (ILAE) proposed in 2005 a conceptual definition of epileptic seizures and epilepsy, and more recently in 2014 a practical clinical definition of epilepsy.(Fisher, Vickrey et al. 2000, Fisher, van Emde Boas et al. 2005, Fisher, Acevedo et al. 2014) Following those guidelines, epilepsy is defined as a “disease” of the brain characterised by a predisposition to have “transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain” (i.e. seizures).(Fisher, van Emde Boas et al. 2005, Fisher, Acevedo et al. 2014) To be diagnosed with epilepsy the patient must have had “at least two unprovoked seizures occurring more than 24 hours apart”, or “one unprovoked seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years”, or had a “diagnosis of an epilepsy syndrome”.(Fisher, Acevedo et al. 2014)

The incidence of epilepsy in most developed countries is between 50 and 100 cases per 100,000 population per year and the prevalence is approximately 5 to 8 cases per 1,000. Although it is estimated that up to 5% of a population will experience non-febrile seizures at some point in life.(Shorvon 1996, Sander 2003)

1.1.2. Seizure types and classifications
During an epileptic seizure, the abnormal function of the neuronal cells can affect one limited area of the brain (inducing a partial seizure) or the whole brain (inducing a primarily generalised seizure).

If during a partial seizure the awareness, memory and consciousness of the patient is preserved the seizure is referred as a simple partial seizure, but if the patient cannot recall the event the seizure is referred as a complex partial seizure. The effects of a partial seizure will be influence by the part of the brain that is affected.

A primarily generalised seizure starts with a bilateral spread of electrical discharges followed by generalised movements and unconsciousness. There are three main types of primarily
generalised seizures: 1) tonic-clonic seizures (or grand mal seizure) characterised by tonic stiffening of the patient followed by clonic jerking movements, 2) absence seizures when the patient stays immobile and seems disconnected from its surrounding for a few seconds to a few minutes, and 3) myoclonic seizures characterised by jerking movements of the patient that last only a few seconds.

1.1.3. Pathophysiology of epilepsy
Brain injuries and developmental abnormalities are two main categories of causes of seizures although 50% of seizures remain idiopathic. The causes of seizures are age dependent with for example children being likely to develop seizures due to birth trauma and congenital abnormalities, whereas middle age patients are likely to develop seizures due to alcohol and medications side effects, and elderly patients are likely to develop seizures due to sequels of strokes and brain tumours.

There is a genetic component that is of particular importance for primarily generalised seizures (tonic-clonic, absence and myoclonic seizures). Multiple gene abnormalities may be involved that will affect the excitability of the brain predisposing the patient to for example chemical imbalances resulting in a higher likelihood of seizure occurrence. (Engel and Pedley 2008)

1.1.4. Diagnosis of epilepsy
An anamnesis can provide strong suggestions of an epileptic activity especially when a clear description of the events is coming from an observer. (Malmgren, Reuber et al. 2012, Tolaymat, Nayak et al. 2015) Although not always feasible, performing an inpatient video recorded electroencephalogram (EEG) will allow confirmation of the diagnosis. (Malmgren, Reuber et al. 2012, Tolaymat, Nayak et al. 2015) Finally, magnetic resonance imaging (MRI) is the current gold standard for non-invasive structural evaluation of the brain and is used to diagnose epilepsy in patients with a clear brain lesion (e.g. contusion, tumour, stroke, dysplasia, etc….). (Urbach 2013, Tolaymat, Nayak et al. 2015)
1.2. Conventional treatments of epilepsy

1.2.1. Anti-epileptic drug (AED) therapy

The diversity of the aetiology of epilepsy is largely responsible for the challenge that faces a clinician when prescribing an anti-epileptic drug (AED). A long term AED treatment is usually not prescribed after a single epileptic seizure event but after determination of the epileptic syndrome that the patient may suffer from. The different epileptic syndromes are associated with different treatment options and the choice of the first line AED will be done in consideration of the safety profile of the AED, the co-morbidities that the patient may suffer from and the concomitant medications prescribed to the patient. (Miller and Goodkin 2014) Changing or adding another AED is usually done if the first line AED at high dose does not control seizures or if it induces severe side effects. Due to the poor understanding of the pathophysiology of epilepsy, AEDs aim at abolishing symptoms but very little progress is made in curing the underlying cause of epilepsy. Considering the great range of AEDs commercialised (more than 24 presently), the success rate of AED treatments remains low with only 50% the patients being seizure free after a first line AED and 66% ultimately controlled after changes in the AED therapy. (Miller and Goodkin 2014) Even though the quality of life of patients may have progressed with the apparition of newer AEDs, the percentage of AED-resistant epilepsy has not significantly decreased. (Sankaraneni and Lachhwani 2015)

As for anaesthetic drugs, the mechanism of action of antiepileptic drugs on the CNS remains mostly unknown. There is evidence that certain AEDs interact with certain receptors and based on those interactions some mechanisms of actions are hypothesised. Some AEDs interact with voltage-gated cation channel (Na⁺, Ca²⁺ and/or K⁺) reducing trans-membrane cation gradient and inhibiting the spread of seizure activity within the motor cortex. Some other AEDs enhance the effects of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) and/or weaken the effects of the excitatory neurotransmitter glutamate. Finally, other potential mechanisms of action have been reported such as inhibition of carbonic anhydrase, although its implication in the anti-epileptic action of AED remains controversial. The speculated characteristics and mechanisms of action of common AEDs are presented in table 1.1. (Rho, Donevan et al. 1994, Perucca 1997, Gurbanova, Aker et al. 2006, Taylor,
Angelotti et al. 2007, Chadwick, Shukralla et al. 2009, Hofler and Trinka 2013, Vajda and Eadie 2014, Sankaraneni and Lachhwani 2015, Calandre, Rico-Villademoros et al. 2016, Chong and Lerman 2016, Vickery, Tillery et al. 2017). The spectrum of activity relates to the type of seizure the particular AED has been reported to treat. Broad spectrum AEDs are used for a wide variety of seizures whereas narrow spectrum AEDs are used for specific types of seizures (e.g. partial, focal, or absence, myoclonic seizures).
## Table 1.1 Main Mechanisms of action of common AEDs.

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Generation</th>
<th>Spectrum</th>
<th>Interaction with voltage-gated cation channel reducing transmembrane cation gradient</th>
<th>Enhancement of the effects of the inhibitory neurotransmitter GABA</th>
<th>Inhibition of the effects of the excitatory neurotransmitter Glutamate</th>
<th>Others / Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>First</td>
<td>Narrow</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>Effects on Na⁺ and Ca²⁺ channels are not typical, allosteric modulation of GABA receptor,</td>
</tr>
<tr>
<td>Diazepam</td>
<td>First</td>
<td>Broad</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>Block thalamic &quot;T&quot; type Ca²⁺ channel</td>
</tr>
<tr>
<td>Ethosuximide</td>
<td>First</td>
<td>Specific absence seizure</td>
<td>✔️</td>
<td></td>
<td></td>
<td>Discontinued in several country</td>
</tr>
<tr>
<td>Ezogabine</td>
<td>Second</td>
<td>Narrow</td>
<td>✔️</td>
<td></td>
<td></td>
<td>Activation of GABA receptor, NMDA receptor blockade</td>
</tr>
<tr>
<td>Felbamate</td>
<td>Second</td>
<td>Broad</td>
<td>✔️</td>
<td></td>
<td>✔️</td>
<td>Binds α2-δ subunit of L-type voltage-regulated Ca²⁺ channels</td>
</tr>
<tr>
<td>Gabapentine</td>
<td>Second</td>
<td>Narrow</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>Presynaptic inhibition of neurotransmission (e.g., GLU, ASP)</td>
</tr>
<tr>
<td>Lacosamide</td>
<td>Third</td>
<td>Broad</td>
<td>✔️</td>
<td></td>
<td></td>
<td>Binds to synaptic vesicle protein 2A</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>Second</td>
<td>Specific absence seizure</td>
<td>✔️ ✔️</td>
<td></td>
<td>✔️</td>
<td>Similar mechanism of action than carbamazepine</td>
</tr>
<tr>
<td>Levitracetam</td>
<td>Second</td>
<td>Broad</td>
<td>✔️</td>
<td></td>
<td></td>
<td>NMDA receptor antagonist, AMPA-receptor non-competitive antagonist</td>
</tr>
<tr>
<td>Oxcarbazepine</td>
<td>Second</td>
<td>Narrow</td>
<td>✔️</td>
<td></td>
<td></td>
<td>Prolong the opening of the Chloride anion channel within the GABA receptor</td>
</tr>
<tr>
<td>Perampanel</td>
<td>Third</td>
<td>Narrow</td>
<td>✔️</td>
<td></td>
<td></td>
<td>Bind alpha, delta subunit of L-type voltage-gated Ca²⁺ channels</td>
</tr>
<tr>
<td>Pregabalinine</td>
<td>Second</td>
<td>Narrow</td>
<td>✔️</td>
<td></td>
<td></td>
<td>Inhibition of GABA reuptake from synaptic cleft</td>
</tr>
<tr>
<td>Tiagabine</td>
<td>Narrow</td>
<td></td>
<td>✔️</td>
<td></td>
<td></td>
<td>Activation of GABA receptor, inhibition of AMPA, carbonic anhydrase inhibitor</td>
</tr>
<tr>
<td>Topiramate</td>
<td>Second</td>
<td>Broad</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>Reduced release and/or effects of excitatory amino acids (NMDA?), modulation of dopaminergic and serotoninergic transmission</td>
</tr>
<tr>
<td>Valproic acid</td>
<td>First</td>
<td>Broad</td>
<td>✔️</td>
<td>✔️</td>
<td>✔️</td>
<td>Irreversible inhibition GABA-transaminase</td>
</tr>
<tr>
<td>Vigabatrin</td>
<td>Third</td>
<td>Narrow</td>
<td>✔️</td>
<td></td>
<td></td>
<td>Carbonic anhydrase inhibitor</td>
</tr>
<tr>
<td>Zonisamide</td>
<td>Second</td>
<td>Broad</td>
<td>✔️</td>
<td>✔️</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AMPA, α-amino-3-hydroxy-5-methyl-4-isoaxolepropionic acid; GABA, Gamma-aminobutyric acid; NMDA, N-methyl-D-aspartate.
1.2.2. Epilepsy neurosurgery

When the patient’s epilepsy is refractory to AED therapy, surgical excision of brain tissue responsible for initiation of the seizure sometimes can be achieved. However this procedure can only be performed on selected patients (35%) depending on the size and the location of the epileptic foci. (Kwan and Sperling 2009, de Flon, Kumlien et al. 2010, Miller and Goodkin 2014) The success of inducing long-lasting seizure remission from epilepsy surgery ranges from a low of 25% for patients exhibiting extra-hippocampal seizure origin to 90% in patients with hippocampal sclerosis. (Kwan and Sperling 2009, Miller and Goodkin 2014) Such surgery also comes with potential psychiatric (e.g. changes in cognitive functions), neurologic (e.g. stroke in up to 4% of the patients) and medical complications (e.g. local infection, venous thrombosis or pulmonary embolism in up to 12% of the patients). (Kwan and Sperling 2009, Miller and Goodkin 2014)

1.2.3. Vagus nerve Stimulation (VNS)

When a patient suffers from an AED refractory epilepsy and is not a suitable candidate for epilepsy neurosurgery, vagus nerve stimulation can reduce the incidence of seizures by 50% although very few patients become seizure free after this treatment. (Uthman, Reichl et al. 2004, Ardesch, Buschman et al. 2007, Aihua, Lu et al. 2014, Orosz, McCormick et al. 2014) In consequence it is used as an adjunct therapy to AEDs. Similar to a cardiac pacemaker, a subclavicular implanted pacer sends intermittent electrical impulse to the vagus nerve. The mechanism of action is unclear although vagus nerve stimulations could disrupt several thalamic and brains stem nuclei synaptic transmissions leading to desynchronization of the thalamocortical connections. (Miller and Goodkin 2013) Most common side effects of the treatment include hoarseness and coughing during stimulations. (Uthman, Reichl et al. 2004, Ardesch, Buschman et al. 2007). Also, a long term follow up study has shown that 44% of the patients wished to have the implant disconnected, when the batteries need to be replaced, due to lack of efficacy. (Uthman, Reichl et al. 2004)

1.2.4. Responsive neurostimulation (RNS)

Only approved by the FDA in 2013, responsive neurostimulation devices send imperceptible electrical stimuli to seizure onset areas with abnormal electrographic patterns. As of 2017, only 1000 of those devices were implanted and moderate-to-low-quality evidence supports the efficacy and safety of RNS. (Boon, De Cock et al. 2018)
Figure 1.1 provides a summary of success and failure rates of the conventional anti-epileptic treatments.

Out of 100 patients diagnosed with epilepsy, 50 will have their seizures controlled after a first line anti-epileptic drug (AED) treatment and another 16 after refinement of the AED treatment. Out of the 34 patients that remain epileptic approximately 12 will be eligible to undergo epilepsy surgery with 7 of whom will be treated successfully. Out of the 27 patients that are still AED resistant, non-eligible for surgery or in whom the surgery failed, 3 will be seizure free as a response to the vagal nerve stimulation. In conclusion, out of the 100 patients diagnosed with epilepsy, 24 will remain epileptic after all the conventional treatments.
1.2.5. Consequences of epilepsy on patient life style

1.2.5.1. Severe impact of epilepsy on the patient life style
A study by Fisher et al. (2000) from the Barrow Neurological Institute and the University of Arizona has shown that patients with epilepsy are less educated, more likely to be unemployed and single, and come from lower income households when compared to the US census bureau norms. (Fisher, Vickrey et al. 2000) Those patients also have a great fear of experiencing another seizure and show ongoing psychological problems. Their lifestyle has important limitations such as the inability to drive and limited socialisation. Those patients have effected cognitive functions which decreased their ability to remember and concentrate. (Fisher, Vickrey et al. 2000) They are also afraid of missing medication and of its consequences for their safety and of those around them. (Fisher, Vickrey et al. 2000)

1.2.5.2. Seizures prediction is becoming reality
Being able to predict when a seizure will occur would greatly improve patient safety and would increase their independence. In a recent multicentre study including 15 drug-resistant epileptic patients, Cook et al. (2013) has shown that “intracranial electroencephalography monitoring is feasible in ambulatory patients with drug-resistant epilepsy” and have established proof of concept that seizure prediction is possible, although larger scale studies will be needed to confirm it. (Cook, O’Brien et al. 2013) The prediction of the seizure likelihood could also lead to new therapeutic strategies including acute AED administration when a seizure is likely to occur. Also, delivering the AED directly over its site of action would increase its efficiency when the time window for drug delivery can be as short as a few seconds.

1.3. AED loaded polymer implant as a novel treatment for epilepsy
A review of the literature investigating AEDs administration using polymer-based drugs was published by Halliday et al. (2012). Up to date summaries are presented in table 1.2 and table 1.3 for in vitro and in vivo experiments respectively.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Polymer</th>
<th>Device type</th>
<th>In vitro release time</th>
<th>Release profile</th>
<th>Paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetazolamide</td>
<td>Eudragit</td>
<td>Injectable</td>
<td>10 hours (EOE) however most within 4 hours</td>
<td>Biphasic</td>
<td>(Duarte, Roy et al. 2007)</td>
</tr>
<tr>
<td>Acetazolamide</td>
<td>Eudragit</td>
<td>Injectable</td>
<td>7 hrs (EOE)</td>
<td>Linear</td>
<td>(Haznedar and Dortunc 2004)</td>
</tr>
<tr>
<td>Adenosine</td>
<td>EVA</td>
<td>Implantable</td>
<td>17 days (EOE) however most within 4 days</td>
<td>Triphasic</td>
<td>(Boison, Scheurer et al. 1999)</td>
</tr>
<tr>
<td>Adenosine</td>
<td>Silk</td>
<td>Implantable</td>
<td>14 days (EOE)</td>
<td>Biphasic</td>
<td>(Wilz, Pritchard et al. 2008)</td>
</tr>
<tr>
<td>Adenosine</td>
<td>Silk</td>
<td>Implantable</td>
<td>14 days</td>
<td>Linear</td>
<td>(Pritchard, Szybula et al. 2010)</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Chitosan</td>
<td>Injectable</td>
<td>3 hours (EOE) however most within 2 hours</td>
<td>Linear</td>
<td>(Gavini, Hegge et al. 2006)</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Chitosan, HPMC</td>
<td>Injectable</td>
<td>5 hours (EOE)</td>
<td>Biphasic</td>
<td>(Filipovic-Grcic, Perissutti et al. 2003)</td>
</tr>
<tr>
<td>Carbazamipine</td>
<td>Eudragit, HPMC</td>
<td>Injectable</td>
<td>Between 30 and 60 minutes</td>
<td>Biphasic</td>
<td>(Dong, Maincent et al. 2007)</td>
</tr>
<tr>
<td>Carbazamipine</td>
<td>Precifix</td>
<td>Injectable</td>
<td>4 hours (EOE)</td>
<td>Linear</td>
<td>(Barakat and Raddan 2006)</td>
</tr>
<tr>
<td>Carbazamipine</td>
<td>PVP/PVA</td>
<td>Injectable</td>
<td>1 hr (EOE)</td>
<td>Linear</td>
<td>(Patterson, James et al. 2008)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>PCL/PEG</td>
<td>Injectable</td>
<td>Up to 250 days (EOE)</td>
<td>Biphasic</td>
<td>(Cho, Han et al. 1999)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>PBLG/PEG</td>
<td>Injectable</td>
<td>8 days (EOE)</td>
<td>Linear</td>
<td>(Jeong, Cheon et al. 1998)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>CEC</td>
<td>Injectable</td>
<td>100 hours (EOE)</td>
<td>NA</td>
<td>(Ryu, Jeong et al. 2000)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>PLGA</td>
<td>Injectable</td>
<td>24 hrs (EOE)</td>
<td>Linear</td>
<td>(Montanari, Cilurzo et al. 2001)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>PLGA</td>
<td>Injectable</td>
<td>8 to 9 days (EOE)</td>
<td>Biphasic</td>
<td>(Benelli, Conti et al. 1998)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>PLGA</td>
<td>Injectable</td>
<td>7 days (EOE)</td>
<td>Biphasic</td>
<td>(Nah, Paek et al. 1998)</td>
</tr>
<tr>
<td>CNF</td>
<td>PLGA (microspheres)</td>
<td>Injectable</td>
<td>70 days</td>
<td>Biphasic</td>
<td>(Nkansah, Tzeng et al. 2008)</td>
</tr>
<tr>
<td>CNF</td>
<td>PLGA (nanospheres)</td>
<td>Injectable</td>
<td>Most after 14 days</td>
<td>Triphasic</td>
<td>(Nkansah, Tzeng et al. 2008)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>EVA</td>
<td>Implantable</td>
<td>21 days (EOE)</td>
<td>Linear</td>
<td>(Haik-Creguer, Dumbar et al. 1998)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>PHBV (microspheres)</td>
<td>Injectable</td>
<td>30 days (EOE)</td>
<td>Triphasic</td>
<td>(Chen and Davis 2002)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>(Diazepam/Gelatine) in PHBV microspheres</td>
<td>Injectable</td>
<td>30 days (EOE)</td>
<td>Biphasic</td>
<td>(Chen and Davis 2002)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>PHBV/Gelatine (microcapsules)</td>
<td>Injectable</td>
<td>30 days (EOE)</td>
<td>Linear</td>
<td>(Chen and Davis 2002)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>PLA</td>
<td>Injectable</td>
<td>NA</td>
<td>NA</td>
<td>(Bohrey and McGinity 1987)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>PDLLA</td>
<td>Injectable</td>
<td>8 days, most after 1 day</td>
<td>Biphasic</td>
<td>(Giunchedi, Conti et al. 1998)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>PLGA</td>
<td>Injectable</td>
<td>Most within 10 h</td>
<td>Linear</td>
<td>(Bohrey, Chourasia et al. 2016)</td>
</tr>
<tr>
<td>Ethosuxamide</td>
<td>PECA</td>
<td>Injectable</td>
<td>4 hrs (EOE), most after 3 hours</td>
<td>Linear</td>
<td>(Fresta, Cavallaro et al. 1996)</td>
</tr>
<tr>
<td>Ethosuxamide</td>
<td>PLGA</td>
<td>Injectable</td>
<td>24 hrs (EOE)</td>
<td>Linear</td>
<td>(Montanari, Cilurzo et al. 2001)</td>
</tr>
<tr>
<td>GABA</td>
<td>EVA</td>
<td>Implantable</td>
<td>24 to 72 hours</td>
<td>Biphasic</td>
<td>(Kokaia, Aebischer et al. 1994)</td>
</tr>
<tr>
<td>GABA</td>
<td>PNP</td>
<td>Injectable</td>
<td>6 hrs (EOE)</td>
<td>Linear</td>
<td>(Yurdas Kimlioglu, Menceloglou et al. 2016)</td>
</tr>
<tr>
<td>Gabapentine</td>
<td>Albumine nanoparticles coated with polysorbate</td>
<td>Injectable</td>
<td>24 hours (EOE)</td>
<td>Biphasic</td>
<td>(Wilson, Lavanya et al. 2014)</td>
</tr>
</tbody>
</table>
Table 1.2. Anti-epileptic drugs delivered using polymer-based devices (in vitro experiments) (cont’d).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Polymer</th>
<th>Device type</th>
<th>In vitro release time</th>
<th>Release profile</th>
<th>Paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lacosamide</td>
<td>PLGA</td>
<td>Injectable/implantable</td>
<td>Up to 250 hours (EOE)</td>
<td>NA</td>
<td>(Chen, Gu et al. 2017)</td>
</tr>
<tr>
<td>Levetiracetam</td>
<td>PLGA</td>
<td>Implantable</td>
<td>Most within 3 days</td>
<td>NA</td>
<td>(Halliday, Campbell et al. 2013)</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>PLGA</td>
<td>Injectable</td>
<td>24 hours (EOE)</td>
<td>Biphasic</td>
<td>(Sharma, Maheshwari et al. 2014)</td>
</tr>
<tr>
<td>MK-801</td>
<td>EVA</td>
<td>Implantable</td>
<td>60 days (EOE) however most within 30 days</td>
<td>Biphasic</td>
<td>(Smith, Cordery et al. 1995)</td>
</tr>
<tr>
<td>Muscimol</td>
<td>LPSPs</td>
<td>Injectable</td>
<td>5 days however most within 20 hours</td>
<td>Biphasic</td>
<td>(Kohane, Holmes et al. 2002)</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>EVA</td>
<td>Implantable</td>
<td>Within 24 hours</td>
<td>Biphasic</td>
<td>(Kokaia, Aebischer et al. 1994)</td>
</tr>
<tr>
<td>Oxpabazine</td>
<td>Chitosan</td>
<td>Injectable</td>
<td>NA</td>
<td>NA</td>
<td>(Rane, Mashru et al. 2007)</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>PLGA</td>
<td>Injectable</td>
<td>NA</td>
<td>NA</td>
<td>(Barichello, Morishita et al. 1999)</td>
</tr>
<tr>
<td>Phenobarbitone</td>
<td>PCL</td>
<td>Injectable</td>
<td>NA</td>
<td>NA</td>
<td>(Berrabah, Andre et al. 1994)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>EVA</td>
<td>Implantable</td>
<td>105 days (EOE)</td>
<td>Biphasic</td>
<td>(Tamargo, Rossell et al. 2002)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>PCL</td>
<td>Injectable</td>
<td>22 days (EOE) however mostly within 12 days</td>
<td>Triphasic</td>
<td>(Li, Li et al. 2007)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>PECA</td>
<td>Injectable</td>
<td>4 hrs (EOE)</td>
<td>Biphasic</td>
<td>(Fresta, Cavallaro et al. 1996)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>PCL</td>
<td>Injectable</td>
<td>30 days (EOE) however mostly within 4 days</td>
<td>Triphasic</td>
<td>(Jiang, Yue et al. 2015)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>ANG-ERHNPs</td>
<td>Injectable</td>
<td>24 hours (EOE)</td>
<td>Triphasic</td>
<td>(Yang, Wang et al. 2014)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>ANG-ERHNP</td>
<td>Injectable</td>
<td>4 hours (EOE) (with electrical field stimulation)</td>
<td>NA</td>
<td>(Wang, Ying et al. 2016)</td>
</tr>
<tr>
<td>Pregabalin</td>
<td>Alginate/HPMC</td>
<td>Injectable</td>
<td>12 hours (EOE)</td>
<td>Biphasic</td>
<td>(Madan, Adokar et al. 2015)</td>
</tr>
<tr>
<td>Primidone</td>
<td>PCL</td>
<td>Injectable</td>
<td>10 hours (EOE) however most within 1 hours</td>
<td>Triphasic</td>
<td>(Ferranti, Marchais et al. 1999)</td>
</tr>
<tr>
<td>Valproate</td>
<td>Hexadecanol</td>
<td>Injectable</td>
<td>3 hours</td>
<td>Biphasic</td>
<td>(Giannola, De Caro et al. 1993)</td>
</tr>
<tr>
<td>Valproate</td>
<td>PLGA</td>
<td>Injectable</td>
<td>NA</td>
<td>NA</td>
<td>(Barichello, Morishita et al. 1999)</td>
</tr>
</tbody>
</table>

ANG, Angiopep; CEC, Triblock copolymers synthesized by non-catalyzed ring opening polymerization of caprolactone in the presence of PEG; CNF, Ciliary neurotrophic factor; EOE, End of experiment; ERHNPs, Electro-responsive hydrogel nanoparticles; EVA, Ethylene vinylacetate; GABA, Gamma amino butyric acid; HPMC, Hydroxypropyl methylcellulose; LPSPs, Lipid protein sugar particles; MK-801, Methyl-10,11-dihydro-SH-dibenzo[a,d]cyclohepten-5,10-imine maleate; NA, Not available; PBLG, Polybenzyl l-glutamate; PCL, Polycaprolactone; PDLLA, Poly(D,L-Lactide); PECA, Polyethylcyanoacrylate; PEO, Poly(ethylene oxide); PEG, Polyethylene glycol; PHBV, Poly(3-hydroxybutyrate-co-hydroxyvalerate); PLA, Poly(dl-lactide); PLGA, Poly-lactide-co-glycolide; PNP, dimethylacrylamide-based pegylated polymeric nanoparticles; PVA, Polyvinyl acetate ; PVP, Polyvinyl pyrrolidone ; SA, Sebacic acid.
<table>
<thead>
<tr>
<th>Drug</th>
<th>Polymer</th>
<th>Device type</th>
<th>Animal model</th>
<th>Delivery</th>
<th>In vivo results</th>
<th>Paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine</td>
<td>EVA</td>
<td>Implantable</td>
<td>Kindled rats</td>
<td>Rod, unilateral implantation in ventricle</td>
<td>1 week seizure reduction</td>
<td>(Boison, Scheurer et al. 1999)</td>
</tr>
<tr>
<td>Adenosine</td>
<td>Silk</td>
<td>Implantable</td>
<td>Kindled rats</td>
<td>Rod, unilateral implantation within the infrahippocampal cleft</td>
<td>Dose-dependent delay in kindling acquisition for 11 days, and reduce after discharge duration</td>
<td>(Wilz, Pritchard et al. 2008)</td>
</tr>
<tr>
<td>Adenosine</td>
<td>Silk</td>
<td>Implantable</td>
<td>Kindled rats</td>
<td>Rod, unilateral implantation within the infrahippocampal cleft</td>
<td>Protection from generalized seizures over a period of 10 days of suppressed seizures, delay in epileptogenesis</td>
<td>(Szybala, Pritchard et al. 2009)</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Chitosan</td>
<td>Injectable</td>
<td>Sheep</td>
<td>Powder Intranasal</td>
<td>Three hours increased drug concentration in the serum when compared to the nasal administration of the pure drug</td>
<td>(Gavini, Hegge et al. 2006)</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Eudragit, HPMC</td>
<td>Injectable</td>
<td>Rabbits</td>
<td>Oral (powder in capsules)</td>
<td>Ten hours increased plasma levels</td>
<td>(Dong, Maincent et al. 2007)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>PCL/PEG</td>
<td>Implantable</td>
<td>Rabbits</td>
<td>IP pellet</td>
<td>Constant plasma concentration of clonazepam for 45 days after initial burst effect.</td>
<td>(Cho, Han et al. 1999)</td>
</tr>
<tr>
<td>GABA</td>
<td>EVA</td>
<td>Implantable</td>
<td>Kindled rats</td>
<td>Bilateral implantation into the mesencephalon dorsal to the substanti nigra</td>
<td>At 48 h, generalized convulsions were prevented</td>
<td>(Kokaia, Aebischer et al. 1994)</td>
</tr>
<tr>
<td>GABA</td>
<td>PNP</td>
<td>Injectable</td>
<td>Rats, PTZ-induced acute seizure model</td>
<td>IP nanoparticles</td>
<td>Shorter seizure and decrease in mortality</td>
<td>(Yurttas Kirmililoglu, Menceloglu et al. 2016)</td>
</tr>
<tr>
<td>Gabapentine</td>
<td>Albumin nanoparticles coated with polysorbate</td>
<td>Injectable</td>
<td>Rats, PTZ and electrochock induced convulsion</td>
<td>IP nanoparticles</td>
<td>Increase gabapentin brain concentration, decrease duration of all phases of convulsion</td>
<td>(Wilson, Lavanya et al. 2014)</td>
</tr>
<tr>
<td>Levetiracetam</td>
<td>PLGA</td>
<td>Implantable</td>
<td>Rats, hippocampal tetanus toxin model of mesial temporal lobe epilepsy</td>
<td>Bilateral implantation above motor and somatosensory cortices</td>
<td>Trend towards a reduction in seizure frequency</td>
<td>(Halliday, Campbell et al. 2013)</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>PLGA</td>
<td>Injectable</td>
<td>Rats</td>
<td>Intranasal</td>
<td>Increased brain concentration for up to 8 hours</td>
<td>(Sharma, Maheshwari et al. 2014)</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>PLGA</td>
<td>Injectable</td>
<td>Sheep nasal mucosa</td>
<td>Ex vivo, biphasic controlled release 58% (24 hours)</td>
<td>Ex vivo, biphasic controlled release 58% (24 hours)</td>
<td>(Sharma, Maheshwari et al. 2014)</td>
</tr>
<tr>
<td>MK-801</td>
<td>EVA</td>
<td>Implantable</td>
<td>Unknown</td>
<td>Implanted over brain area for 28 to 65 days followed by in vitro release studies</td>
<td>Still releasing drug after 60 days in vivo (EOE). The in vitro release profiles appears to predict accurately the in vivo release.</td>
<td>(Smith, Cordery et al. 1995)</td>
</tr>
</tbody>
</table>
Table 1.3 Anti-epileptic drugs delivered using polymer-based devices (*in vivo* experiments) (cont’d).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Polymer</th>
<th>Device type</th>
<th>Animal model</th>
<th>Delivery</th>
<th>In vivo results</th>
<th>Paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscimol</td>
<td>LPSPs</td>
<td>Injectable</td>
<td>Rats, hippocampal injection of pilocarpine</td>
<td>Unilateral injection into the hippocampus (i.e. seizure focus)</td>
<td>Prevents seizures formation for up to 120 min (EOE)</td>
<td>(Kohane, Holmes et al. 2002)</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>EVA</td>
<td>Implantable</td>
<td>Rats, interventricular treatment with 6-hydroxydopamine</td>
<td>Bilateral implantation of matrices and electrode in the hippocampus</td>
<td>No change in kindling acquisition</td>
<td>(Kokaia, Aebischer et al. 1994)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>EVA</td>
<td>Implantable</td>
<td>Cobalt-induced rat model of epilepsy</td>
<td>Unilateral implantation in cortical seizure focus</td>
<td>reduction in seizure activity for 6 days (EOE)</td>
<td>(Tamargo, Rossell et al. 2002)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>EVA</td>
<td>Implantable</td>
<td>Rat</td>
<td>Two weeks in vitro release study following 365 days intracortical</td>
<td>NA</td>
<td>(Tamargo, Rossell et al. 2002)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>PCL</td>
<td>Injectable</td>
<td>Rats, hippocampal injection of tetanus toxin</td>
<td>Unilateral implantation into cortical seizure focus</td>
<td>Significant reduction in epileptic events for 3 days with no observed clinical side effects.</td>
<td>(Jiang, Yue et al. 2015)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>ANG-ERHNPs</td>
<td>Injectable</td>
<td>Amygdala-kindled seizures in rats</td>
<td>IP nanoparticles</td>
<td>PHT high concentrations in the hippocampus, amygdale, cerebellum, and brainstem regions, decrease in the kindling-stimulation-induced seizures</td>
<td>(Ying, Wang et al. 2014)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>ANG-ERHNPs</td>
<td>Injectable</td>
<td>Mices, electrical- (maximal electrical shock) and chemical-induced</td>
<td>IP nanoparticles</td>
<td>lowered the effective therapeutic doses of PHT and demonstrated the improved anti-seizure effects</td>
<td>(Wang, Ying et al. 2016)</td>
</tr>
<tr>
<td>Piperine</td>
<td>Chitosan/STPP NPs</td>
<td>Injectable</td>
<td>Mices, PTZ-induced kindling model</td>
<td>IP nanoparticles</td>
<td>Enhance the neuroprotection and ameliorate the astrocytes activation</td>
<td>(Anissian, Ghasemi-Kasman et al. 2017)</td>
</tr>
<tr>
<td>TRH</td>
<td>FAD/SA</td>
<td>Implantable</td>
<td>Kindled rats</td>
<td>Unilateral implantation in amygdala</td>
<td>Reduced seizure duration for 50 days (EOE)</td>
<td>(Kubek, Liang et al. 1998)</td>
</tr>
<tr>
<td>Valproate</td>
<td>PCL</td>
<td>Implantable</td>
<td>Rats, necocortical injected tetanus toxin model</td>
<td>Implanted above the cortical seizure focus</td>
<td>Decrease in epileptiform potential however the model failed to induce consistent seizures.</td>
<td>(Rassner, Hebel et al. 2015)</td>
</tr>
</tbody>
</table>

ANG, Angiopep; EOE, End of experiment; ERHNPs, Electro-responsive hydrogel nanoparticles; EVA, Ethylene vinylacetate; FAD, Fatty acid dimer; GABA, Gamma amino butyric acid; HPMC, Hydroxypropyl methylcellulose; IP, Intraperitoneal; LPSPs, Lipid protein sugar particles; MK-801, Methyl-10,ll-dihydro-SH-dibenzo[a,d]cyclohepten-5,10-imine maleate; NA, Not available; NPs, Nanoparticles; PCL, polycaprolactone; PEG, Polyethyleneglycol; PLGA, Poly-lactide-co-glycolide; PNP, dimethylacrylamide-based pegylated polymeric nanoparticles; PTZ, Pentylenetetrazole; SA, Sebacic acid; STTP, Sodium tripolyphosphate; TRH, Thyrotropin-releasing hormone.
1.3.1. The blood brain barrier, an obstacle for drug delivery to the brain parenchyma

The central nervous system (CNS) is a delicate organ that can only maintain its normal function within a narrow homeostatic range that is achieved through a strict control of all exchanges between the blood and the neural tissue. (Serlin, Shelef et al. 2015) Three key elements play a major role in that control: 1) the blood brain barrier (BBB) with an extremely specific endothelial cells layer, 2) the blood-CSF barrier (BCSFB) with a cerebro-spinal fluid (CSF) secreting choroid plexus epithelium and 3) the blood-subarachnoid CSF barrier (i.e. arachnoid epithelium). (Reese and Karnovsky 1967, Abbott, Ronnback et al. 2006, Serlin, Shelef et al. 2015). Those barriers play an important protective role for the brain but also prevent easy access for drugs targeting the CNS.

The specialised endothelial cells of the BBB have continuous tight junctions, no fenestrae, efflux transporter expression and poor pinocytotic uptake. (Correale and Villa 2009, Abbott, Patabendige et al. 2010, Hartz and Bauer 2011, Daneman 2012, Lajoie and Shusta 2015) To be able to diffuse freely across the BBB, a molecule must have a low molecular weight (<400 Da) and be lipid soluble (forms less than 8 hydrogen bonds). (Pardridge 2012) Larger drug molecules and even smaller ones often do not comply with those characteristic making them unable to reach the brain parenchyma. (Lipinski 2000) However, the drug could cross the BBB through carrier-mediated transport systems (e.g. large neutral amino-acid transporter type 1 in the case of the two following examples) if a particular endogenous substrate has a similar structure to the drug (e.g. Gabapentine) or if the drug can be conjugated with a particular substrate (e.g. with the thiol group of cysteine). (Killian, Hermeling et al. 2007, Pardridge 2012) Larger molecules can also be engineered using a molecular Trojan horse to access receptor mediated transport systems within the BBB. (Lajoie and Shusta 2015) Although feasible for some drug molecules, those alternatives to cross the BBB remain inaccessible for most.
1.3.2. Polymer helping AEDs to cross the blood brain barrier

The encapsulation of some AEDS in biodegradable polymers has also allowed better BBB penetration. Albumin nanoparticles coated with polysorbate 80 and loaded with gabapentin improved gabapentin brain concentration by up to 3 times when compared to the free drug administration, after intraperitoneal (IP) administration.(Wilson, Lavanya et al. 2014) Furthermore, the IP administration of gabapentin encapsulated nanoparticles significantly reduced the duration of all phases of convulsion in a model of Pentylentetrazole (PTZ) induced convulsions.(Wilson, Lavanya et al. 2014) The encapsulation of phenytoin (PHT) in Angiopep-Conjugated Electro-Responsive Hydrogel Nanoparticles (ANG-ERHNP) administered intraperitoneally lowered the effective therapeutic doses of PHT and demonstrated an improved anti-seizure effect when compared with free PHT solution in several models of epilepsy (i.e. amygdala kindling model, maximal electroshock and PTZ induced convulsion models)(Ying, Wang et al. 2014, Wang, Ying et al. 2016). Those nanoparticles are of a particular interest as rapid PHT release could be achieved following the application of an electrical current.(Ying, Wang et al. 2014) Other IP administrations of encapsulated AEDs in nanoparticles (i.e. piperine and GABA) have demonstrated positive results in in vitro release study and/or in in vivo models of epilepsy. (Yurtdas Kirimlioglu, Menceloglu et al. 2016, Anissian, Ghasemi-Kasman et al. 2017)

Polymer based formulation of AEDs for intranasal or oral administration has also been studied. (Gavini, Hegge et al. 2006, Dong, Maincent et al. 2007, Sharma, Maheshwari et al. 2014) Using gamma scintigraphy studies, Sharma et al. (2014) found that the brain/blood concentration ratio of lorazepam was higher when the rats were administered intranasally the Poly(D,L-lactide-co-glycolide (PLGA) loaded with lorazepam nanoparticles compared to the free lorazepam formulation.(Sharma, Maheshwari et al. 2014) Also, the pregabalin oral gel formulation enabled controlled release of the AED for up to 12 hours and the carbamazepine/Eudragit oral formulation increase plasma concentration of carbamazepine for up to 10 hours. (Dong, Maincent et al. 2007, Madan, Adokar et al. 2015)
1.3.3. Polymer allowing AEDs delivery to the brain parenchyma while bypassing the blood brain barrier

If crossing the BBB is proven difficult, there are ways to bypass it. These include 1) administration of AEDs into the CSF and 2) direct topical application of the AEDs onto/into the brain parenchyma. The administration of drugs directly into/onto the brain parenchyma and into the CSF is invasive and cannot be repeated frequently unless a cannula is permanently implanted or a mini-osmotic pump is used. These techniques permanently compromise the isolation of the brain parenchyma, leaving patients prone to infection and injury and are not recommended for use in humans. In consequence, polymer devices that can store and release the drugs over time were implanted onto/into the brain parenchyma and in the ventricles. This approach has shown some successes in the treatment of animal models of several neurological disorders such as Parkinson’s disease, Huntington’s disease and Alzheimer’s disease. (Halliday and Cook 2009) The approach also offers the advantages of achieving high concentration of drug in the brain with minimal systemic toxicity and would eliminate the need of the patient for strict compliance to regular drug intake. However in the field of epilepsy, the report of such techniques remains rare.

1.3.3.1. Implantable polymers delivering AEDs into the cerebrospinal fluid

The administration of drug into the CSF is often as efficient as intravenous drug administration. The BCSFB produce a constant and rapid flow of CSF allowing molecules to be eliminated from the brain and into the blood stream. The result is a poor molecular transport from CSF to brain parenchyma and an efficient molecular transport from CSF to blood. (Pardridge 2012) Nevertheless, Boison et al. (1999) have demonstrated that ethylene vinylacetate (EVA) rods loaded with adenosine implanted in the ventricles of kindled rats induced a 75% reduction of grade 5 seizures over 7 days and a reduction of the amplitude and duration of after-discharges. (Boison, Scheurer et al. 1999)

1.3.3.2. Implantable polymers delivering AEDs into/onto brain parenchyma

Monolithic or implantable polymers (e.g. rods and sheets) are solid devices that can be implanted onto the brain parenchyma or into the brain parenchyma adjacent to deeper seizure focuses. Out of 22 in vivo experiments investigating polymer-based drug delivery devices
For the treatment of epilepsy, 12 used implantable polymers, including the first original study.

To the best of the author’s knowledge, the first prototypes were studies by Kokaia et al. in 1994. In that study, EVA polymer matrices were used to deliver GABA into the mesencephalon of rats subjected to amygdala kindling as a model of epilepsy. (Kokaia, Aebischer et al. 1994) The implantation of the GABA loaded polymer significantly reduced the severity of seizures at 48 h post-surgery when compared to the control polymer. The authors concluded that the GABA loaded polymer was not only inhibiting epileptogenesis but also dampened seizure generalisation in already established kindling epilepsy. The kindled rats model was also used in 3 other publications evaluating the antiepileptic efficacy of implantable polymer loaded with adenosine or thyroid releasing hormone (TRH). In 2 studies by Wiltz et al. (2008) and Szybala et al. (2009), adenosine loaded silk polymers implanted within the infrahippocampal cleft protected rats from general seizure for a period of up to 11 days. (Wilz, Pritchard et al. 2008, Szybala, Pritchard et al. 2009) In the third study, by Kubeck et al. (1998), utilizing a kindling model of temporal lobe epilepsy, biodegradable polyanhydride copolymers loaded with TRH and implanted into the seizure focus (i.e. amygdala) achieved a reduction in clonus duration for a period of 50 to 60 days. (Kubek, Liang et al. 1998)

Finally, Tamargo et al. (2002) demonstrated a reduction in seizure activity for 6 days using EVA polymer loaded with phenytoin in a cobalt-induced rat model of epilepsy. (Tamargo, Rossell et al. 2002)

1.3.3.3. Injectable polymers delivering AEDs into/onto brain parenchyma

Although implantation of monolithic polymers deeper into the brain parenchyma is feasible, there is a significant risk of disrupting superficial layers of the cortex inducing permanent post-implantation neurological deficits. Injectable polymers are particles of different sizes (i.e. nano or micro-particles) that can be reconstituted in solution and injected at a precise location into the brain parenchyma. Injectable polymers allow a controlled delivery of AEDs adjacent to deep seizure focuses that cannot be surgically resected due to multiple reasons. (Halliday, Moulton et al. 2012).
To the best of the author’s knowledge only 2 studies have been published reporting the use of injectable polymers for *in vivo* delivery of AEDs into the brain parenchyma. The first by Kohane *et al.* (2002) studied the antiepileptic effects of muscimol delivered adjacent to the hippocampal seizure focus using lipid protein sugar particles (LPSPs). (Kohane, Holmes *et al.* 2002) Rats were pre-treated with saline loaded LPSPs or muscimol loaded LPSP, and seizures were induced with hippocampal injections of pilocarpine. That study demonstrated that muscimol loaded LPSPs prevented seizure formation during the entire experimentation period of 120 minutes.

A more recent study by Jiang *et al.* (2015) studied the antiepileptic effects of phenytoin loaded polycaprolactone (PCL) microspheres. (Jiang, Yue *et al.* 2015) Phenytoin-PCL or blank-PCL microspheres were injected into the hippocampus of a rat tetanus toxin model of temporal lobe epilepsy. The results demonstrated that phenytoin-PCL microspheres induced a significant reduction in epileptic events for 3 days with no observed clinical side effects.

**1.4. Initial development of an implantable drug delivery device for the treatment of epilepsy**

As previously discussed, to the best of the author’s knowledge there is no report demonstrating that polymer devices loaded with commonly used AEDs and implanted onto or into the brain parenchyma can decrease seizure activity for a sustained period. Most reports of positive effects lasted from a few hours to a few days. The implantation of a polymer-based drug delivery device onto or into the brain parenchyma requires an invasive neurosurgery. The repetition of such a procedure can only exacerbate the associated risks encountered by the patient such as infection, stroke, brain tissue damage and haemorrhage, all of which can endanger the life of the patient or lead to long term neurological deficits. In consequence, the AED/polymer device will not only need to be biocompatible and efficacious but also capable of controlled and long term release of the AED.

The present thesis *aims to report the initial development of polymer based biodegradable implantable drug delivery devices loaded with commonly used AEDs and tested in an animal model of absence epilepsy.*
1.4.1. **Animal model used: GAERS**

Genetic Absent Epilepsy Rats from Strasbourg (GAERS) is a strain of rats that 100% of the animals present with recurrent generalized non-convulsive seizures. (Marescaux, Vergnes et al. 1992) This animal model has become the gold standard to study the mechanisms underlying absence epilepsy. GAERS’ EEGs present typical and very consistent spike and wave discharges (SWDs) of 0.3 to 1 mV in amplitude and 7 to 11 SWDs per second in spike rate. (Marescaux, Vergnes et al. 1992, Danoer, Deransart et al. 1998) Typical absence seizures originate from abnormal thalamocortical networks including the thalamocortical neurons of sensory thalamic nuclei and their main inhibitory input (GABAergic neurons). (Marescaux, Vergnes et al. 1992, Danoer, Deransart et al. 1998) With age the number, frequency and duration of SWDs increase, to achieve maximum number at the age of 6 months and full maturity in frequency and duration at the age of 18 months. (Marescaux, Vergnes et al. 1992)

1.4.2. **Antiepileptic drugs used: Phenytoin and Lacosamide**

Phenytoin anticonvulsant properties were described for the first time by Putnam and Merritt in animals in 1937 and a year later in human patients. (Putnam and Merritt 1937, Merritt and Putnam 1984) After eighty years, the antiepileptic mechanism of action of phenytoin remains a mystery although phenytoin can be classified as a sodium and calcium channel blocker, reducing membrane cations’ gradient and inhibiting the spread of seizure activity within its supposedly primary site of action, the motor cortex. (Keppel Hesselink and Kopsky 2017) The fact that the structure of phenytoin has two phenyl rings at the C5 position of an hydantoin molecule seems responsible for phenytoin’s anticonvulsant properties without inducing sedation. (Keppel Hesselink and Kopsky 2017) The solubility of phenytoin (71 mg/L in aqueous solution) suggests that the elution of the phenytoin from the PLGA polymer should be slow, prolonging the release of the AED from the polymer. Phenytoin may be an “old “drug but it remains as efficacious as new generation AEDs. (Kwan and Brodie 2000, Kellinghaus, Berning et al. 2014) Phenytoin has a narrow epileptic spectrum and has been shown to aggravate absence epilepsy when administered systemically. Nevertheless, it decreases or abolishes brain epileptic activity in two rats model (including GAERS) when administered directly onto the cortex. (Gurbanova, Aker et al. 2006)
Lacosamide is a third generation broad spectrum AED that was only approved in 2008 in the European Union and USA, and in 2009 in Australia. It was first administered as an adjunct AED in patient with partial–onset seizure however because of its availability as an intravenous formulation its use for the treatment of status epilepticus is increasing. (Bauer, Willems et al. 2017) Studies report the rate of successful treatment of status epilepticus is up to 100 % in adults treated with lacosamide. (Hofler, Unterberger et al. 2011) In a study comparing lacosamide and phenytoin, both drugs had similar success rates (33% and 40% respectively) in treating status epilepticus after failure of benzodiazepines and levetiracetam. (Kellinghaus, Berning et al. 2014) However lacosamide was associated with significantly fewer side effects.

Lacosamide is a functionalised amino acid that stabilizes neuronal membranes through the enhancement of slow inactivation of voltage-gated sodium channel. (Hofler and Trinka 2013, Bauer, Willems et al. 2017) The solubility of lacosamide (465 mg/L in aqueous solution) is superior to that of phenytoin which could potentially contribute to a relatively faster elution of lacosamide from the polymers.

1.4.3. Polymers used: PLGA and PPy
The first polymers to be studied for AEDs delivery (i.e. EVA) were non-biodegradable. The intra-cranial implantation of non-biodegradable polymer may require the removal of the polymer, submitting the patient to another neurosurgery and its associated risks. Newer biodegradable polymers that offer good brain biocompatibility and that are intended for intracranial implantation are often made of PLGA, PCL, or polylactic acid (PLA) in the form of implantable sheets or injectable microspheres. (Halliday and Cook 2009, Halliday, Moulton et al. 2012, Halliday, Campbell et al. 2013, Jiang, Yue et al. 2015, Rassner, Hebel et al. 2015) In the present thesis two kinds of biodegradable polymer will be used: 1) PLGA polymer sheets used as devices that continuously deliver AED and 2) Polypyrrole (PPy) polymer sheets used as devices that can be triggered to deliver AED.

1.4.3.1. PLGA polymer sheets
PLGA copolymers (i.e. lactide and glycolide) are physically strong, biocompatible and biodegradable. The degradation products of PLGA are glycolic acid and lactic acid, both of which can be eliminated from the body as carbon dioxide and water. (Bohrey, Chourasiya et al. 2016) PLGA polymers have been extensively used and are approved by the United States
Drug Administration. (Halliday and Cook 2009, Halliday, Moulton et al. 2012, Bohrey, Chourasiya et al. 2016, Wang, Qu et al. 2017) The structure of the polymer includes a hydrophilic surface and a hydrophobic core enabling solubilization in aqueous solution and drug encapsulation that is later released in a continuous way. The rate of the drug delivery can be altered by modifying the drug concentration and the ratio of lactide to glycolide. (Makadia and Siegel 2011)

The PLGA sheets were produced using a coaxial electrospinning method (see Chapter 4 and 5 of the present thesis for detail descriptions). Briefly, electrospinning is a relatively simple and versatile fibre production method consisting of drawing a polymer through a needle onto a collecting plate using electrostatic forces. (Halliday, Moulton et al. 2012) In a coaxial electrospinning method an outer fluid is drawn with an inner fluid simultaneously (Figure 1.2 A) In our case, the result is a three dimensional porous sheets made of randomly oriented fibres (Figure 1.2 B).

![Diagram of coaxial electrospinning process](image)

Figure 1.2 Poly-lactide-co-glycolide polymer sheets produced by coaxial electrospinning method
(A) Illustration of the co-axial electrospinning process. (B) Scanning electron micrographs of PLGA coaxially electrospun polymer mats. Scale bar represents 10 µm.

1.4.3.2. PPy polymer sheets
There are more than 25 PPy based conducting polymers used in direct contact with biological tissues reported in the literature and they all have a conjugated structure of alternating
carbon–carbon double bonds (figure 1.3). (Ateh, Navsaria et al. 2006) Upon intermittent application of an electrical current, the ionic and hydrophobic properties of these polymers vary resulting in actuation of the polymers. (Wallace 2009) In cases where drugs are impregnated within the polymers, the expansion and/or contraction of the structure may be responsible for some drug release. (Hutchison, Lewis et al. 2000, Wallace and Wallace 2003) The mechanism of drug release has been reported for a wide range of incorporated drugs such as dexamethasone, fos-phenytoin and the anti-inflammatory neuropeptide α-melanocyte stimulating hormone (α-MSH). (Zhong and Bellamkonda 2005, Moulton, Imisides et al. 2008, Muller, Yue et al. 2016)

Synchronizing the AED release from a polymer with a seizure advisory system could be technically feasible when using a conductive polymer such as PPy. PPy sheets have been shown in vitro to be well tolerated by biological tissues, including brain tissues, and they seem to be as compatible as Teflon® when implanted into the cerebral cortex. (George, Lyckman et al. 2005, Ateh, Navsaria et al. 2006) Nevertheless in vivo biocompatibility needs first to be proven. The PPy sheets were produced using an electro-polymerization method (Figure 1.3). The pyrrole is oxidized before being polymerized. The PPy polymer is then peeled off the anode. Details of the method used are provided in chapter 6.

![Pyrrole and Polypyrrole](image)

Figure 1.3 Polypyrrole polymer sheets produced by electro-polymerization method

**1.4.4. Proposed steps for the initial development of an implantable drug delivery device for the treatment of epilepsy**
The following steps are proposed in this research project to investigate the application of polymers as an implantable drug delivery device for the treatment of epileptic seizures.

1) Establishment and evaluation of an automated spike and wave complexes detection algorithm applied to GAERS EEGs: EEG analysis is time consuming and subjective and the goal of the study described in chapter 2 is to develop a time efficient and accurate method for analysing days of EEG recordings.

2) Influence of enrofloxacin on GAERS epileptic activity: Craniotomies are invasive surgeries that require supportive treatment such as prophylactic antibiotic administration and the goal of chapter 3 is to evaluate whether enrofloxacin affects the GAERS epileptic activity as it has been reported in other species.

3) Testing of the anti-epileptic effects of phenytoin loaded PLGA polymers implanted sub-durally in GAERS.

4) Testing of the anti-epileptic effects of lacosamide loaded PLGA polymers implanted sub-durally in GAERS.

5) Evaluation of the in vivo biocompatibility of PPy polymers implanted sub-durally in GAERS.
Chapter 2 - Evaluation of an automated spike and wave complexes detection algorithm applied to GAERS EEGs

2.1 Outline

The assessment of the anti-epileptic efficacy of novel treatments in animal model relies on the evaluation of typically many hours of electroencephalogram (EEG) recordings. Manual evaluation of those recordings is time consuming and is very subjective while the development of automatic seizures detection methods can make those analyses quicker and more reproducible. The aim of the study presented in chapter 2 was to develop and evaluate an automated SWD algorithm applied to GAERS’ EEGs.

The algorithm used to automatically mark the onset and termination of SWDs was implemented using MATLAB® (The MathWorks, Inc., Natick, Massachusetts, USA) and was based on the well-defined, consistent and described morphology of the GAERS’SWD. (Marescaux, Vergnes et al. 1992, Rudolf, Bihoreau et al. 2004) A previously published rigorous assessment method of performance was used. (Navakatikyan, Colditz et al. 2006) A total of 6 hours and 20 minutes of EEG recording were analysed and by comparing manual (reference) versus automatic (test) markings, the study demonstrated that the tested algorithm sensitivity, specificity, positive predictive value and negative predictive value were all over 94%, which is comparable to published methodologies based on analysing changes in the EEG in the frequency domain. (Sitnikova, Hramov et al. 2009, Van Hese, Martens et al. 2009, Ovchinnikov, Luttjohann et al. 2010) The algorithm described in this chapter analyses the changes in the EEG in the time domain mimicking manual marking while decreasing subjectivity. In consequence, it is a good performing alternative to scientists and researchers without engineering background.

The use of this algorithm allowed substantial time savings and improved reproducibility when performing EEG analysis during the in vivo experiments of the following chapters.

2.2 Manuscript

This chapter is presented as the author-accepted version of a peer-reviewed manuscript published in Neuroscience Bulletin: Neurosci Bull. 2015 Oct;31(5):601-10. The figures
and tables’ numbers and titles of the author-accepted version of the manuscript may have been changed to follow the formatting of the thesis.
Evaluation of an automated spike-and-wave complex detection algorithm in the EEG from a rat model of absence epilepsy

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Abstract

The aim of this prospective blinded study was to evaluate an automated algorithm for spike-and-wave discharge (SWD) detection applied to EEGs from genetic absence epilepsy rats from Strasbourg (GAERS). Five GAERS underwent four sessions of 20-min EEG recording. Each EEG was manually analyzed for SWDs longer than one second by two investigators and automatically using an algorithm developed in MATLAB®. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for the manual (reference) versus the automatic (test) methods. The results showed that the algorithm had specificity, sensitivity, PPV and NPV >94%, comparable to published methods that are based on analyzing EEG changes in the frequency domain. This provides a good alternative as a method designed to mimic human manual marking in the time domain.

Keywords: GAERS; seizure detection; epilepsy.

Introduction

Epilepsy is a chronic neurological condition characterized by recurrent seizures. Its incidence in most developed countries is between 50 and 100 cases per 100 000 per year⁴⁵. As treatment with conventional anti-epileptic drugs provides adequate seizure control in only 2/3 of patients, more translational research in this field is urgently needed⁴⁵. The efficacy
assessment of novel treatments in animal models typically relies on the evaluation of many hours of electroencephalogram (EEG) recordings. Manual evaluation of these recordings is time-consuming and very subjective, while the development of automatic seizure-detection methods can make these analyses quicker and more reproducible.

Genetic absence epilepsy rats from Strasbourg (GAERS) is a strain in which 100% of the animals exhibit recurrent generalized non-convulsive seizures[3]; and it has become the gold standard for studying the mechanisms of absence epilepsy This model is characterized by the development of well-defined and consistent spike-and-wave discharges (SWDs) even though their duration and numbers vary between colonies[3, 4]. Spontaneous SWDs (7–11 Hz, 330-1 000 μV, 0.5–75 s) start and end abruptly in a normal background EEG[3].

The algorithm to automatically mark the onset and termination of SWDs was implemented using MATLAB® and Signal Processing Toolbox™ R2010b (The MathWorks, Inc., Natick, Massachusetts, USA). Many groups have published methods that automatically detect seizures, but they are predominantly designed for studies in humans[5-9]. The morphology of SWDs in GAERS is noticeably more consistent than seizures in humans, so we hypothesized that an algorithm based on the definition of SWD (referred to as the ‘SWD detection algorithm’) would mark the onset and termination of SWD accurately.

The aim of the present study was to determine whether the SWD detection algorithm can successfully replace the manual marking of EEG recording with high accuracy. A time-domain-based assessment of algorithm performance was used to compare the SWD detection algorithm with human markers[10].

MATERIALS AND METHODS

The study was designed as a prospective controlled masked experiment. All experiments were approved by St Vincent’s Hospital (Melbourne) Animal Ethics Committee and conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004).
Animals

Six-month-old female GAERS, obtained from the University of Melbourne (Parkville, Victoria, Australia), were used in experiments on novel techniques for the delivery of anti-epileptic drugs. The first five animals randomly allocated to the control group in that study were also used in the present study. These animals underwent surgery for the implantation of EEG recording electrodes and their epileptic activity was evaluated for 8 weeks. They were housed individually under a 12-h light/dark regime with ad libitum access to food and water.

Recording Electrode Implantation

Immediately before surgery, the rats were weighed and anaesthetized with an intraperitoneal injection of ketamine (75 mg/kg) and xylazine (10 mg/kg). Following the induction of anesthesia, each rat was placed in a stereotaxic apparatus and given isoflurane (0.5 to 1% in oxygen, 1 L/min) via a nose-cone, along with subcutaneous carprofen (5 mg/kg) for pain relief and 0.9% sodium chloride (2 mL) for cardiovascular support. Then the EEG recording electrodes were implanted as follows: after preparation of the scalp, a single incision was made down the midline, the skull cleared of tissue, and the exposed bone dried with 3% hydrogen peroxide. Four extradural electrodes, consisting of small jeweler’s screws, were implanted cranial to the interaural line (two on each side of the sagittal suture) and one caudal to the interaural line to the right of the sagittal suture (Fig. 2.1A). The electrodes were then connected to an adaptor secured with dental cement. The skin was sutured leaving only part of the dental cement exposed and each animal was placed on a heating pad for recovery. Post-operative treatment included subcutaneous buprenorphine (0.03 mg/kg, twice/day), saline (2 mL, once/day) and carprofen (5 mg/kg, once/day) for up to 3 days.

Electroencephalographic Recording

Beginning on day 7 or 8 after surgery, rats were monitored for at least 60 min (30 min for recovery from anesthesia/acclimation and 30 min for EEG recording), on at least 2 days per week for the following 7 weeks. For the purpose of developing of the semi-automatic detection algorithm, only the first two and the last two recordings were analyzed (Fig. 2.1C) and only the first 20 min of each recording were studied. At each monitoring session, rats were briefly anesthetized with isoflurane (4% in oxygen, 2 L/min) in an induction cage, and

- 27 -
shielded cables were used to connect the recording electrodes to the EEG acquisition system (TDT processors; Tucker-Davis Technologies, Alachua, FL) and high-impedance head-stages driven by custom-designed software. The EEGs were sampled at 3051.76 Hz.

![Diagram](image)

**Figure 2.1 Experimental protocol**

(A) Schematic diagram illustrating the positioning of epidural recording screw electrodes (1-4) and the reference electrode (R). (B) Comparisons were made between the manual EEG recording evaluations of the two researchers, and between the manual evaluations and the semi-automated SWD detection algorithm. (C) Experimental design: 20-min EEG recording began at day 7 or 8 after surgery and 2 days per week for the following 7 weeks; for the purpose of developing of the semi-automatic detection algorithm only the first two and the last two recordings were analyzed.

The rats were allowed to fully recover from anesthesia before recording. During the recording sessions, their behavior was observed and the EEGs were visualized using a custom-designed MATLAB® program. If the rats were perceived as being asleep and after confirmation of no seizure activity on the EEG, noise stimuli of 94 and 98 dB were delivered
At the end of each recording session, the rats were briefly anaesthetized with isoflurane (4% in oxygen, 2 L/min) for disconnection from the shielded cable.

Figure 2.2 Example of rats EEGs when active, sleeping or seizing.

During a recording session, the EEGs were visualized using a custom-designed MATLAB® program. The visualization of the EEGs allowed confirmation of the status of the rat: active (A), sleeping (B), or seizing (C). If the rats were perceived as being asleep and seizure activity on the EEG was confirmed, a noise stimulus of 94 to 98dB was applied by knocking on the Plexiglas cage. The three examples were recorded at the same scale. Calibration 1 s, 1 mV.

**Evaluation of EEG Recordings**

**Preparation of raw data** The EEG data were transcribed using a graphical interface developed in MATLAB®. They were first band-pass filtered between 3 and 30 Hz using a second-order Butterworth filter, and then filtered in both the forward and reverse directions to
avoid introducing a delay in the signal, which effectively doubled the filter order to fourth order. During the second step, all recorded EEG channels were displayed for the observer to select the one with the best signal for SWD detection (i.e., largest SWD amplitude relative to background EEG amplitude) and without signal dropout (i.e., poor physical connection resulting in occasional flat lines) (Fig. 2.3A). The selected channel was used for manual and semi-automated marking.

**Manual analysis of EEG data** EEG recordings were independently analyzed manually by two experienced researchers. While reviewing the recorded EEG, the researcher marked the beginning and the end of all identified SWDs that were more than one second in duration (Fig. 2.3 B) and an Excel data sheet reporting the start and end times of all the SWDs was generated. After completing the manual marking, each researcher applied the semi-automated SWD detection algorithm to the selected channel of EEG recording.

**Semi-automated SWD detection algorithm** The semi-automated SWD detection algorithm was developed within the same graphical interface as that noted above. This algorithm required human intervention to select the best EEG channel and to set an amplitude threshold for SWD detection. In the latter step, non-parametric thresholds were generated by evaluating the empirical cumulative distribution function of the voltage magnitude (from 90% to 100% at 0.5% intervals) and were plotted against the filtered EEG data, so that the observer could select, by visual inspection, a percentile threshold that would best differentiate the SWD activity from the background EEG (Fig. 2.3).
Figure 2.3 Selection the EEG channel and determination of amplitude threshold for SWD detection

EEG analysis using a semi-automated SWD detection requiring human intervention to select the best EEG channel/electrode and set an amplitude threshold for SWD detection. (A) Example of EEG recording: in this example, channel four was selected by the investigator as having the best signal-to-noise ratio, so subsequent analysis was done using this channel. (B) Example of manual marking: the EEG was analyzed using a 10-s window and the start and end of the SWD were noted. (C) Example of threshold selection: the traces show the EEG of the selected channel (channel 4 in this example) with superimposed automatically-calculated thresholds. To be selected, the threshold red line had to be just above the baseline EEG signal. In this example a threshold of 0.71 mV was selected (middle panel).

Once the threshold was selected, the commands of the program were to: (1) construct a binary array corresponding to whether the filtered EEG amplitude was above the user-set threshold (i.e., 0 for below and 1 for above); (2) evaluate the derivative of the binary array to identify spike rises and spike falls; (3) iterate through the derivative array, data-point by data-point, and alternatively search for SWD onsets and terminations; and (4) when the end of the
data set was reached, to evaluate the number of SWDs (number of SWD onsets) and their durations (time between onset and termination) (Fig. 2.4).

Figure 2.4 Flowchart of the semi-automated algorithm
The first sample of the 1-s observation window was classified as a SWD onset if it met the following criteria (Fig. 2.5): (1) the first data point of the window corresponded to a spike rise; (2) the number of spike rises within the window was between 5 and 13; and (3) the interspike intervals were between 40 and 300 ms. The first data point of the 1-s observation window was classified as a SWD termination if it met the following criteria (Fig. 2.5): (1) the first sample of the window corresponded to a spike fall; and (2) apart from the first sample, all other samples in the window were below the threshold amplitude. So, a SWD event had to be at least 1 s in duration and two SWD events were considered separate if they were >1 s apart.

Figure 2.5 The process of automatic marking.

Horizontal red line, the threshold level. The left green arrow indicates the first 1-s observation window classified as a SWD onset (yellow crosses represent spike rises going above the threshold level), and the right green arrow indicates the first 1-s observation window classified as a SWD termination (yellow cross represents a spike fall). See text for detailed criteria of classification.

Data Analysis and Performance Metrics
Comparisons were made between the manual evaluations of EEG recordings by the researchers and evaluation by the semi-automated SWD detection algorithm. A previously-published, rigorous method of assessing performance was used (Fig. 2.6), where only the time intersection of an automatically detected event with one that was manually marked as a positive SWD was considered a true positive time window\cite{10}. Likewise, only when the automatic time intersection of the absence of an event coincided with a manually marked negative SWD was considered a true negative time window. When a time window was evaluated to be SWD-positive by the detection algorithm, but not by the reviewer, it was considered to be a false-positive. When the detection algorithm evaluated a time window as SWD-negative, but the reviewer evaluated it as SWD-positive, it was considered to be a false-negative. Then, the performance metrics of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated using equations 1 to 4. The performance metrics were assessed to a precision of 0.1 s.

\[
\text{Sensitivity} = \frac{\text{TruePositive}}{\text{TruePositive} + \text{FalseNegative}} \quad (1)
\]

\[
\text{Specificity} = \frac{\text{TrueNegative}}{\text{TrueNegative} + \text{FalsePositive}} \quad (2)
\]

\[
\text{PositivePredictiveValue} = \frac{\text{TruePositive}}{\text{TruePositive} + \text{FalsePositive}} \quad (3)
\]

\[
\text{NegativePredictiveValue} = \frac{\text{TrueNegative}}{\text{TrueNegative} + \text{FalseNegative}} \quad (4)
\]
Figure 2.6 Performance metrics

SWD negative, EEG not showing spike-and-wave discharges (SWDs) as evaluated by the researcher (human) or the algorithm (auto); SWD positive, EEG showing SWDs as evaluated by the researcher (human) and the algorithm (auto); true negative, neither human nor auto detected SWDs; false negative, human-detected SWDs whereas auto did not; true positive, both human and auto detected SWDs; false positive, human did not detect SWDs whereas auto did.

Comparisons were also made between the two researchers using the same approach (with one being considered the detection algorithm and the other as the reviewer). In total, three comparisons were made for each EEG recording (Fig. 2.1B).

Data were analyzed with Microsoft Excel 2010 and graphic representations generated with GraphPad Prism 6. The computer used to run the detection algorithm was a desktop PC (twelve Intel® Xenon® CPUs X560 at 3.47 GHz) running Windows 7, 64-bit).
RESULTS

The first recording from one of the rats was missing, so only 19 EEG recordings were evaluated, representing a total of 6 h and 20 min of recording. The median number of SWDs over 20 min ranged from 40 to 46 and the mean duration of SWDs from 8.9 to 11.4 s for each evaluator and method, with extreme values resulting from the two manual markings (Table 1). The calculated sensitivity, specificity, PPV, and NPV was >91% for all (Table 2).

Table 2. 1 Median number and mean duration of spike-and-wave discharges (SWDs).

<table>
<thead>
<tr>
<th></th>
<th>Investigator A</th>
<th>Automatic A</th>
<th>Investigator B</th>
<th>Automatic B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median number of SWDs</td>
<td>40 (30-56)</td>
<td>41 (29-58)</td>
<td>46 (37-63)</td>
<td>42 (29-54)</td>
</tr>
<tr>
<td>Mean duration of SWD (s)</td>
<td>11.4 (±2.9)</td>
<td>10.7 (±2.9)</td>
<td>8.9 (±2.8)</td>
<td>10.2 (±2.8)</td>
</tr>
</tbody>
</table>

Automatic A and B represent the semi-automated algorithm run by investigators A and B. Median (min-max), mean (± SD).

Table 2. 2 Sensitivity, specificity, positive predictive value (PPV), and negative predicted value (NPV)

<table>
<thead>
<tr>
<th></th>
<th>Investigator A versus</th>
<th>Investigator B versus</th>
<th>Investigator A versus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Automatic A</td>
<td>Automatic B</td>
<td>investigator B</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>0.96 ± 0.02</td>
<td>0.95 ± 0.03</td>
<td>0.91 ± 0.05</td>
</tr>
<tr>
<td>Specificity</td>
<td>0.96 ± 0.03</td>
<td>0.97 ± 0.02</td>
<td>0.98 ± 0.02</td>
</tr>
<tr>
<td>PPV</td>
<td>0.94 ± 0.04</td>
<td>0.94 ± 0.05</td>
<td>0.97 ± 0.03</td>
</tr>
<tr>
<td>NPV</td>
<td>0.97 ± 0.01</td>
<td>0.97 ± 0.02</td>
<td>0.95 ± 0.03</td>
</tr>
</tbody>
</table>

Automatic A and B represent the semi-automated algorithm run by investigators A and B. Mean ± SD.
DISCUSSION

The algorithm was demonstrated to have good sensitivity, specificity, PPV, and NPV for detecting SWDs with durations ≥1 s. The study was not randomized, as the EEG data were always analyzed manually by the investigators first and then by automatic detection. This was done so that the investigators’ manual analysis would not be biased by the automatic detection results.

The characteristics of the SWDs of GAERS used to build the algorithm were slightly modified from Maurescaux et al. (1992)\[^3\]. Indeed only SWDs of ≥1 sec duration were detected and the reported SWD frequency range of 7 to 11 Hz was extended to include SWDs ranging from 5 to 13 Hz. The larger frequency range provided tolerance as, for example, spikes can sometimes be below threshold resulting in a lower spike count.

Twenty-minute recording was used for the analysis as this was the duration reported in the original paper describing the EEG of GAERS (Marescaux et al., 1992). Comparisons were made between the manual EEG recording evaluations by the two researchers to establish a baseline range of sensitivity, specificity, PPV and NPV. Comparison between the manual and that investigator’s evaluation using the semi-automated SWD detection algorithm resulted in a higher sensitivity when compared to baseline (95 to 96% versus 91%) while retaining a high specificity (96–97%). These results are comparable to the performance of other high-quality detection algorithms for SWDs in rodents\[^11\]–\[^13\]\]. In these three publications, the analyses were based on transforming the EEG from the time domain to the frequency domain to quantify changes. The methodology reported by Ovchinnikov et al. 2010, only detect the onset of SWDs (evaluating the number of leading edges without taking the length of the detector event into consideration) and is based on wavelet analysis\[^11\]. The methodology reported by Van Hese et al. 2009 is based on spectral and variant analysis and that of Sitnikova et al. 2009 on spectral and wavelet analysis\[^12\, 13\]. In contrast, the methodology described in the present study analyzes the EEG in the time domain, mimicking manual marking, while decreasing the subjectivity and allowing the results of the EEG analysis to be more reproducible. In consequence, it provides a good alternative for researchers. Nelson et al. 2011 developed an SWD detection system in GAERS based on time-series analysis, gauging changes in amplitude and/or frequency\[^14\]. This system only
allowed the detection of the start of the SWD in order to trigger therapy. Subsequent EEG analyses were performed manually.

The median number of SWDs over 20 min and the mean duration of one SWD were similar in the different evaluations, with the results of the manual recordings showing the extreme values. This could indicate that the semi-automated algorithm provides more accurate results than manual marking. The frequency of SWDs (~2/min) was slightly higher than that described in the original paper (1.5/min) [3]. This is easily explained by the fact that the seizure activity in GAERS is colony- and age-dependent [3].

Although spontaneous SWDs start and end abruptly on a normal background EEG and are quite easy to isolate, the EEG patterns seen during sleep make it more difficult to differentiate the beginning and end of an SWD. During the present experiments, rats were stimulated when seen to be sleeping, to improve seizure detection.

Although not all of the manual analyses were timed, it took the researchers on average 15 min to analyze one EEG recording. In comparison, it took the computer 95–100 sec to analyze one EEG recording using the algorithm. If we consider that some studies can include >400 EEG recordings (3 groups of 6 rats undergoing 3 recordings a week for 8 weeks), the use of the algorithm can avoid 88 h of laborious work. The computer used in this study was certainly more powerful than average, but subsequent analysis using a laptop computer resulted in similar durations.

The system requires manual intervention to select the best EEG channel/electrode and set an amplitude threshold for SWDs. These steps may cause inter-individual variability in seizure detection; however, the use of the present algorithm is still an improvement in inter-individual variation compared to manual marking. Also, the manual intervention is relatively short and allows control of the quality of the EEG recording.

Another limitation of the study is the absence of an automatic artefact-rejection step. Nonetheless, the performance of the algorithm was as expected from such a system and the manual channel-selection allowed rejection of recordings that included too many artefacts.
The semi-automatic SWD detector algorithm described here allowed analysis of the EEG of GAERs with sensitivity, specificity, PPV, and NPV >94% compared to manual analysis. The use of this algorithm would reduce the time necessary to analyze such data and the subjectivity of the results, as well as providing a good performance alternative for researchers without an engineering background.

Acknowledgements

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REFERENCES


Chapter 3 - Seizures induced by oral administration of enrofloxacin in GAERS.

3.1. Outline
Antibiotics have known adverse effects on the nervous system such as antibiotic induced seizures that have not been intensively investigated. The literature mainly describes case reports of antibiotic induced seizures in predisposed patients. (Lerner, Smith et al. 1967, Arcieri, Becker et al. 1989, Grondahl and Langmoen 1993, Lode 1999, Kim, Kim et al. 2013, Bhattacharyya, Darby et al. 2014, Tsubouchi, Ikematsu et al. 2014) Fluoroquinolones are one of the antibiotic classes most commonly associated with seizures and are thought to provoke seizures through inhibitory effects on GABA transmission. (Bhattacharyya, Darby et al. 2014) Enrofloxacin is a broad spectrum potent fluoroquinolone that can be administered orally and that is routinely used post-operatively in rats. (Adams 2001)

GAERS express typical absence seizures originating from abnormal thalamocortical networks including the thalamocortical neurons of sensory thalamic nuclei and their main inhibitory input (GABAergic neurons). (Noebels and Jasper 2012) Consequently it was hypothesized that GAERS could be predisposed to fluoroquinolone induced seizures, and that administration of enrofloxacin would increase their seizure activity. To test this hypothesis, five GAERS were monitored for spike and wave discharges (SWD) activity before and after addition of enrofloxacin into their drinking water.

Although no effect of enrofloxacin on GAERS’ SWD activity was demonstrated with statistical significance, its administration coincided with the appearance of tonic seizures, which is abnormal in this model of epilepsy. The incidence of tonic seizures (n=2) remained uncommon when compared to the number of non-convulsive seizures recorded (n>500) during the same recording period, however two rats out of five were affected representing 40% of the sample population. Also, considering that the alteration of the blood brain barrier is a risk factor for antibiotic induce seizure and that certain quinolones have synergistic inhibition with non-steroidal anti-inflammatory drugs at the GABA receptor, the administration of enrofloxacin peri-operatively when performing craniotomies could result in
a higher incidence and more severe clonic seizures and should be avoided. (Lode 1999, 
Bhattacharyya, Darby et al. 2014)

To the best of the authors’ knowledge, this is the first study looking at the effect of 
enrofloxacin in GAERS. The results also allowed the author to refine the experimental 
protocol and provided evidence to change the standard operative procedures of the institution 
for the in vivo studies presented in the following chapters.

3.2. Manuscript
This chapter is presented as the author-accepted version of a peer-reviewed manuscript 
published in Comparative Medicine, 2016;66(3):220-4. The figures’ numbers and titles of 
the author-accepted version of the manuscript may have been changed to follow the 
formatting of the thesis.
Clonic Seizures in GAERS Rats after Oral Administration of Enrofloxacin

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Enrofloxacin induces clonic seizures in GAERS

The aim of this study was to evaluate the effect of oral enrofloxacin on the epileptic status of Genetic Absence Epilepsy Rats from Strasbourg (GAERS). Five adult female GAERS rats, with implanted extradural electrodes for EEG monitoring, were declared free of clonic seizures after an 8-wk observation period. Enrofloxacin was then added to their drinking water (42.5 mg in 750 mL), and rats were observed for another 3 days. The number of spike-and-wave discharges and mean duration of a single discharge did not differ before and after treatment, but 2 of the 5 rats developed clonic seizures after treatment. Enrofloxacin should be used with caution in GAERS rats because it might induce clonic seizures.

GABA, γ-aminobutyric acid; GAERS, genetic absence epilepsy in rat from Strasbour; SWD, spike-and-wave discharge
The potential adverse effects of antibiotics on the nervous system include antibiotic-induced seizures that have not been investigated intensively. With a few exceptions, the literature includes only case reports of antibiotic-induced seizures in predisposed patients. Fluoroquinolones are one of the antibiotic classes most commonly associated with seizures and are thought to provoke seizures through inhibitory effects on gamma-aminobutyric acid (GABA) transmission. Enrofloxacin is a fluoroquinolone antimicrobial drug that is rapidly bactericidal against a wide variety of clinically important bacterial organisms. Enrofloxacin is potent, well tolerated by animals, and can be administered through a variety of routes (orally by tablet and drinking water, subcutaneously, and intramuscularly).

The GAERS (Genetic Absence Epilepsy in Rat from Strasbourg) strain is a genetic Wistar rat model of absence epilepsy that displays electroclinical, behavioral, and pharmacologic features of absence seizures and has become a ‘gold standard’ to study the mechanisms underlying absence epilepsy. The EEG of GAERS presents typical spike-and-wave discharges (SWD) of 0.3 to 1 mV in amplitude and 7 to 11 SWD per second in spike rate. Typical absence seizures originate from abnormal thalamocortical networks with cellular elements that include pyramidal cells and interneurons of different cortical layers, the thalamocortical neurons of sensory thalamic nuclei and their main inhibitory input (GABAergic neurons of the nucleus reticularis thalami).

Consequently and for the purpose of a pilot study, we hypothesized that GAERS could be predisposed to fluoroquinolone-induced seizures and that oral administration of enrofloxacin would increase their seizure activity. During that pilot study, some rats developed clonic seizures. To our knowledge, no description of antibiotic-induced clonic seizure in GAERS has been published, the aim of the present case study was to document clonic seizures induced by oral administration of enrofloxacin in GAERS.

Materials and Methods

The pilot study was designed as a prospective, blinded experiment. The experiments were approved by St Vincent’s Hospital (Melbourne) Animal Ethics Committee and conducted in
accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004).

Female GAERS rats (*Rattus norvegicus*; age, 6 mo) with a clean colony pathogen status (tested for 17 different virus, 18 bacteria and fungi, and 13 ectoparasites and endoparasites) were obtained from the Royal Melbourne Hospital’s Biologic Research Facilities, The University of Melbourne (Parkville, Victoria, Australia), to take part in an 8-wk experiment investigating novel techniques to deliver antiepileptic drugs. The first 5 rats randomly allocated to the control group were also used in the present pilot study.

The GAERS were housed individually in high-top rat cages (RB2, Wiretainers, Melbourne, Victoria, Australia) with recycled paper bedding (Fibrecycle, Yatala, Queensland, Australia). The temperature and humidity of the room were kept at 22 °C and 55%, respectively. The rats were maintained under a 12:12-h light:dark cycle with free access to food (Barastoc GR2 pellets, Ridley, Melbourne, Victoria, Australia) and tap water.

All 5 rats underwent surgery for the implantation of EEG recording electrodes (Figure 3.1A). Immediately before surgery, the rats were weighed and anesthetized with an intraperitoneal injection of ketamine (75 mg/kg; ketamine hydrochloride 100 mg/mL, Ceva Animal Health, Glenorie, New South Wales, Australia) and xylazine (10 mg/kg; xylazine hydrochloride 20 mg/mL, Troy Laboratories, Glendenning, New South Wales, Australia). After the induction of anesthesia, each rat was placed in a stereotaxic apparatus (Kopf, Stoelting, Wood Dale, IL) and given isoflurane (0.5% to 1% in oxygen, 1 L/min; Isoflo, Abbott Australasia, Botany, New South Wales, Australia) by nose-cone, subcutaneous carprofen (5 mg/kg; 50 mg/mL, Parnell Australia, Alexandria, New South Wales, Australia) for pain relief, and 0.9% sodium chloride (2 mL; Baxter, Old Toongabbie, New South Wales, Australia) for cardiovascular support. The EEG recording electrodes were implanted as follows: after aseptic preparation of the scalp, a single incision was made down the midline, the skull cleared of tissue, and the exposed bone dried with 3% hydrogen peroxide (Sanofi, Victoria, Queensland, Australia). Four extradural electrodes, consisting of small jewelers’ screws (diameter, 3.2 mm; Plastics One, Roanoke VA), were implanted cranial to the interaural line (2 on each side of the
Figure 3.1 Experimental protocol

(A) Schematic diagram illustrating the positioning of the epidural recording screw electrodes (1 through 4) and reference electrode (R). (B) Experimental design. EEG recording (30-min session twice weekly) began on day 7 or 8 after surgery and continued for the following 7 wk. On day 61, the drinking water was replaced with enrofloxacin-containing water (1.7 mL of enrofloxacin 25 mg/mL in 750 mL of drinking water), and additional EEG recordings were performed on days 62 and 64. The enrofloxacin treatment was stopped on day 65. (C) The clonic seizure of rat 5 started on day 64 after 308 s of EEG recording and lasted for 40 s. The intensity of the clonic seizure was classified as stage 4 (clonic seizure in a seating position). The EEG recordings presented SWD that were similar in amplitude (0.3 to 0.7 mV) and frequency (average, 7 SWD per s) to that observed during absence seizure.

sagittal suture), and one screw was implanted caudal to the interaural line to the right of the sagittal suture. The electrodes were connected to an adaptor secured with dental repair material (Cold-curing acrylic, Vertex, Zeist, The Netherlands). The skin was sutured (3-0 Monosyn, B Braun, Rubi, Spain), leaving only part of the dental cement exposed, and each rat was placed on a heating pad for recovery from anaesthesia. Postoperative treatment
included subcutaneous buprenorphine (0.03 mg/kg BID; Temgesic, 0.324 mg/mL buprenorphine hydrochloride, Reckitt Benckiser Healthcare, Hull, United Kingdom), 0.9% sodium chloride (2 mL daily), and carprofen (5 mg/kg, daily) as needed for a maximum of 3 d.

Beginning at day 7 or 8 after surgery, rats were monitored for at least 60 min (30 min for recovery from anaesthesia and acclimation, 30 min for EEG recording) daily and on at least 2 d each week for the following 7 wk. At day 61, regular drinking water was replaced with water containing enrofloxacin (42.5 mg in 750 mL of drinking water; Baytril, 25 mg/mL, Bayer Australia, Pymble, New South Wales, Australia), and additional EEG recordings were obtained on days 62 and 64 (Figure 3.1A). Each EEG recording session began 4 to 6 h after the start of the light cycle. Treatment with enrofloxacin stopped on day 65. The rats were weighed (sensitivity, ± 1 g; model KD-200, Tanika, Kowloon, Hong Kong) on days 61, 62, and 64.

For each monitoring session, rats were anesthetized in an induction cage by using isoflurane in oxygen for 3 min to connect the recording electrodes through shielded cables to the EEG acquisition system, which consisted of TDT processors (Tucker-Davis Technologies, Alachua FL) and high-impedance head stages driven by TDT’s programmable software. The EEG was sampled at 3051.76 Hz, and recording began only after the rats had returned to normal behavior (moving around without ataxia, eating, cleaning itself) and presented absence seizures. During the recording sessions, the rat’s behaviour was observed, and the EEG was visualized by using a custom-designed MATLAB program. When clonic seizures occurred, their intensity was classified according to the modified Racine scale. At the end of a recording session, the rats were briefly anesthetized with isoflurane (4% in oxygen, 2 L/min) to be disconnected from the shielded cable.

To evaluate the effects of the enrofloxacin treatment on the GAERS SWD, the median number of SWD, duration of a single SWD, and cumulative duration of SWD during 60 min were compared between weeks 8 and 9 (Figure 3.1B) by using the Wilcoxon signed ranks test (IBM SPSS Statistics, version 20, IBM Corp, Armonk, NY USA). The significance level was set at 5%. The researcher performing EEG analysis was blinded regarding treatment and used a GAERS-specific automated SWD detection algorithm.
Results

All 5 rats were declared free of clonic seizures during the first 8 wk of EEG recordings. On day 61, the median weight of the rats was 189 g (range, 185 to 204 g). The median weight gains were 0 g (range, −1 to 2 g) and −0.2 g (range, −1 to 1 g) on days 62 and 64, respectively, when compared with day 61. Although the duration of SWD showed an increasing trend (p=0.34), the number of SWD, duration of a single SWD, and cumulative duration of SWD during 60 min did not differ significantly (Figure 3.2). However, 2 rats each manifested a single episode of clonic seizures. The first observed episode occurred on day 62 (24 h after the start of treatment) and lasted 31 s, whereas the second episode occurred on day 64 (72 h after the start of treatment) and lasted 40 s. During both episodes, the affected rats had convulsions interpreted as clonic seizures while they were in a sitting position. The first signs were trembling of the whiskers, shortly followed by spasms and jerking of the neck muscles and some flexion of the legs. Both seizures were classified as intensity stage 4 seizures.

The EEG recordings obtained during the clonic seizures presented SWD of similar amplitude (0.3 to 0.7 mV) and frequency (7 SWD per s) to those observed during absence seizures (Figure 3.1C).
Figure 3. 2 Box-and-whisker plots of the epileptic activity before enrofloxacin treatment (n = 5) and afterward (posttreatment, n = 5).

(A) Number of SWD. (B) Median duration of a single SWD. (C) Total duration of SWD during 60 min of recording time each week. ° represent a yellow card zone outlier (distance between the outlier and the interquartile range box superior to 1.5 times the interquartile range box length) and * represent...
a Red card zone outlier (distance between the outlier and the interquartile range box superior to 3 times the interquartile range box length).

Discussion

The GAERS strain is a genetic rat model of absence epilepsy of which all rats present recurrent generalized nonconvulsive seizures accompanied with behavioural arrest, staring, and sometimes twitching of the vibrissae.\textsuperscript{12} Although the small number of rats in the present study precluded definitive determination of the effect of enrofloxacin on SWD activity, the administration of the antibiotic coincided with the appearance of clonic seizures, which are abnormal in this model of epilepsy.\textsuperscript{12}

To our knowledge, the neurologic circuits and biochemical substrates of absence seizures and clonic seizures are similar. However, why some similar patterns of abnormal brain activity result in absence compared with clonic compared with tonic–clonic seizures is unknown. The incidence of clonic seizures \((n = 2)\) remained uncommon when compared with the number of nonconvulsive seizures recorded \((n > 500)\) during the same recording period, but 2 of the 5 rats were affected, representing 40\% of the sample population.

In addition, considering that alteration of the blood–brain barrier is a risk factor for antibiotic-induced seizures (by allowing for increased concentration of the antibiotic in the brain) and that certain quinolones have synergistic inhibition with NSAID at the GABA receptor, the administration of enrofloxacin perioperatively when performing craniotomies might result in a higher incidence and more severe clonic seizures than those observed in the present study.\textsuperscript{5,10}

The 5 GAERS rats used in this pilot experiment were control animals in a separate study investigating novel techniques to deliver antiepileptic drugs. Because the recording period for the antiepileptic drug experiment was 8 wk, enrofloxacin couldn’t be administered earlier. In addition, GAERS epileptic activity is age-dependent; therefore, the SWD activity that occurred during week 9 (after the start of enrofloxacin treatment) was compared only with week 8 (pretreatment) data.\textsuperscript{12} Specifically, the number and duration of SWD increase with age, with the maximal number of SWD at the age of 6 mo.\textsuperscript{12}
One limitation of the current study is that the water consumption was not measured. However, rats were weighed daily, and all rats maintained appropriate weight during the antibiotic treatment, implying a normal hydration status.

When combined with a nonseizure-modulating dose of theophylline, ciprofloxacin and norfloxacin increased seizure activity in amygdaloid-kindled rats. However, when given as a sole agent in that model, enrofloxacin decreased seizure activity. In another experiment, ciprofloxacin ($\geq 50$ mg/L) showed moderate to marked epileptogenic effects when tested on slices of rat hippocampus, which have a low threshold for the development of epileptiform activity.

The $\text{GABA}_A$ receptor plays a major role in the epileptogenic activity of fluoroquinolones, and (in vitro) the N-methyl-D-aspartate receptor is directly involved in this process as a target for the excitatory effect of fluoroquinolones. The substituent at the C7 position of fluoroquinolones appears to be an important predictor of GABA binding and seems to be, at least partially, responsible for the differences in seizure incidence among fluoroquinolones. To our knowledge, the C7 substituent of enrofloxacin has not been studied for its epileptogenic activity.

The EEG of GAERS rats presents typical SWD that originate from abnormal thalamocortical networks, including its GABAergic neurons. In contrast, kindling is a local process whereby an electrical stimulus typically delivered to a limbic brain structure evokes an electrographic after-discharge capable of spreading and initiating a generalized convulsive seizure. The kindled rat model of epilepsy doesn’t involve the GABAergic neurons in the initiation of the seizure, and this might be the reason for the different effects of enrofloxacin between the GAERS and kindled rat models. In addition, amygdala-kindled events are focal as compared with the primarily generalized nature of the seizures in GAERS rats; consequently, these processes have different mechanisms from the pharmacologic perspective.

Isoflurane was used briefly when connecting and disconnect the recording apparatus. In previous experiments, we found that some rats showed stress behavior (for example, vocalization) during handling for connection and disconnection; consequently we opted for brief general anaesthesia or sedation to increase animal wellbeing. Rats recovered quickly.
from the anaesthetic episodes, and it is unlikely that, after the 30-min acclimation period, isoflurane would still be present to interfere with epileptic activity.

Finally, no postmortem brain histologic analyses were performed on the rats and, although improbable, it cannot be excluded that the appearance of the clonic seizures coincided with the development of an intracranial pathology unrelated to administration of enrofloxacin. Similarly, it cannot be excluded that the isoflurane anaesthesia affected the appearance of SWD; however the 30-min recovery period prior to recording likely reduced isoflurane effects to being negligible.

Within the described experimental conditions, enrofloxacin did not appear to change the duration or frequency of SWD in GAERS rats. However, this antibiotic should be administered with caution because it might induce clonic seizures in GAERS rats, especially during the perioperative period, when aggravating factors such as disruption of the blood–brain barrier and coadministration of NSAID may be present.

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References


Chapter 4 – Anti-epileptic effects of Phenytoin loaded polymers implanted subdurally in GAERS

4.1. Introduction
Before undergoing human clinical trials, polymer-based drug delivery devices need to go through thorough investigations reviewing product characterisation, *in vivo* long term efficacy, and biocompatibility and safety animal studies. In the following investigation, PLGA sheets loaded with the commonly used AED phenytoin were developed and their ability to decrease seizure activity was investigated in the GAERs. As mentioned in chapter 1, phenytoin has been used for the treatment of epilepsy for almost 80 years and PLGA polymers have been approved by the United State Drug Administration after extensive investigations of its safety profile.

The aim of the study presented in this chapter is to provide some *in vitro* product characterizations and to investigate the effect of subdural implantation of biodegradable PLGA polymers loaded with phenytoin on seizure activity in the GAERS animal model of epilepsy. The hypothesis was that subdural implantation of AED-loaded PLGA polymers can decrease seizure activity of the GAERS. The novelty of the study would reside in 1) the *in vitro* release profile of phenytoin from PLGA polymer and 2) the demonstration that focal delivery of AEDs can achieve sustained anti-epileptic effect in an animal model of generalized epilepsy.

4.2. Materials and methods
The in vitro experiments were designed to characterise the structure of the polymers and the *in vitro* release profile of phenytoin. Those experiments were performed at the Intelligent Polymer Research Institute and ARC Centre of Excellence for Electromaterials Science (Wollongong).

The *in vivo* study was designed as a randomized controlled masked experiment. PLGA polymer loaded with the AEDs phenytoin was investigated. All experiments were approved
by St Vincent’s Hospital (Melbourne) Animal Ethics Committee and conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004).

### 4.2.1. PLGA polymer mat production

Phenytoin-laden polymer mats were produced using a coaxial electrospinning method (Viry et al., 2012), with the core composed of phenytoin and 75:25 PLGA (lactide/glycolide = 75:25), and the shell composed of 85:15 PLGA (lactide/glycolide = 85:15). The core solution was prepared at 17% w/v 75:25 PLGA in dimethylformamide (DMF), to which phenytoin was added to a final concentration of 10% w/w relative to the polymer. The shell solution was prepared at 20% w/v 85:15 PLGA in a binary solvent system comprising dichloromethane and DMF (dichloromethane/DMF = 7/3). Coaxial electrospinning was conducted using a nano-electrospinning system (NANON-01A, MECC CO. Ltd) at an applied DC voltage of 23 kV. A coaxial spinneret with a diameter of 0.2 mm for the core and 0.8 mm for the sheath nozzles was connected to the core and shell solutions and a feed rate of 0.3 mL h⁻¹ and 1.2 mL h⁻¹ was applied for the core and shell solutions respectively. Fibre mats were collected on a grounded plate collector that was set 15 cm away from the spinneret tip. The drug-free, coaxially spun polymer mats were also prepared using the above procedure. The polymer mats containing drug are denoted as ‘phenytoin-loaded polymers’ whilst the drug-free mats are denoted as ‘blank polymers’. All the samples were finally dried in a vacuum oven at 40 °C for 72 h to remove residual solvent and were stored at -20 °C prior to subsequent physiochemical characterization and animal studies.

### 4.2.2. Morphology Study

The morphology of the electrospun mats (with and without drug) was examined using a field emission scanning electron microscope (FESEM, JEOL JSM-7500FA). The samples were sputter-coated with gold prior to FESEM to avoid sample charging.

### 4.2.3. Determination of drug loading in polymer mats

An extraction method was used to determine the drug loading in the electrospun mats. Briefly, each sample (1 cm × 1 cm) was weighed and placed into 1 mL methanol for 12 hours after which the methanol was removed and replenished with 1 mL of fresh methanol. This
extraction procedure was repeated four times with each methanol sample allowed to evaporate to leave residual drug behind which was reconstituted using artificial cerebrospinal fluid (aCSF). Each aCSF sample was then analysed for drug using high performance liquid chromatography (HPLC) (see below). The fourth reconstituted sample showed no presence of drug indicating that the entire drug had been extracted from the electrojetted sample.

4.2.4. Silicone sheets production
Silicone sheets were prepared using implant grade liquid silicone rubber (Cochlear Ltd), LSR 30, which comprises a polymer precursor base and a crosslinker. The polymer precursor base and crosslinker were dissolved in hexane at a ratio of 10:1, cast in a Teflon mould at room temperature, and cured by crosslinking at 110 °C for 30 min and then at 200 °C for 4 h. The silicone sheets (approximately 1 mm thick) were then extracted with hexane for 72 h to remove any residual oligomers, and dried in vacuo at 60 °C overnight.

4.2.5. In vitro drug release
The phenytoin-loaded polymers (1 cm × 1 cm) were suspended in 1 mL of aCSF, and the release experiment was conducted at 37 °C in a shaker water bath (Julabo Pty. Ltd.). The aCSF contained NaCl (0.866% w/v), KCl (0.224% w/v), CaCl2-2H2O; (0.0206% w/v) and MgCl2-6H2O (0.0164% w/v) in 1 mM phosphate buffer (pH 7.4). (Halliday, Campbell et al. 2013) For each sample, the release medium solution was collected and replenished with fresh aCSF at various time points, and stored at -20 °C prior to HPLC analysis being undertaken. The eluted samples were analysed by HPLC using a modified method on an Agilent 1260 Infinity HPLC system. (Lin, Hsieh et al. 2010) An Atlantis® T3 C18 column (250 mm × 4.6 mm, 5 μm) was employed as the analytic column and set at 40 °C. The mobile phase was composed of water, acetonitrile and methanol (65:26.2:8.8, v/v/v), the injection volume was 10 μL with the flow rate of 0.8 mL/min. The eluting phenytoin was detected using a UV-vis detector set at a wavelength of 203 nm (λmax of phenytoin). To convert the UV-vis absorbance to drug concentration a standard curve was established by plotting the UV-vis peak areas against respective concentrations of standard solutions.

4.2.6. Animals
Adult female GAERS were obtained from the University of Melbourne (Parkville, Victoria, Australia) and housed individually in 12-hour light/dark cycles with ad libitum access to food.
and water. The rats were randomly allocated to a control group (no implant; n=6), blank polymer group (bilateral implantation of blank PLGA polymers not containing AED; n=6) or treatment group (bilateral implantation of phenytoin loaded PLGA polymers; n=6). The randomization was performed using the random function in Microsoft Excel 2007. Later, a silicone group (bilateral implantation of silicone sheets; n=4) was added to test the effects of the products resulting from the polymer biodegradation. The group attribution list was kept concealed from the researchers performing the EEG analysis.

4.2.6.1 Implantation surgery
Immediately prior to surgery, rats were weighted and anaesthetized with an intra-peritoneal injection of ketamine (75 mg/kg) and xylazine (10 mg/kg). Following anaesthesia induction rats were placed in a stereotaxic apparatus, and administered isoflurane (0.5 to 1% in oxygen, 1 L/min) via a nose-cone, and were administered subcutaneous (SQ) carprofen (5 mg/kg) for pain relief and 0.9% sodium chloride (2 mL) for cardiovascular support. Rats from the control group underwent surgery for EEG recording electrode implantation whereas surgeries for the rats from the blank polymer group, the treatment group and the silicone group also included bilateral craniotomies for subdural placement of two identical implants. Over the scalp of all rats, the hair was clipped and the skin aseptically prepared. A single incision was made down the midline, the skull cleared of tissue and the exposed bone dried with 3% hydrogen peroxide. Five extradural electrodes, consisting of small jeweller’s screws, were implanted caudal to the intended polymer implantation sites through burr holes. Four were implanted cranially to the interaural line (two on each side of the sagittal suture) and one caudally to the interaural line on the right side of the sagittal suture (figure 4.1 A and B). The electrodes were then connected to an adaptor and secured with dental cement.
Figure 4. 1 Polymers and electrodes implantation sites, and timeline of the experiment.

Schematic diagram illustrating the positioning of epidural recording screw electrodes (1-4), reference electrode (R) and polymer mats (A); Picture of the actual surgery (B); Timeline of the experimental design (C): For a minimum of three days post-surgery the rats were monitored for weight loss once a day, and for mobility and grooming twice a day. Thirty minute electroencephalogram recordings began at day 7 or 8 post surgery and for the following 7 weeks for 2 days per week during experiment one, and for 3 days per week during experiment two. For each rat, the mean desired value (number of SWDs, duration of each SWD or cumulative duration of SWDs over 20 min) were calculated for eight recording blocks, each block being 1 week of duration.

Implants measuring 3 mm by 4 mm were cut from the polymer sheets described above. Placements of the implants were performed after 5 mm by 4 mm craniotomies were created bilaterally at the level of the coronal suture (over the motor cortices) and after incising the dura to expose the brain surface (Figure 4.1A and B). Following implant placements, the dura was flapped-back over the polymer and the skull removed from craniotomy sites replaced. The craniotomy sites were then sealed with an alginate-based hydrogel, the entire surgical site covered with dental cement and the skin sutured leaving exposed only part of the dental cement. The animals were placed on heat pads for recovery. Post-operative treatment included SQ buprenorphine every twelve hours (0.03mg/kg, twice a day), saline (2mL, once a day) and caroprofen SID (5mg/kg, once a day) for up to 3 days.
4.2.6.2 Electroencephalograph recording and analysis

At day 7 or 8 post surgery and at least 2 days per week for the following 7 weeks, rats were monitored for one hour (30 to 40 min anaesthesia recovery/acclimation time, 20 min recording time) (Figure 4.1C). At each monitoring session, rats were briefly anaesthetized in an induction cage with isoflurane (4% in oxygen, 2 L/min), and shielded cables were used to connect the recording electrodes to the EEG acquisition system, which consisted of TDT processors and high impedance head stages driven by custom-designed software (Tucker Davis Technologies, USA). The rats were allowed to recover from the anaesthetic before recordings began. The EEGs were visualized using a custom-designed MATLAB program (The MathWorks, Inc., U.S.A.). During EEG recording, if the rats were perceived as being asleep, and after confirmation of no seizure activity on the EEG, a noise stimulus between 94 and 98 decibels was applied. At the end of a recording session, the rats were again briefly anaesthetized with isoflurane (4% in oxygen, 2 L/min) to be disconnected from the shielded cable.

4.2.7. Primary outcome: epileptic activity

For each rat, the median value and interquartile range for number of SWDs, duration of one SWD and cumulative duration of SWDs over 30 min were calculated for eight recording blocks, each block representing 1 week of recording. The first block only included one recording whereas the following 7 blocks included 2 recordings (Figure 4.1C).

4.2.8. Secondary outcome: Post-operative health monitoring

For a minimum of three days post-surgery and until full recovery from the surgery, the rats were monitored for weight loss once a day, and for mobility and grooming twice a day.

4.2.9. Statistical analysis

Data were analysed using commercially available software (IBM SPSS Statistic 22; Stata 13.0; StataCorp, 2013). The number of SWDs, mean duration of each SWD and cumulative duration of SWDs were compared between the three groups using individual Kruskal-Wallis tests for each block of the study. When significant values were found, post-hoc pairwise comparisons were conducted for this block, comparing the control versus AED polymer, and
blank polymer versus AED polymer conditions using the Mann-Whitney U test. Significance level was set at 5%.

4.3. Results
The FESEM images obtained from the PLGA mats with and without Phenytoin (Figure 2A and 2B respectively) show a smooth and regular morphology characteristic of PLGA electrospun fibres and indicated that there was no difference between the fibre morphology and diameter range. Measured by visual inspection of the FESEM images, the fibre diameter ranged between 2 µm and 5 µm.

![Figure 4. 2 Scanning electron micrographs of coaxially electrospun polymer mats.](image)

Blank PLGA polymer mat (A), Phenytoin loaded PLGA polymer mat (B). Scale bar represents 10 µm.

The in vitro release results for the PLGA-phenytoin mats are presented in figure 4.3 and show linear release of phenytoin over a 20-day time with the release not demonstrating substantial plateau off. A total of approximately 11% of the incorporated phenytoin has been released by day 20.
Figure 4. 3 In vitro release data are presented for the phenytoin polymer (A). Bars are representing standard deviation.

Epileptic activity monitoring results for the groups control, blank polymer and phenytoin polymer are presented in figure 4.4. On week 1, 2 and 3 the mean numbers of SWD did not significantly differ among the groups, however the mean duration of each SWD and the cumulative time spent having SWD was significantly lower in phenytoin polymer group compared to the control group ($p=0.006, 0.016, 0.004$ and $p=0.006, 0.25, 0.25$ respectively). During that same period the mean duration of individual SWD and the cumulative time spent having SWD were not significantly different in the phenytoin polymer group compared to the blank polymer group. For week 4 to 8, no significant differences were seen between any of the groups on any of the variables tested.
Figure 4.4 Comparison of the epileptic activity monitoring results (not including the silicone group)

Epileptic activity monitoring results were obtained for groups control (n=6), blank polymer (n=6) and phenytoin polymer (n=6). SWD: Spike and wave discharge; IQR: Interquartile range; Results are reported as median; * Phenytoin polymer group significantly different when compared to control group (p<0.05).
As the rats from the silicone group were on average 2 months older and not randomly allocated, their epileptic activity monitoring results are presented in a different figure (figure 5). The silicone group showed a similar decreased in seizure activity immediately following surgery when compared to the blank polymer group however those results were not tested for statistical significance.

No post-operative complication was detected in any of the rats and they were all fully recovered within 3 days.
Figure 4.5 Comparison of the epileptic activity monitoring results from group silicone versus group blank polymer.

Epileptic activity monitoring results obtained for groups blank polymer (n=6) and silicone sheet (n=4). SWD: Spike and wave discharge; IQR: Interquartile range; Results are reported as median. The silicone group showed a similar decreased in seizure activity following surgery when compared to the blank polymer group however those results were not tested for statistical significance.
4.4. Discussion

Drug release from electrospun polymeric structures typically follows zero order kinetics. (Viry, Moulton et al. 2012) Zero order kinetics implies a homogeneous drug distribution and a release profile governed by the wetting properties of the material and encapsulation of hydrophilic and neutrally charged drugs of low molecular weight can be problematic for these types of structures. (Ishihara and Mizushima 2010, Jyothi, Prasanna et al. 2010, Kluge, Mazzotti et al. 2010, Tiwari, Tzezana et al. 2010) The interaction of these types of drug molecules with the polymer is usually very poor and their rate of diffusion is often faster than the rate of polymer erosion. This fast rate of diffusion has detrimental effects on drug release from electrospun polymer structures. Several factors could be responsible for variations in release, namely the solubility of the drug in the mat, the morphology of the fibres within the mat and the distribution of the drug throughout the fibres (i.e., the degree of drug encapsulation within the core of the coaxial spun mats). The low percentage of released phenytoin at day 20 (Figure 4.3) is most likely, in part, the result of its solubility. As discussed above the less soluble a drug is in aqueous solutions (when all other parameters are constant between the mats such as polymer type, polymer concentration and fabrication conditions) the more likely it is for this drug to elute from the structure at a slower rate. The solubility of phenytoin in aqueous solution is 71 mg/L therefore suggesting that the elution of the phenytoin should be slow which is what is reflected in the release profile presented in Figure 4.3.

Biodegradable PLGA polymer sheets containing a large amount of phenytoin were implanted above the motor cortices of GAERS rats. In this strain of rats, 100% of the animals present recurrent generalized non-convulsive seizures characterized by bilateral and synchronous SWD accompanied with behavioural arrest, staring and sometimes twitching of the vibrissae. (Marescaux, Vergnes et al. 1992) Although depth EEG recordings and lesion experiments show that SWD in GAERs depend on cortical and thalamic structures with a possible rhythmic triggering by the lateral thalamus, more recent studies indicate a seizure initiation site within the peri-oral region of the somatosensory cortex (S1po) as well as the somatosensory cortex forelimb region (S1FL) (Marescaux, Vergnes et al. 1992, Manning, Richards et al. 2004, Gurbanova, Aker et al. 2006) Neurophysiological, behavioural,
pharmacological and genetic studies have demonstrated that spontaneous SWD in GAERS fulfill all the requirements for an experimental model of absence epilepsy. (Marescaux, Vergnes et al. 1992) Twenty-minute recordings were used during the experiment as this is the duration used by the original paper describing GAERS EEGs. (Marescaux, Vergnes et al. 1992)

Although spontaneous SWD start and end abruptly on a normal background EEG and are quite easy to isolate, the EEG patterns seen during sleep make it more difficult to differentiate start and stop of SWD. During the experiment, rats were stimulated when seen sleeping to improve seizure detection. This intervention could be reduced by inverting the light cycle of the rats. The EEG recordings of those diurnal animals would then be recorded during the time of maximal activity reducing sleep time EEG interferences. SWDs usually occur at a mean frequency of 1.5 per min when the animals are in a state of quiet wakefulness and have duration of 0.5 to 75 s. (Marescaux, Vergnes et al. 1992)

During the first 3 weeks of the experiment, the phenytoin polymer group had shorter duration of SWDs when compared to the control group however there were no significant differences when compared to the blank polymer group. In consequence, the phenytoin was not responsible for this effect, which could be attributed to brain injuries resulting from the surgery or the PLGA polymers and their degradation products. The phenytoin was chosen for its lipophilic properties allowing easy drug loading within the polymer and its proven efficacy in GAERS when applied directly over S1po and S1FL. (Gurbanova, Aker et al. 2006) However, the mats were placed over the motor cortex and the delivery of phenytoin even only 2 mm cranially to S1po and S1FL resulted in no changes in duration and numbers of SWDs. The minimal amount of drug release observed in the in vitro experiment may also have been a contributing factor to the negative results.

The presence of an anti-epileptic effect after local administration and the absence of effect after systemic injections may be due to phenytoin’s different selectivity of sodium channels in the cortex and other brain areas and have already been discussed elsewhere. (Gurbanova, Aker et al. 2006)
To better understand the effect seen during the first 3 weeks, silicone sheets were implanted in four older GEARS from the same colony. Although the SWD activity in GAERS is age dependent, preventing statistical comparison with the previous groups (blank polymer and phenytoin loaded polymer groups), the post-surgical transient decrease in SWD’s duration was observed again. We could conclude that the surgery itself, and not the PLGA sheets, was responsible for the decreased duration of SWDs, which is in agreement with previous publications reporting that PLGA-based implants are very well tolerated by the brain in animal models of other neurological disorders.(McRae, Hjorth et al. 1991, McRae and Dahlstrom 1994, Gouhier, Chalon et al. 2000, Menei, Pean et al. 2000, Pean, Menei et al. 2000, Arica, Kas et al. 2005)

We have demonstrated that phenytoin loaded PLGA polymer can provide constant delivery of phenytoin for at least 20 days (end of experiment). However, we haven’t been able to demonstrate that phenytoin loaded PLGA polymer sheets implanted on the surface of the cortex could decrease seizure activity in GAERS for a sustained period. In the next chapter we will endeavour to improve the experimental protocol and test a more soluble and broad spectrum AED (i.e. lacosamide).
Chapter 5 – Anti-epileptic effects of lacosamide loaded polymers implanted subdurally in GAERS

5.1 Outline
Following the negative in vivo results obtained in chapter 4, a more water soluble and broad spectrum AED was selected and modifications in the experimental protocol were made. The daylight cycle of the rats was inverted in order to record EEGs during a period of more intense activity and in consequence reduce the artefacts produce by sleep EEG pattern. Only three groups of rats were selected: control group (no implant; n=6), blank polymer group (bilateral implantation of PLGA polymers not containing AED; n=6) or treatment group (bilateral implantation of lacosamide loaded PLGA polymers; n=6). The surgical technique was refined, the piece of the dura exposed following craniotomy was removed and the skull was repositioned directly above the polymer. The EEG recording time at each recording session was extended to 30 minutes and the EEG sessions occurred 3 days a week instead of two. Finally, for a minimum of three days post-surgery and until full recovery from the surgery, the rats were monitored for weight loss once a day, and for mobility and grooming twice a day, and scores were attributed accordingly.

The aim of the study in chapter 5 remains similar to one presented in chapter 4: to provide some in vitro product characterization and to investigate the effect of subdural implantation of biodegradable PLGA polymers loaded with lacosamide on seizure activity in the GAERS animal model of epilepsy. The hypothesis was that subdural implantation of AEDs loaded PLGA polymers can decrease seizure activity of the GAERS.

5.2 Manuscript
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Anti-epileptic Effects of Lacosamide Loaded Polymers Implanted Sub-durally in GAERS Rats

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ABSTRACT

The current experiment investigated the ability of coaxial electrospun Poly(D,L-lactide-co-glycolide) (PLGA) biodegradable polymer implants loaded with the anti-epileptic drugs (AED) lacosamide to reduce seizures following implantation above the motor cortex in the Genetic Absence Epilepsy Rat from Strasbourg (GAERS).

In this prospective, randomized, masked experiments, GAERS underwent surgery for implantation of skull electrodes (n=6), skull electrodes and blank polymers (n=6), or skull electrodes and lacosamide loaded polymers (n=6). Thirty-minute electroencephalogram (EEG) recordings were started at day 7 post-surgery and continued for eight weeks. EEGs were analyzed using an automated detection algorithm. The number of SWDs and mean duration of one SWD were compared week-by-week between the three groups using Kruskal-Wallis tests and when significant values were found, post-hoc pairwise comparisons were conducted using the Mann-Whitney U test. Significance level was set at 5%.

There was no difference in the number of SWD between any of the groups. However, the mean duration of one SWD was significantly lower in the lacosamide polymer group for up to 7 weeks when compared to the control group (0.004 <p< 0.038). The mean duration of one seizure was also lower at week 3, 5, 6 and 7 when compared to the blank polymer group (p= 0.016, 0.037, 0.025 and 0.025 respectively).

We have demonstrated that AED loaded PLGA polymer sheets implanted on the surface of the cortex could affect seizure activity in GAERS by decreasing the mean duration of the SWDs events for a sustained period of up to 7 weeks.

Keywords: Absent seizure; Antiepileptic Drug Delivery; Biodegradable; Epilepsy; PLGA; SWD.
Introduction

Epilepsy is a chronic neurological condition characterized by recurrent seizures. The incidence of epilepsy in most developed countries is between 50 and 100 cases per 100,000 population per year although it is estimated that up to 5% of a population will experience non-febrile seizures at some point in life (Shorvon 1996, Sander 2003). Individuals with medically untreatable epilepsy often have impaired ability to work or function socially (e.g. inability to drive, difficulty at attending school, losing jobs and friends, anxiety regarding the possibility of having seizure in potentially hazardous conditions) (Swarztrauber, Dewar et al. 2003). Treatment with conventional anti-epileptic drugs (AEDs; e.g.: phenytoin and lacosamide administered orally) results in only 33% of the patients having no seizure recurrence (Shorvon 1996, Sander 2003). Alternatively, neuro-stimulation based therapy has also shown to reduce seizure activity but has typical reductions of seizure frequency of approximately 40% acutely and 50–69% after several years (Fisher and Velasco 2014). Surgical resection of the seizure focus can be performed in the case of focal seizures, however this procedure can only be applied on selected patients depending on the localization of the epileptic foci (Kwan and Sperling 2009). Indeed, the success of inducing long-lasting seizure remission from epilepsy surgery range from a low of 25% for patients exhibiting extrahippocampal seizure origin to 70% in appropriately selected candidates (Kwan and Sperling 2009).

The mechanisms by which resistance to AEDs treatment develops are not fully understood, some evidence suggests that this may be due to a lack of effective penetration into the brain parenchyma, however the drug side effects prevent large increase in the posology (Loscher 2005). Alternative therapies aiming at improving the availability of AEDs such as the
intracranial implantation of polymer-based drug delivery systems are being investigated (Halliday, Moulton et al. 2012, Halliday, Campbell et al. 2013). This targeted drug delivery approach has shown some success in the treatment of animal models of several neurological disorders such as Parkinson’s disease, Huntington’s disease and Alzheimer’s disease (Halliday and Cook 2009). Also, Halliday et al. (2013) used Levetiracetam-loaded biodegradable polymer implants in the tetanus toxin model of temporal lobe epilepsy in rats; the results of this study indicated that drug-eluting polymer implants represent a promising evolving treatment option for intractable epilepsy, however important limitations of the study were that the effects could only be seen for a week and only a single group of control animals were investigated. These animals received an injection of tetanus toxin and a sham craniotomy, without the implantation of a polymer sheet (Halliday, Campbell et al. 2013).

Poly(D,L-lactide-co-glycolide) (PLGA) is the most commonly used biodegradable polymer as it is highly biocompatible, easily engineered and has been approved for drug delivery purposes by the United States Food and Drug Administration (Halliday, Campbell et al. 2011). It has been used in numerous applications including bone and skin tissue engineering, ocular treatment, vaccine, cancer therapy, nerve regeneration and many more (Allahyari and Mohit 2015, Cao, Chen et al. 2015, Groynom, Shoffstall et al. 2015, Yu, Zhao et al. 2015, Zhang, Song et al. 2015). PLGA polymers have also been successfully used for intracranial drug delivery in animal models of neurological disorders, showing no evidence of toxic injury or immune-mediated inflammation when implanted subdurraly above the motor cortex in rats (Halliday, Campbell et al. 2011).

Genetic Absent Epilepsy Rats from Strasbourg (GAERS) is a strain of rats where 100% of the animals present with recurrent generalized non-convulsive seizures (Marescaux, Vergnes
et al. 1992). This animal model has become the gold standard to study the mechanisms underlying absence epilepsy (Marescaux, Vergnes et al. 1992). In the present investigation, PLGA sheets loaded with the commonly used AED lacosamide were developed and their ability to decrease seizure activity was investigated in the GAERs. Lacosamide stabilizes neuronal membranes through enhancing slow inactivation of voltage-gated sodium channel and is effective in different rodent seizure models including generalized seizure (Beyreuther, Freitag et al. 2007).

The aim of the study is to investigate the effect of sub-dural implantation of biodegradable polymers (PLGA) loaded with lacosamide on seizure activity in an animal model of epilepsy (GAERS); the hypothesis is sub-dural implantation of lacosamide loaded PLGA polymers can decrease seizure activity of the GAERS. Our results demonstrated that focal delivery of lacosamide can achieve partial sustained anti-epileptic effect in an animal model of generalized epilepsy.

**MATERIALS AND METHODS**

The study was designed as a randomized controlled masked experiments. The experiment was approved by St Vincent’s Hospital (Melbourne) Animal Ethics Committee and conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004).
1. PLGA polymer mat production

Lacosamide-laden polymer mats were produced using a coaxial electrospinning method (Viry, Moulton et al. 2012), with the core composed of lacosamide and 75:25 PLGA (lactide/glycolide = 75:25), and the shell composed of 85:15 PLGA (lactide/glycolide = 85:15). The core solution was prepared at 17% w/v 75:25 PLGA in dimethylformamide (DMF), to which lacosamide was added to give a range of final concentration of 2.5, 12.5 or 20% w/w relative to the polymer. The shell solution was prepared at 20% w/v 85:15 PLGA in a binary solvent system comprising dichloromethane and DMF (dichloromethane/DMF = 7/3). Coaxial electrospinning was conducted using a Nano-electrospinning system (NANON-01A, MECC CO. Ltd) at an applied DC voltage of 23 kV. A coaxial spinneret with a diameter of 0.2 mm for the core and 0.8 mm for the sheath nozzles was connected to the core and shell solutions and a feed rate of 5 µL min⁻¹ and 20 µL min⁻¹ for the core and shell solutions respectively. Fibre mats were collected on a grounded plate collector that was set 15 cm away from the spinneret tip. The drug-free, coaxially spun polymer mats were also prepared using the above procedure.

Depending on lacosamide loading in the core, the as-prepared, coaxially electrospun polymer mats were denoted as PLGA-2.5%, PLGA-12.5% and PLGA-20%. The drug-free mats are denoted as blank polymers. All the samples were finally dried in a vacuum oven at 40°C for 72 h to remove residual solvent and were stored at -20°C prior to subsequent physiochemical characterization and animal studies.
2. Morphology study

The morphology of the electrospun mats (with and without drug) was examined using a field emission scanning electron microscope (FESEM, JEOL JSM-7500FA). The samples were sputter-coated with gold prior to FESEM to avoid sample charging.

3. Determination of drug loading in polymer mats

An extraction method was used to determine the drug loading in the electrospun mats. Briefly, each sample (1 cm × 1 cm) was weighed and placed into 1 mL methanol for 12 hours after which the methanol was removed and replenished with 1 mL of fresh methanol. This extraction procedure was repeated four times with each methanol sample allowed to evaporate to leave residual drug behind which was reconstituted in methanol (1 mL), diluted 20 times with the HPLC mobile phase (see below) and filtered through a 0.2 µm syringe filter. The fourth reconstituted sample showed no presence of drug indicating that the entire drug had been extracted from the electrojetted sample.

4. In vitro drug release

The lacosamide-loaded polymers (1 cm × 1 cm) were suspended in 1 mL of aCSF, and the release experiment was conducted at 37 °C in a shaker water bath (Julabo Pty. Ltd.). The aCSF contained NaCl (0.866% w/v), KCl (0.224% w/v), CaCl$_2$-2H$_2$O; (0.0206% w/v) and MgCl$_2$-6H$_2$O (0.0164% w/v) in 1 mM phosphate buffer (pH 7.4) (Halliday, Campbell et al. 2013). For each sample, the release medium solution was collected and replenished with fresh aCSF at various time points, and stored at -20 °C prior to HPLC analysis being undertaken. The eluted samples were analyzed by HPLC using a modified method on an Agilent 1260 Infinity HPLC system (Lin, Hsieh et al. 2010). An Atlantis® T3 C18 column (250 mm × 4.6 mm, 5 µm) was employed as the analytic column and set at 40 °C. The mobile
phase was composed of water, acetonitrile and methanol (65:26.2:8.8, v/v/v), the injection volume was 10 µL with the flow rate of 0.8 mL/min. The eluting lacosamide was detected using a UV-vis detector set at a wavelength of 230 nm ($\lambda_{\text{max}}$ of lacosamide). To convert the UV-vis absorbance to drug concentration a standard curve was established by plotting in triplicate the UV-vis peak areas against respective concentrations of standard solutions (10, 20, 50, 100, 200, 500µM Lacosamide).

5. Animals

Adult female GAERS were obtained from the University of Melbourne (Parkville, Victoria, Australia) and housed individually in inverted 12-hour light/dark cycles (the light was turn off between 6 am and 6 pm) with ad libitum access to food and water. Six-month-old rats were randomly allocated to a control group (no implant; n=6), blank polymer group (bilateral implantation of blank PLGA polymers not containing lacosamide; n=6) or treatment group (bilateral implantation of lacosamide loaded PLGA polymers; n=6). The randomization was performed using the random function in Microsoft Excel 2007. The group attribution list was kept concealed from the researchers performing the EEG analysis.

5.1. Implantation surgery

Immediately prior to surgery, rats were weighted and anaesthetized using a balanced anaesthesia protocol including an intra-peritoneal injection of ketamine (75 mg/kg) and xylazine (10 mg/kg). Following anaesthesia induction rats were placed in a stereotaxic apparatus, and administered isoflurane (0.5 to 1% in oxygen, 1 L/min) via a nose-cone as needed, and were administered subcutaneous (SQ) carprofen (5 mg/kg) for pain relief and 0.9% sodium chloride (2 mL) for cardiovascular support.
Rats from the control group underwent surgery for EEG recording electrode implantation whereas surgeries for the rats from the blank polymer group, the treatment group and the silicone group also included bilateral craniotomies for subdural placement of two identical implants. Over the scalp of all rats, the hair was clipped and the skin aseptically prepared. A single incision was made down the midline, the skull cleared of tissue and the exposed bone dried with 3% hydrogen peroxide. Five extradural electrodes, consisting of small jeweller’s screws, were implanted caudal to the intended polymer implantation sites through burr holes. Four were implanted cranially to the interaural line (two on each side of the sagittal suture) and one caudally to the interaural line on the right side of the sagittal suture (Figure 1A and B). The electrodes were then connected to an adaptor and secured with dental cement.

Implants measuring 3 mm by 4 mm were cut from the polymer sheets described above. Placements of the implants were performed after 5 mm by 4 mm craniotomies were created bilaterally at the level of the coronal suture (over the motor cortices) and after excising the dura to expose the brain surface (Figure 1A and B). Following implant placements, the skull removed from craniotomy sites was replaced. The craniotomy sites were then sealed with an alginate-based hydrogel, the entire surgical site covered with dental cement and the skin sutured leaving exposed only part of the dental cement. The animals were placed on heat pads for recovery. Post-operative treatment included SQ buprenorphine every twelve hours (0.03mg/kg, twice a day), saline (2mL, once a day) and carprophen SID (5mg/kg, once a day) for up to 3 days.
5.2. Electroencephalograph recording and analysis

At day 7 or 8 post surgery and at least 3 days per week for the following 7 weeks, rats were monitored for one hour (half an hour anaesthesia recovery/acclimation time, half an hour recording time) (Figure 1C). At each monitoring sessions, rats were briefly anaesthetized in an induction cage with isoflurane (4% in oxygen, 2 L/min, for 2 to 3 minutes), and shielded
cables were used to connect the recording electrodes to the EEG acquisition system, which consisted of TDT processors and high impedance head stages driven by custom-designed software (Tucker Davis Technologies, USA). The rats were allowed to recover from the anesthetic before recordings began. The EEGs were visualized using a custom-designed MATLAB program (The MathWorks, Inc., U.S.A.). During EEG recording, if the rats were perceived as being asleep, and after confirmation of no seizure activity on the EEG, a noise stimulus between 94 and 98 decibels was applied. At the end of a recording session, the rats were again briefly anaesthetized with isoflurane (4% in oxygen, 2 L/min) to be disconnected from the shielded cable. The researcher performing EEG analysis was masked to the treatment and used a GAERS specific automated spike-and-wave discharges (SWDs) detection algorithm. (Bauquier, Lai et al. 2015)

6. Primary outcome: Epileptic activity
For each rat, the median value and interquartile range for number of SWDs, duration of one SWD and cumulative duration of SWDs over 30 min were calculated for eight recording blocks, each block representing 1 week of recording. The first block only included one recording at day 6 or 7 post-surgery (total of 30 minutes of EEG recording) whereas the following 7 blocks included 3 recordings per week (total of 90 minutes of EEG recording per week) (Figure 1C).

7. Secondary outcome: Post-operative health monitoring
For a minimum of three days post-surgery and until full recovery from the surgery, the rats were monitored for weight loss once a day, and for mobility and grooming twice a day. Those observations were given scores (Table 5.1). For each day a debilitation score was calculated by cumulating the highest grooming and mobility scores with the weight loss score (Table 1).
A debilitation score of 0, 1 to 3, 4 to 6 and 7 to 9 meant that the rat health was not affected, mildly affected, moderately affected, severely affected by the surgery/polymer implantation respectively. The highest debilitation score of each rat over the post-operative period is reported.

**Table 5.1 Post-operative health monitoring chart.**

<table>
<thead>
<tr>
<th>Score</th>
<th>Percentage of weight loss</th>
<th>Mobility Score</th>
<th>Grooming Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No weight loss</td>
<td>Rat moving normally</td>
<td>No decrease in grooming activity</td>
</tr>
<tr>
<td>1</td>
<td>less than 10% weight loss</td>
<td>Rat ataxic moving at a normal speed</td>
<td>Middle decrease in grooming activity</td>
</tr>
<tr>
<td>2</td>
<td>10% to less than 20% weight loss</td>
<td>Rat ataxic and moving slowly</td>
<td>Moderate decrease in grooming activity</td>
</tr>
<tr>
<td>3</td>
<td>20% or more weight loss</td>
<td>Rat recumbent</td>
<td>No grooming activity</td>
</tr>
</tbody>
</table>

**Total score = debilitation score**

0-9

For a minimum of three days post-surgery and until full recovery from the surgery, the rats were monitored for weight loss once a day, and for mobility and grooming twice a day. For each day a debilitation score was calculated by cumulating the highest grooming and mobility scores with the weight loss score. A debilitation score of 0, 1 to 3, 4 to 6 and 7 to 9 meant that the rat health was not affected, middle affected, moderately affected, severely affected by the surgery/polymer implantation respectively.
8. Statistical analysis

Data analysis was performed using a commercially available software (IBM SPSS Statistic 22; Stata 13.0; StataCorp, 2013). The number of SWDs, mean duration of one SWD and cumulative duration of SWDs were compared between the three groups using individual Kruskal-Wallis tests for each block of the study. When significant values were found, post-hoc pairwise comparisons were conducted for this block, comparing the control versus lacosamide polymer, and blank polymer versus lacosamide polymer conditions using the Mann-Whitney U test. The debilitation scores of group lacosamide were compared to group control and group blank polymer using a 2-tailed Mann-Whitney exact test. Significance level was set at 5%.

RESULTS

The FESEM images obtained from the PLGA mats without lacosamide (figure 2A) show a smooth and regular morphology characteristic of PLGA electrospun fibres. However when lacosamide was incorporated into the PLGA the fibres within the mats demonstrated a mixture of porous and non-porous morphology when examined under FESEM (figure 2B). Measured by visual inspection of the FESEM images, the fibre diameter ranged between 2 µm and 5 µm. These observations of the PLGA-12.5% samples shown in figure 2C were also observed in the other PLGA-2.5% and PLGA-20.0% samples.

The in vitro release results for the PLGA-lacosamide mats are presented in figure 2C. The polymer containing 20% lacosamide showed a cumulative drug release above 40% after only 7 days. The polymers containing 2.5 and 12.5 % lacosamide both had a reduction in release after around 42 days (6 weeks) however the total drug release was higher for 12.5%
lacosamide polymer. In consequence, the polymer containing 12.5% lacosamide was chosen to be implanted for the in vivo experiment.

Figure 5. 2 Electron micrographs of coaxially electrospun polymer mats and in vitro release data.

Scanning electron micrographs of coaxially electrospun polymer mats of PLGA Blank (A) and PLGA-12.5% lacosamide (B) (Scale bar represents 10 µm). In vitro release data of lacosamide polymers with final core lacosamide concentration of 2.5, 12.5 and 20% w/w relative to the polymer (C). Bars are representing standard deviation.
The results for the postoperative debilitation score are presented in figure 3. One rat from the blank polymer group and 3 rats from the lacosamide had a debilitation score that were classified as moderate. One rat from the blank polymer group and 3 rats from the lacosamide group had a debilitation score that were classified as severe. The debilitation scores were significantly increased for the lacosamide polymer group when compared to the control and blank polymer groups (p=0.002 and p=0.041 respectively).

**Figure 5. 3 Post-operative debilitation scores**

Post-operative debilitation scores obtained for groups control (n=6), blank polymer (n=6) and lacosamide polymer (n=6). Rats from the lacosamide polymer group had debilitation scores that were higher when compared to the 2 other groups (*: p=0.002; **: p=0.041). The box indicates the interquartile range (25th to 75th percentile), and the whiskers indicate the range.
The results of the measurements performed to evaluate the epileptic activity for the groups control, blank polymer and lacosamide polymer are presented in figure 4. The difference in the number of SWDs between the groups was not statistically significant. However, the mean duration of one SWD was significantly lower in the lacosamide polymer group for up to 7 weeks when compared to the control group (p=0.037, 0.004, 0.01, 0.025, 0.037 and 0.016 for week 1, 2, 3, 4, 6, and 7 respectively). The mean duration of one seizure was also lower at week 3, 5, 6 and 7 when compared to the blank polymer group (p= 0.016, 0.037, 0.025 and 0.025 respectively). The cumulative duration of SWDs of the lacosamide group was significantly lower when compared to the control group at week 1 and 5 (p=0.010 and p=0.055 respectively) and when compared to the blank polymer group at week 3 and 5 (p=0.010 and p=0.055 respectively). Examples of EEG recordings are presented on figure 5.
Figure 5. 4 Comparison of the epileptic activity monitoring results

Comparison of the measurements obtained to evaluate the epileptic activity of groups control (n=6), blank polymer (n=6) and lacosamide polymer (n=6). SWD: Spike and wave discharge; IQR: Interquartile range; Results are reported as median. * and † represent the time points at which the results from the lacosamide polymer group were significantly different to the control and blank polymer groups respectively (p<0.05).
Figure 5. Example of EEG recordings

Example of EEG recording from 3 rats belonging to group control (A), blank polymer (B) and lacosamide polymer (C). Figures D and E are amplifications of spike and wave discharges observed during the recording shown and figure A and C respectively.
DISCUSSION

Drug release from electrospun polymeric structures typically follows zero order kinetics (Viry, Moulton et al. 2012). Zero order kinetics implies a homogeneous drug distribution and a release profile governed by the wetting properties of the material and encapsulation of hydrophilic and neutrally charged drugs of low molecular weight can be problematic for these types of structures (Ishihara and Mizushima 2010, Jyothi, Prasanna et al. 2010, Kluge, Mazzotti et al. 2010, Tiwari, Tzezana et al. 2010). The interaction of these types of drug molecules with the polymer is usually very poor and their rate of diffusion is often faster than the rate of polymer erosion. This fast rate of diffusion has detrimental effects on drug release from electrospun polymer structures.

Several factors are responsible for the variations in the release profiles of AEDs, namely the solubility of the drug in the mat, the morphology of the fibres within the mat and the distribution of the drug throughout the fibres (i.e., the degree of drug encapsulation within the core of the coaxial spun mats). The solubility of lacosamide (465 mg/L) contributes to the relatively fast elution of that drug from the polymer. Also, the morphology of the fibers within the electrospun mats shows a porous nature for the PLGA-Lacosamide structures which increases the rate at which the release media (aCSF) can infuse into the internal region of the fibers and promote the elution of the drug.

The initial rapid drug release observed in the in vitro experiment for the polymer mats tested with a final core lacosamide concentration of 12.5 % (figure 2C) coincided with the post-surgical debilitation seen in most animals of the lacosamide polymer group. It is unlikely that the surgery alone was responsible for these adverse events as this debilitation was not
observed to such extent in the blank polymer group. Debilitated rats were treated with fluid therapy and analgesic administration and they all recovered well. Looking at the results retrospectively, one could argue that implanting the PLGA-2.5% may have been a better choice as the initial release of drug is not as abrupt and the constant lacosamide release seem to last longer. Indeed the lack of effect of lacosamide after 7 weeks correlate with the almost absence of release of the lacosamide from the polymer. Variations in the release profiles observed in figure 5.2.C from the samples prepared with varying amounts of Lacosamide indicates that the interaction of the drug with the polymer (and hence its propensity to be released from the structures) is influenced by the drug loading. It has previously been shown that the amount of drug loaded into electrospun fibres and drug-polymer-electrospinning solvent interactions has an effect of the release profiles (Chew, Wen et al. 2005, Zeng, Yang et al. 2005, Cui, Li et al. 2006, Xie 2009).

Biodegradable PLGA polymer sheets containing a large amount of lacosamide were implanted above the motor cortices of GAERS rats. In the GAERS model of epilepsy, the rats present recurrent generalized non-convulsive seizures characterized by bilateral and synchronous SWD accompanied with behavioral arrest, staring and sometimes twitching of the vibrissae (Marescaux, Vergnes et al. 1992). Furthermore, the GAERS rats were at least 6 months old, time at which 100% of the GAERS should present SWDs and at which the numbers of SWDs are at their maximum.(Marescaux, Vergnes et al. 1992) Although depth EEG recordings and lesion experiments show that SWD in GAERs depend on cortical and thalamic structures with a possible rhythmic triggering by the lateral thalamus, more recent studies indicate a seizure initiation site within the peri-oral region of the somatosensory cortex (S1po) as well as the somatosensory cortex forelimb region (S1FL) (Marescaux,

Neurophysiological, behavioral, pharmacological and genetic studies have demonstrated that spontaneous SWD in GAERS fulfill all the requirements for an experimental model of absence epilepsy (Marescaux, Vergnes et al. 1992). Although twenty-minute recording were used by the original paper describing GAERS EEGs, to try to improve the performance of the EEG analysis 30 min recording were used during the present experiment (Marescaux, Vergnes et al. 1992).

Although spontaneous SWD start and end abruptly on a normal background EEG and are quite easy to isolate, the EEG patterns seen during sleep make it more difficult to differentiate start and stop of SWD (Bauquier, Lai et al. 2015). In previous experiments, rats were stimulated when seen sleeping to improve seizure detection (Bauquier, Lai et al. 2015). This intervention was reduced during the present experiment by inverting the light cycle of the rats. The EEG recordings of those diurnal animals were then recorded during the time of maximal activity reducing sleep time EEG interferences. SWDs usually occur at a mean frequency of 1.5 per min when the animals are in a state of quiet wakefulness and have duration ranging from 0.5 to 75 s (Marescaux, Vergnes et al. 1992).

Isoflurane was used for a short period to connect and disconnect the recording apparatus before and after each recording. The authors found in previous experiments that during that setup without the use of anaesthesia some rats were showing stress behaviour (like crying) and in consequence the authors opted for a short general anaesthesia/sedation to improve animal welfare. Isoflurane was chosen for its low blood:gas solubility (1.4) allowing quick elimination. Indeed, rats recovered quickly from those short anaesthesia episodes and,
although it cannot be excluded, it is unlikely that after 30 minutes isoflurane could still be interfering with their epileptic activity (Bauquier, Lai et al. 2016).

Implantation of the lacosamide sheets led to shorter seizures for up to seven weeks after implantation compared to rats that did not receive the implant. During the first 2 weeks of the experiment, the blank polymer group also demonstrated decrease duration of SWDs when compared to the control group and to better understand the effect seen, silicone sheets were implanted in four older GAERS from the same colony (unpublished data). Although the SWD activity in GAERS is age dependent, preventing statistical comparison with the present experiment, the post-surgical transient decrease in SWD’s duration was observed again. We could assume that the PLGA sheets were not themselves responsible for the decreased duration of SWDs seen in the control blank polymer group but could be attributed to brain injuries resulting from the surgery. This assumption is in agreement with previous publication reporting that PLGA-based implants are very well tolerated by the brain in animal models of other neurological disorders (McRae, Hjorth et al. 1991, McRae and Dahlstrom 1994, Gouhier, Chalon et al. 2000, Menei, Pean et al. 2000, Pean, Menei et al. 2000, Arica, Kas et al. 2005).

Only one EEG recording per rat was performed during the first week at day 6 or 7 while there were three recordings per week performed during the following seven weeks. This study design allowed time for good surgical wound healing before some traction could be applied to the electrodes ‘adaptor.

The lacosamide was chosen for its lipophilic properties allowing easy drug loading within the polymer and its proven efficacy in treating absence epilepsy (Sodemann, Moller et al. 2014).
Knowing that the polymer mats were implanted over the motor cortex, and that previous investigations have shown that substances released from intraparenchymally implanted polymers are able to penetrate around 3 mm, it is possible that the lacosamide released from the sheets in our study may not have reached the seizure triggering focus in high enough concentration to stop seizure from happening (Krewson, Klarman et al. 1995, Krewson, Dause et al. 1996, Saltzman, Mak et al. 1999, Bensadoun, Pereira de Almeida et al. 2003). However, lacosamide reduces the ability of epileptic neurons to endure extended firing burst by enhancing slow inactivation of voltage-gated sodium channel (Kellinghaus 2009). This would explain the unchanged number of SWDs but significant decrease in SWDs duration. The total duration of SWDs during the recording SWD reflects the arithmetic product of the number of seizures and the mean duration of SWDs. Another AED alternative could have been the use of valproic acid which is one of the drugs of choice for the treatment of absence seizure. Its use could have provided additional support for the validity of the novel delivery method. Lastly, now that it has been shown that a lacosamide loaded PLGA polymer sheets implanted on the surface of the cortex could affect the duration of the individual SWDs in GAERS, performing dose-response experiments in order to determine optimal concentrations of the drug in PLGA would be required to effectively improve the treatment and the novel delivery method.

From the statistical perspective, Bender and Lange (2001) recommended that data of exploratory studies be analyzed without multiplicity adjustment (Bender and Lange 2001). However, the lack of adjustment for multiple comparisons as well as the pilot nature of our study means that caution needs to be exercised when interpreting the results of this exploratory study. A larger study is needed to confirm these findings.
Although temporary side-effects were seen, we have demonstrated that lacosamide loaded PLGA polymer sheets implanted on the surface of the cortex could affect seizure activity in GAERS by decreasing the mean duration of the SWDs events for a sustained period of up to 7 weeks. With improvements in polymer technologies and episodic release offering potentially much longer lasting release durations, intracranial polymer-based drug delivery systems may provide an effective therapeutic strategy for chronic epilepsy.

**Conflict of interest/disclosures**

The authors declare that they have no financial or other conflicts of interest in relation to this research and its publication.

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REFERENCES


Chapter 6 – Evaluation of the biocompatibility of polypyrrole polymers implanted sub-durally in GAERS

6.1 Outline
In chapter 5 the evidence suggested that passive release AED loaded PLGA polymers can decrease seizure activity. We will focus in chapters 6 on PPy polymers as they offer the advantage of being reactive to electrical stimulation and could potentially serve as active release implantable devices. Upon intermittent application of an electrical current, the ionic and hydrophobic properties of PPy polymers vary resulting in the expansion and/or contraction of the structure and this actuation is responsible for the release of impregnated drug.(Hutchison, Lewis et al. 2000, Wallace and Wallace 2003) The mechanism of drug release is the same for all polypyrrole despite the chemical composition of the polymer and has been reported for a wide range of incorporated drugs such as dexamethasone, fosphenytoin and the anti-inflammatory neuropeptide α-melanocyte stimulating hormone (α-MSH).(Zhong and Bellamkonda 2005, Moulton, Imisides et al. 2008, Muller, Yue et al. 2016)

PPy sheets have been shown in vitro to be well tolerated by biological tissues, including brain tissues, and they seem to be as compatible as Teflon when implanted into the cerebral cortex.(George, Lyckman et al. 2005, Ateh, Navsaria et al. 2006) Nevertheless thorough biocompatibility and safety study in an animal model are required during the development of any implantable medical device. The results of the study presented in chapter 6 showed that implanted PPy mats on top of the brain cortex of GAERS did not induce obvious inflammation, trauma, gliosis and neuronal toxicity. Therefore, the results are demonstrating in vivo that the PPy used offered good histocompatibility with central nervous system cells and that PPy sheets could be used as a drug delivery device implanted onto the surface of the brain parenchyma.

6.2 Manuscript
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The figures’ numbers and titles of the author-accepted version of the manuscript may have been changed to follow the formatting of the thesis.
Evaluation of the biocompatibility of polypyrrole implanted sub-durally in GAERS

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Abstract

This blinded controlled prospective randomized study investigated the biocompatibility of polypyrrole (PPy) polymer that will be used for intracranial triggered release of anti-epileptic drugs (AEDs). Three by three millimetres PPy were implanted subdurally in six adult female GAERS (Genetic Absence Epilepsy Rat from Strasbourg). Each rat had a polymer implanted on one side of the cortex and a sham craniotomy performed on the other side. After a period of 7 weeks, rats were euthanized and parallel series of coronal sections were cut throughout the implant site. Four series of fifteen sections were histological (H&E) and immunohistochemically (NeuN, GFAP and ED1) stained and evaluated by 3 investigators. The results showed that implanted PPy mats did not induce obvious inflammation, trauma, gliosis and neuronal toxicity. Therefore we conclude the PPy used offer good histocompatibility with central nervous system cells and that PPy sheets could be used as intracranial, AED delivery implant.

A brain slice from a rat with a confirmed cortical abscess
1. Introduction

The incidence of epilepsy ranges from 50 and 100 cases per 100,000 population per year in most developed countries though it is estimated that up to 5% of a population will experience non-febrile seizures at some point in life.\textsuperscript{[1-3]} Because treatment with conventional anti-epileptic drugs (AEDs) provides adequate seizure control in only two third of patients, more novel therapy is urgently needed.\textsuperscript{[1-3]}

Alternative therapies aiming at improving the availability of AEDs such as the intracranial implantation of polymer-based drug delivery systems are being investigated.\textsuperscript{[4, 5]} This targeted drug delivery approach has shown some successes in the treatment of animal models of several neurological disorders such as Parkinson’s disease, Huntington’s disease and Alzheimer’s disease.\textsuperscript{[6]} This approach has been published once for the treatment of epilepsy.\textsuperscript{[4]} The results of this study indicated that drug-eluting poly(D,L-lactide-co-glycolide) (PLGA) polymer implants represents a promising evolving treatment option for intractable epilepsy. The PLGA polymers used is a biodegradable copolymer that is very well tolerated by the brain.\textsuperscript{[7]} However this polymer can only be used as a passive release vehicle.

Work by Cook et al, 2013 has shown that prediction of seizure likelihood with a long-term, implanted seizure advisory system in patients with drug-resistant epilepsy is becoming reality.\textsuperscript{[8]} Synchronizing the AED release from a polymer with the seizure advisory system could be technically feasible when using a conductive polymer such as polypyrrole (PPy) that can change physical properties when being applied electrical stimulation. The physical changes associated with the mechanism for release is proposed to involve electrostatic interactions as the application of electrical stimulation protocol increases the amount of drug release. However, both the ionic and hydrophobic
properties of polypyrrole have been shown to vary upon application of electrical stimulus.\(^9\) The expansion and contraction (actuation) of polypyrrole is also a well-documented property of polypyrrole upon oxidation and reduction and these processes may be involved in the release of incorporated drug.\(^{10, 11}\) As these properties change simultaneously it is not possible to separate them to determine which process is the driving force for release. These mechanisms of drug release are the same for all polypyrrole despite the chemical composition of the polymer and has been reported for a wide range of incorporated drugs such as dexamethasone, fos-phenytoin \(^\) and the anti-inflammatory neuropeptide α-melanocyte stimulating hormone (α-MSH).\(^{12-14}\) PPy sheets have been shown to be well tolerated by the brain tissue in vitro and they seem to be as compatible as Teflon when implanted into the cerebral cortex.\(^{15}\) The aim of the present study is to test the in vivo biocompatibility of PPys implanted sub-durally in GAERS (Genetic Absent Epilepsy Rat from Strasbourg) on top of their cerebral cortex.

2. Experimental Section

The study was designed as blinded controlled prospective randomized experiments. To evaluate the in vivo biocompatibility of the PPy sheets, the rats underwent surgery for bilateral craniotomy; a PPy sheet was implanted subdurally on one side of the cortex and the other side was used as a sham control. After a period of 7 weeks, the rats were euthanized and parallel series of coronal sections were cut throughout the implant site. Hematoxylin and Eosin (H&E) staining was used to evaluate inflammation, and general histological and architectural changes.\(^{16}\) Immunohistochemical (IHC) staining for glial fibrillary acidic protein (GFAP) was used to evaluate possible materials glial reaction
(gliosis), whilst the ED1 (CD68) antibody and the neural marker NeuN antibody were used to evaluate rat macrophages and neuronal toxicity respectively.\[^{16-18}\]

### 2.1. Evaluated materials

Pyrrole (Py) was obtained from Merck (Frenchs Forest, NSW, Australia) and distilled prior to use. The electrolytes used for electropolymerisation were 2 mg/mL dextran sulphate (DS, M\(_w\) 9000-20,000, Sigma–Aldrich, Castle Hill NSW, Australia). The PPy was synthesized from a monomer solution containing 0.2 M pyrrole and the aforementioned electrolyte. The synthesis method employed was galvanic polymerisation using a current density of 0.2 mA/cm\(^2\) applied for 180 min using a 3 electrode cell containing an auxiliary electrode (stainless steel mesh – 5cm x 5cm), reference electrode (RE: Ag/AgCl - 3.0M NaCl) and a working electrode (WE: polished stainless steel plate 5cm x 5cm). After polymerisation, the WE was rinsed with Milli-Q water overnight to remove any unreacted monomer and dopant. The PPy film standing film was obtained by carefully cutting around the edges of the deposited PPy film and gently removing it from the WE. The free standing film was then laid down on a KimWipe\(^\text{TM}\) tissue sheet that was pre-wet with Milli-Q water. The films were left on the tissue paper and placed into a petri dish, covered and left at 2\(^\circ\)C until required for implantation.

The morphology of the as-prepared PPy film was examined using a Field Emission Scanning Electron Microscope (FESEM, JEOL JSM-7500FA). The thickness of the sample was determined by analyzing the cross sectional micrographs of the sample (n = 20). The mean static contact angle of the polypyrrole free standing film was measured using a Dataphysics optical contact angle goniometer. A total of 20 measurements were
made on the sample using deionized water droplets of 1 µl for the reported value.

2.2. Animals

Six 2-month-old female GAERS were obtained from the University of Melbourne (Parkville, Victoria, Australia) and were housed individually in 12 hours light/dark cycles with *ad libitum* access to food and water. All experiments were approved by St Vincent’s Hospital (Melbourne) Animal Ethics Committee and conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004).

2.3. Animal surgery

Immediately prior to surgery, rats were weighed and anaesthetized with an intra-peritoneal injection of ketamine (ketamine hydrochloride 100 mg/mL, Ceva Animal Health, Glenorie NSW, Australia) (75 mg/kg) and xylazine (Ilum xylazine hydrochloride 20 mg/mL, Troy Laboratories Pty Ltd, Glendenning NSW, Australia) (10 mg/kg). Following anaesthesia induction, rats were placed in a stereotaxic apparatus, and administered isoflurane (Isoflo™, Abbott Australasia Pty Ltd, Botany NSW, Australia) (0.5 to 1% in oxygen, 1 L/min) via a nose-cone, and were administered subcutaneous (SQ) carprofen (Tergive carprofen 50 mg/mL, Parnell Australia Pty Ltd, Alexandria NSW, Australia) (5 mg/kg) for pain relief and 0.9% sodium chloride (Baxter, Old Toongabble NSW, Australia) (2 mL) for cardiovascular support. Over the scalp of the rats, the hair was clipped and the skin aseptically prepared. A single incision was made down the midline, the skull cleared of tissue and the exposed bone dried with 3% hydrogen peroxide (Sanofi, Virginia QLD, Australia).
Implants measuring 3 mm by 3 mm were cut from the polymer sheets described above. Placements of the implants were performed after 5 mm by 4 mm craniotomies were created bilaterally at the level of the coronal suture (over the motor cortices) and after excising the dura to expose the brain surface. Only one of the two craniotomy sites was used to implant the polymer. The site of the polymer implantation was randomly allocated using the random function in Microsoft Excel 2007. Following implant placements, although neither the dural flap nor the bone pieces were replaced, an approximately 20 mm by 10 mm silicone sheet was placed on top of the skull to cover both craniotomy sites and the scalp was sutured closed (3/0 Monosyn® B Braun, Rubi, Spain). Silicone sheets were prepared using implant grade liquid silicone rubber (Cochlear Ltd), LSR 30, which comprises a polymer precursor base and a crosslinker. The polymer precursor base and crosslinker were dissolved in hexane at a ratio of 10:1, cast in a Teflon mould at room temperature, and cured by crosslinking at 110 °C for 30 min and then at 200 °C for 4 h. The silicone sheets were finally extracted with hexane for 72 h to remove any residual oligomers, and dried in vacuo at 60 °C overnight. The animals were placed on heat pads for recovery. Post-operative treatments included SQ buprenorphine (Temgesic® 0.324 mg/mL buprenorphine hydrochloride, Reckitt Benckiser Healthcare Ltd, Hull, UK) every twelve hours (0.03mg/kg, twice a day), saline (2mL, once a day) and carprofen (5mg/kg, once a day) for up to 3 days.

2.4. Animal post-operative health monitoring

For a minimum of three days post-surgery and until full recovery from the surgery, the rats were monitored for weight loss, mobility and grooming twice a day (Table 6.1). At each assessment, a debilitation score was calculated by cumulating weight loss score,
mobility scores and grooming score. A debilitation score of 0, 1 to 3, 4 to 6 and 7 to 9 meant that the rat health was not affected, mildly affected, moderately affected, severely affected by the surgery/polymer implantation respectively.

Table 6.1 Post-operative health monitoring chart.

<table>
<thead>
<tr>
<th>Post-operative health monitoring chart</th>
<th>Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Percentage of weight loss</strong></td>
<td></td>
</tr>
<tr>
<td>No weight loss</td>
<td>0</td>
</tr>
<tr>
<td>Less than 10% weight loss</td>
<td>1</td>
</tr>
<tr>
<td>Weight loss between 10 and 20%</td>
<td>2</td>
</tr>
<tr>
<td>Weight loss of more than 20%</td>
<td>3</td>
</tr>
<tr>
<td><strong>Mobility Score</strong></td>
<td></td>
</tr>
<tr>
<td>Normal mobility</td>
<td>0</td>
</tr>
<tr>
<td>Rat ataxic moving at a normal speed</td>
<td>1</td>
</tr>
<tr>
<td>Rat ataxic and moving slowly</td>
<td>2</td>
</tr>
<tr>
<td>Recumbent rat</td>
<td>3</td>
</tr>
<tr>
<td><strong>Grooming Score</strong></td>
<td></td>
</tr>
<tr>
<td>Normal grooming activity</td>
<td>0</td>
</tr>
<tr>
<td>Grooming activity mildly decreased</td>
<td>1</td>
</tr>
<tr>
<td>Grooming activity significantly decreased</td>
<td>2</td>
</tr>
<tr>
<td>No grooming activity</td>
<td>3</td>
</tr>
</tbody>
</table>

Debilitation score 0-9

For a minimum of three days post-surgery and until full recovery from the surgery, the rats were monitored for weight loss, mobility and grooming twice a day. A debilitation score of 0, 1 to 3, 4 to 6 and 7 to 9 meant that the rat health was not affected, mildly affected, moderately affected, severely affected by the surgery, respectively.

2.5. Sacrifice and sample collection

Seven weeks after surgery, rats were weighed, anesthetized with isoflurane in oxygen and euthanized with pentobarbital (Lethabarb, pentobarbitone sodium 325 mg/mL, Virbac Pty Ltd, Milperra NSW, Australia) (100 mg/kg, intraperitoneal). The brain was immediately carefully dissected and frozen in liquid nitrogen-cooled isopentane and
stored at -80°C. At a later stage, brains were sectioned using a Leica CM 1850 cryostat. Parallel series of coronal sections were cut at 10 µm throughout the implant site, mounted onto chrome-gel-alum subbed microscope slides, air-dried then stored at -80°C until further processing.

2.6. Histological and immunohistochemical analysis

For each of the histological or IHC staining, the sections from all brains were processed simultaneously to ensure similarity in the experimental conditions.

2.6.1. H&E staining

H&E staining was performed similarly to previously published protocol.[16] In brief, slices were removed from the -80°C freezer and leave to air dry for 10 to 30 min. They were then placed into room temperature (RT) 10% Neutral Buffered Formalin (NBF, Sigma–Aldrich, Castle Hill NSW, Australia) for 15 minutes, rinsed with tap water (3*1 min), dipped in Hematoxylin (Gills 3, Sigma–Aldrich, Castle Hill NSW, Australia) for 4 minutes, rinsed with tap water for 5 minutes, differentiated by dipping in ethanol 70% (containing 0.5% HCl) for 20 seconds, rinsed in tap water (1min) and allowed bluing for 15s (0.1% ammonium hydroxide solution in distilled water). After another rinse in tap water (3 min), the slides were dipped into ethanol (50 and 70 %, 1 min each), stained in Eosin 0.6% (Alcoholic, Fisher Scientific, Pittsburgh, PA) for 30 seconds and dipped in ethanol (95% and 100 % for 3 minutes and 2 *1 minute respectively). Finally the slices were cleared in Xylene (Ajax Finechem, Taren Point NSW, Australia) for 2* 5 minutes, mounted in DPX (Sigma–Aldrich, Castle Hill NSW, Australia), and coverslipped.
2.6.2. NeuN/GFAP-IHC staining

NeuN/GFAP staining was performed similarly to previously published protocol.[7] In brief, immediately after taking the slices out of the freezer, they were placed into room temperature (RT) 10% NBF for 15 minutes. Sections were then washed in 0.1M phosphate-buffered saline (PBS, pH=7.4) (3 x 5 min) on a rocking platform and incubated in 10% normal goat serum (NGS) for 1 h RT in a humidified chamber. The slices were washed again in PBS (3 x 5 min) on a rocking platform, before overnight incubations in primary antibodies for neuron-specific nuclear protein (NeuN) and glial fibrillary acidic protein (GFAP) NeuN/GFAP (1/500 and 1:1000 respectively; NeuN - mouse monoclonal MAB377 and GFAP – rabbit polyclonal AB5804; both from Millipore Merck Millipore, Bayswater VIC, Australia) at 4°C in a humid chamber. The following day slides were washed in PBS (3 x 5 min) on a rocking platform, incubated in two secondary antibodies (each at 1/1500; goat anti-mouse IgG (H+L) secondary Antibody, Alexa Fluor® 594 conjugate and goat anti-rabbit IgG (H+L) secondary antibody, Alexa Fluor® 488 conjugate; both from Life technologies, Scoresby VIC, Australia) for 1 h RT in a dark humid chamber, and washed again in PBS (3 x 5 min) on a rocking platform. The slices were then incubated in 4',6-diamidino-2-phenylindole (DAPI, Sigma–Aldrich, Castle Hill NSW, Australia ; 1/5000) for 5 min in the dark on a rocking platform and washed again in PBS (3 x 5min) on a rocking platform. Finally the slices were coverslipped with an aqueous mounting medium (Fluoromont™, Sigma–Aldrich, Castle Hill NSW, Australia) and stored in the dark.
2.6.3. **ED1-IHC staining**

The ED1 staining was performed following the protocol than NeuN/GFAP staining except for the following points: The overnight incubation was performed in primary antibody for anti-CD68 (ED1) (1/1000; mouse monoclonal from Serotec, Bio-Rad Laboratories Pty, Gladesville NSW, Australia) at 4°C in a humid chamber and the secondary antibody used was GaM Alexa Fluor 594 (1/1500). A brain slice from a rat with a confirmed cortical abscess was also ED1 stained and used as a positive control.

2.6.4. **Histological and IHC evaluations**

Images were obtained using a Dot Slide Olympus system using an Olympus BX51 microscope (Olympus Corporation, Tokyo, Japan) and captured using Virtual Slide System Software (VS-ASW-S1 FL 2.5, Olympus Soft Imaging Solution GmbH, Hamburg, Germany).

Both sides (polymer implantation side and sham surgery side) of 15 cortexes’ parallel coronal sections cut throughout the surgery site were evaluated independently by three investigators for each histological and immunohistochemical staining. At the time of the evaluations, the investigators were unaware of the side of the polymer implantation. H&E stained sections were evaluated to assess macroscopic consequences of polymer implantation. The degree of damage was rated following a published scale: minor = incomplete disruption of the meninges and disruption of molecular cortical layer; moderate = complete disruption of the meninges and molecular cortical layer; severe = damage extending beyond the meninges and molecular cortical layer.[7]
Immunohistochemically stained sections were subjectively rated from 0 to 3 (0 = no change in immunoreactivity, 1 = subtle changes in immunoreactivity, 2 = clear changes in immunoreactivity but only affecting a very limited part of the cortex, 3 = clear changes in immunoreactivity affecting an extended area of the cortex).

2.7. Statistical methods

Differences in immunoreactivity between the two sides of the cortexes were evaluated using a multilevel mixed-effects ordered logistic regression using commercially available statistical software (Stata 11.0; StataCorp, College Station, TX). Graphics were made using commercially available software (Prism 6 for Windows, GraphPad Inc., San Diego CA, USA). P-Value of 0.05 was used to distinguish statistically significant differences. Unless specified, the results are reported as median and range.

3. Results and discussion

The thickness of the PPy mats was determined to be 3.1 ± 0.3 µm (Figure 6.1) with a contact angle of 67.8 ± 3.9 deg (n = 20) measured for the free standing film.
The median of the post-operative health monitoring scores was 0.5 (0 to 4) with only
one rat receiving a score above 2 (i.e. score of 4 at the first evaluation of day 2 post-operatively). The median pre-surgical and pre-euthanasia weight of the 6 rats were 152 g (136 to 156) and 186.5 g (168 to 207) respectively representing a weight gain over the 7 weeks implantation period of 24 % (21 to 33). Samples of parallel series of coronal sections representative of the changes seen after H&E, NeuN, GFAP and ED1-IHC staining are presented in Figure 6.2.

3.1. **Histological changes in H&E staining:**

The three evaluators were in agreement for the following results: there was no appreciable differences between the sham and implanted sides of the cortex, and besides the fact that part of the stratum 1 was missing on some of the sections from both sides of the cortex (and not limited to the surgical sites), no additional changes occurred that could have been attributed to the implantation of the polymers (i.e. no evidence of necrosis, infection or inflammation).

3.2. **Neuronal toxicity**

The median NeuN-IHC scores were 0 (0 to 3) (average [± standard error] 0.60 ± 0.05) and 0 (0 to 2) (average score [± standard error] 0.52 ± 0.05) for the sham and implanted polymer sides of the cortexes respectively (Figure 6.3). Overall, there were subtle changes in neuronal density in the boundary layer and the surrounding brain tissues in the two sides of the cortexes and with the sham surgery sides being more affected (p=0.005).
Samples of parallel series of coronal sections that were cut throughout the cortex of a rat with PPy sheets implanted subdurally for a period of 7 weeks. The rat had a polymer implanted on one side of the cortex (A, B, C, D) and a sham craniotomy performed on the other side (A’, B’, C’, D’). Those samples are representative of the changes seen after H&E (A, A’), NeuN (B, B’), GFAP (C, C’) and ED1 (red)/DAPI (blue) (D, D’) immunohistochemistry (IHC) staining. A brain slice from a rat with a confirmed cortical abscess was also ED1 (red)/DAPI (blue) stained (D”) and used as a positive control for ED1 staining. The stratum one is missing from the sham surgery side of the cortex and changes in IHC staining were minor on both sides of the cortex.
Figure 6. 3 Summary of the immunohistochemical scores (NeuN, GFAP and ED1)

Average (and standard error) scores attributed to cortical sections immunohistochemically (ICHIHC) stained with NeuN, GFAP and ED1. PPy mats were implanted subdurally in six adult female GAERS rats. Each rat had a polymer implanted on one side of the cortex and a sham craniotomy performed on the other side. After a period of 7 weeks the rats were euthanized and parallel series of coronal sections were cut throughout the implant site. Fifteen sections per rat for each immunohistochemical stain (NeuN, GFAP and ED1), were subjectively rated from 0 to 3 (0 = no change in immunoreactivity, 1 = subtle changes in immunoreactivity, 2 = clear changes in immunoreactivity but only affecting a very limited part of the cortex, 3 = clear changes in immunoreactivity affecting an extended area of the cortex) by 3 investigators. Differences in immunoreactivity between the two sides of the cortex were evaluated using multilevel mixed-effects ordered logistic regression. *: p=0.005; **: p=0.002.
3.3. **Glial reaction**

The median GFAP-IHC scores were 0 (0 to 2) (average [± standard error] 0.61 ± 0.04) and 0 (0 to 2) (average score [± standard error] 0.44 ± 0.04) for the sham and implanted polymer sides of the cortexes respectively (Figure 6.3). Overall, the increases in GFAP-IHC staining observed were subtle and affected significantly more the sham surgery sides of the cortexes \( (p=0.002) \).

3.4. **Presence of macrophages**

The median ED1-IHC scores were 0 (0 to 3) (average [± standard error] 0.45 ± 0.05) and 0 (0 to 2) (average score [± standard error] 0.40 ± 0.04) for the sham and implanted polymer sides of the cortexes respectively (Figure 6.3). Overall, although ED1-IHC showed significant amounts of macrophages in the positive control brain tissue, only very few macrophages were present in rats implanted with PPy sheets. No difference was observed between the 2 sides of the cortexes \( (p=0.248) \).

PPys were implanted subdurally for a period of 7 weeks in adult female GAERS and using the histological and immunohistological methods (i.e. H&E staining, and NeuN, GFAP and ED1-IHC staining) described above we demonstrated in vivo histobiocompatibility of the PPy with central nervous system (CNS) cells. These results confirmed previously published in vitro work.\(^{[15]}\)

All rats recovered well and rapidly following the surgery. They all gain significant weight during the 7 weeks of the implantation period showing very little impact of the PPys on the growth and vital functions of GAERS.
To be declared histobiocompatible, an implant must: 1) not be toxic to surrounding tissues and 2) not be recognized as foreign material by the immune system. In the presence of acute toxicity, neurons will appear karyopyknotic and in the presence of chronic toxicity, macrophages/monocytes and astrocytes will be activated by necrotic cellular debris resulting from neurons necrosis. Furthermore, as fibroblasts are absent from within the CNS, the scar tissue will originate from the astrocytes forming gliotic scarring. This scarring will also be seen if the implant is recognized as a foreign material. In this case, astrocytes will undergo gliosis and microglia and monocytes/macrophages will initiate a local inflammatory reaction resulting in the formation of multinucleate giant cells (i.e. fusions of multiple macrophages).

The Stratum 1 (or microgyric layer) of the cortex was missing from some of the sections. This variability in brain implant outline was attributable to mechanical procedures during sample preparation. Also, no difference between the sheets implantation side or the sham surgery side of the cortex were seen, the loss of the stratum 1 represents a limitation of the study. Loss of portions of the boundary layer adhering to the implant during its removal from the brain for histological procedures prevents precise examination of interface tissues. This problem could be avoided by keeping the implant in place during all stages of tissue preparation. Nevertheless, on the slices where the stratum 1 is present no major changes were observed (e.g. Figure 6.2 A, B, C, D).

The IHC scores observed on the PPy implanted side of the cortices strongly favor the histobiocompatibility of the PPy. The median IHC scores (0 for all three IHC staining)
shows that there were no evidence of neuronal degeneration, glial reaction or of the presence of macrophages in the majority of the slices rated. However, a previous study reported middle to moderate acute inflammatory reactions after the subcutaneous implantation of PPy in rats followed by a resolution of the inflammatory response.\textsuperscript{22} Our study did not analyze the brain tissue immediately after implantation and may have missed the opportunity to observe an acute inflammatory reaction.

The low average IHC scores (0.40 to 0.52) observed demonstrated that when some IHC staining changes occurred, they were of minimal extent and of lower or similar amplitude than the one observed on the sham surgery sides of the cortexes. The statistically significant differences in IHC scores observed between the PPy implantation sites demonstrate that it was not the lack of statistical power that allowed the conclusion that the PPy does not induce more IHC staining changes compared to a sham surgery. The subjectivity of scale used for grading IHC related changes was taken into consideration during the planning of the study and it was decided that at least 3 evaluators were to grade every brain section. For each of the 6 rats and for each of the 3 IHC staining, 3 series of 15 sections were graded twice (PPy and sham surgery sides) by 3 investigators making a total 1620 IHC related data points that allowed establishment of statistical significances. The absence of a control group (rat not undergoing craniotomy) prevented comparison with normal brain tissue however all evaluator were trained and experimented in IHC evaluation allowing them to have reasonable assumption on what a normal brain tissue should look like.

Any surgery will induce tissue trauma resulting in cell death and tissue scarring. The comparison of NeuN-IHC and GFAP-IHC scores reveals that the amount of neuronal death and gliosis was significantly less in the PPy side than the sham surgery side of the
cortexes ($p$ values of 0.005 and 0.002 respectively) implying that the application of PPy could protect the CNS tissue after surgery. In the present experiment, neither the dura nor the skull flaps were replaced. In a pilot study (unpublished data) we found that during the craniotomy the careful dissection of the dura to maintain a flap intact was performed at the detriment of the brain tissue situated just below. In consequence the dura was peeled away and not replaced. The brain tissue also presented some edema resulting in an expansion of the cortex partially protruding through the craniotomy and in consequence the skull window was not replaced to avoid more mechanical damages. Although the PPy may have just been an additional layer of protection, the potential neuro-protective effects of PPys need to be studied further. Several publications have demonstrated the benefice of the use of PPy to allow tissue proliferation/regeneration. Indeed PPy is an advantageous substrate to enhance nerve cell interaction with potential application as nerve guidance channels to bridge the gap between severed nerve ends.$^{[23]}$ Furthermore, using an ischemia-reperfusion rat myocardial infarction model researchers demonstrated that PPy enhanced infiltration of myofibroblasts into the infarct area highlighting the potential clinical benefit of using this PPy as an injectable scaffold to repair ischemic myocardium after myocardial infarction.$^{[24]}$ The softness of the polymer has probably also contributed to the apparent reduction in local reactive response but the absence of animals implanted with a relatively stiff implant as a negative control prevents firm conclusion. The PPy’s friable nature prevented appropriate retrieval of the implant for adequate explant analysis. Also, very little fibrin was visually detected, on a macroscopic scale, around the implant during brain dissection. The absence of explant analysis prevents to exclude the possibility of a formation of a fibrin capsule which could prevent the polymer to release drug into the
central nervous system.

Due to the absence of evidence of toxic injury or immune mediated inflammation, the authors conclude that PPys offer good histocompatibility with CNS cells and that PPy sheets could potentially be used as intracranial AED delivery implant.

Acknowledgements
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Keywords: cerebral cortex implants, conductive polymers, toxicity, biocompatibility
References


Alternative therapies aiming at improving the availability of anti-epileptic drugs (AEDs) such as the intracranial implantation of polymer-based drug delivery systems have shown some promising results. This blinded controlled prospective randomized study investigated the biocompatibility of polypyrrole (PPy) polymer that will be used for intracranial triggered release AED delivery. We conclude the PPy used offer good histocompatibility with cortical cells.


Corresponding Author*

A brain slice from a rat with a confirmed cortical abscess
Chapter 7 – Conclusion

The aim of the thesis was to report the initial development of polymer based biodegradable implantable drug delivery devices loaded with commonly used AEDs, and test these in an animal model of absence epilepsy. After validating an automated spike and wave complex detection algorithm applied to GAERS EEGs and demonstrating some effects of enrofloxacin on the GAERS epileptic activity, in vitro characterisation and in vivo testing of the antiepileptic efficacy of PLGA polymer loaded with either phenytoin or lacosamide were performed. Tables 1.2 and 1.3 of Chapter 1 were updated to take into account the results presented in Chapters 4 and 5 (Appendix 1).

7.1 Development of the PLGA Polymers

Phenytoin loaded PLGA polymer provided constant delivery of phenytoin for the entire experimentation period of 20 days. This is the first report of the combination of PLGA and phenytoin, and the release period observed exceeded that of most other combinations studied (Appendix 1 Table 1.2). When using EVA polymer loaded with phenytoin, Tamargo et al. (2002) achieved a release period of 105 days corresponding to the experimental period. (Tamargo, Rossell et al. 2002) Additional measurements performed after the polymers were implanted for 365 days allowed establishment of a calculated release period of 3.5 years. However, that prolonged release period was only associated with an in vivo demonstrated reduction in seizure activity over a period of 6 days in a cobalt-induced rat model of epilepsy. (Tamargo, Rossell et al. 2002) In that experiment, the phenytoin loaded EVA polymers were implanted precisely where the cobalt was applied to the cortex.

In Chapter 4, the researchers haven’t been able to demonstrate that phenytoin loaded PLGA polymer sheets implanted on the surface of the cortex of GAERS could decrease seizure activity in GAERS for a sustained period. The phenytoin was chosen for its lipophilic properties and its proven efficacy in GAERS when applied directly over S1po and S1FL. (Gurbanova, Aker et al. 2006) The poor water solubility allowed easy drug loading within the polymer and prolong release of the AED. The minimal amount of
phenytoin released in the in vitro experiment may have been a contributing factor to the negative results. The phenytoin mats were placed over the motor cortex and the delivery of phenytoin even only 2 mm cranially to S1po and S1Fl could have also contributed to the absence of changes in duration and numbers of SWDs. Throughout the different experiment

The in vitro release results for the lacosamide loaded PLGA mats presented in Chapter 5 showed a prolonged release of lacosamide over the entire experimentation period of 100 days with more than 90% of the drug being released within 60 days (i.e. PLGA-12.5% lacosamide). By comparison, Chen et al. (2017) only reported a release period of up to 250 hours depending on the nature of the lacosamide loaded PLGA device used. (Chen, Gu et al. 2017) In that experiment, three PLGA/lacosamide (w/w, 10/1) solutions prepared with the PLGA concentration varying from 1.5 wt%, 4.5 wt%, to 14.0 wt% were electrospun and resulted in the formation of microspheroids, microspheres, and microfibers respectively. The microfibers demonstrate the least initial burst release while the spheroids exhibit the most rapid release characteristics. The polymer used in Chapter 5 was made using a coaxial electrospinning method meaning that the core of the polymer was composed of 75:25 PLGA (lactide/glycolide = 75:25), and had a very similar structure to the one used in the Chen et al. (2017) study; and a shell composed of 85:15 PLGA (lactide/glycolide = 85:15) which likely allowed a more prolonged release of the lacosamide. Nevertheless, the Chen et al. (2017) study confirms the author’s choice to use PLGA fibres rather than microspheres for long term constant AED delivery. To the best of the author’s knowledge, no other polymer containing lacosamide has been described in the peer-reviewed scientific literature.

The lacosamide loaded polymer implanted subdurally on the surface of the motor cortex of GAERS in Chapter 5 affected seizure activity in GAERS by decreasing the mean duration of the SWDs events for a sustained period of up to 7 weeks. The evidence suggests that intracranial implantation of polymer-based drug delivery systems could be used successfully in the treatment of epilepsy. This decrease in seizure duration was however associated with significant side effects during the post-operative period. Just before implantation, the PLGA sheets were cut with scissors, potentially leaving the
core of the fibres of the implants open to leakage. It was speculated that the high debilitation scores encountered could have been the result of a significant initial leakage of lacosamide from the polymer. To test that theory another experiment was designed using laser cut lacosamide loaded PLGA mats. The hypothesis of that experiment was that by laser cutting the polymer and consequently sealing the edges of the polymer mats, the more porous core of the polymer would not be able to release as much lacosamide immediately after polymer implantation. Because no in vitro study was performed to characterise those implants, the experiment was not included as a chapter in the thesis but is presented in Appendix 2. The experimental protocol was similar to the one described in Chapter 5. The number of seizures, the mean duration of a seizure and the total duration of seizures were not significantly different except during week one when they were all significantly lower in the lacosamide polymer group compared to the control group. None of the rats from the lacosamide polymer group were moderately or severely affected by the surgery. As it has been demonstrated in Chapter 4, the craniotomy itself can be responsible for a decrease in seizure activity for up to three weeks following the surgery. In consequence there was no evidence of any beneficial or negative effects of the lacosamide and it can be assumed that by laser cutting the polymer and consequently sealing the edges of the polymer mats, the more porous core of the polymer likely did not release as much lacosamide immediately after polymer implantation. The speculated reason for the high debilitation scores for the lacosamide polymer group observed in Chapter 5 is also plausible although additional experiments would need to be performed to confirm this.

The experiments presented in Chapters 4 and 5 took the common approach of testing a drug and reporting the effects. Retrospectively, an alternative approach could have been to first determine the minimum lacosamide intravenous dose that produces the desired effect in 90% of the GAERS, and to determine the corresponding lacosamide concentrations achieved in the different parts of the brain as a reference. Once these are established, lacosamide loaded polymers could be implanted subdurally, and brain tissue concentrations of lacosamide measured at different time points and compared to the theoretical target. Based on these comparisons, the structure of the composition of the polymer sheets could have been optimised and the antiepileptic effects tested. In
other words, one major limitation of the studies presented in Chapters 4 and 5 is the unknown pharmacokinetics and pharmacodynamics of lacosamide within the central nervous system when administered using polymer devices implanted subdurally.

This alternative approach would not have been suitable for the phenytoin polymers, as phenytoin has no anti-epileptic effect when administered systemically in GAERS. Also, as mentioned in the introduction, little is known about the mechanism of action of AEDs and we do not know with certainty which part of the brain should be targeted. Consequently, we could potentially have measured lacosamide tissue concentrations in areas of the brain that have no significance in relation to the anti-epileptic effects of the drug.

The implantation of the AED loaded polymers comes with significant medical risk such as meningitis, stroke, brain tissue damage and haemorrhage, and shouldn’t be repeated frequently. In practice, the patient would probably receive a generic polymer delivering a certain amount of drug that may or may not be adequate. The AED and the associated polymer to be implanted may be chosen based on the type of seizures, medical history, size of the patient and other objective criteria; however the clinician is unlikely to know for a particular patient the exact amount of drug that will be required for the treatment of the seizures. The rate at which the AED is released from an implanted passive release can only be modified by removing or implanting additional devices requiring potentially several additional invasive procedures.

The lack of flexibility in the AED delivery rate is a major limitation of the PLGA polymer and is the reason why after establishing proof of concept that intracranial implantation of polymer-based drug delivery systems could be used successfully in the treatment of epilepsy, the resources were directed towards active release polymers such as PPy.

### 7.2 Development of the PPy polymers

Drugs can be incorporated into the polymer as dopant anions to counterbalance the positive charge associated with the oxidative polymerization (Chapter 1.4.3.2) or loaded after polymerization through ion exchange by redox cycling the polymer in appropriate media.(Uppalapati, Boyd et al. 2016) In reverse, upon intermittent application of an
electrical current, the electrostatic interactions between the polymer and the negatively charged drug change resulting in release of the drug. This advantage of being reactive to electrical stimulation would allow an endogenous or exogenous electrical current to control the release of an AED. The electrical firing of the brain during a seizure could be such endogenous stimulation although the release of the AED would treat and not prevent the seizure from happening. An exogenous electrical stimulation coming from an implanted seizure advisory system would offer more flexibility by also providing treatment during periods of fair or high likelihood of a seizure event and by adjusting the intensity of the electrical stimulation and therefore the amount of drug being released. Electrical stimuli can be applied through tissues and therefore the electrodes delivering the current could be positioned over the dura while the polymer itself could be placed under the dura. No wire or tubing will permanently compromise the isolation of the brain as it is the case with a mini-osmotic pump (Chapter 1.3.3). However, one limitation remains, which is that the nature of the polymer requires the drug to be charged for incorporation, and therefore a significant number of commonly used AEDS would not be suitable for administration through this device. For example, phenytoin could not be loaded into a PPy polymer, although its pro-drug fosphenytoin could. The effectiveness of fosphenytoin when administered directly onto the brain parenchyma will need to be studied as it is unknown if the hydrolysis of fosphenytoin to phenytoin would happen and if other residual products such as formaldehyde could negatively impact the brain.

The results of the study presented in Chapter 6 showed for the first time that implanted PPy mats on top of the brain cortex of GAERS did not induce obvious inflammation, trauma, gliosis and neuronal toxicity. Therefore, the results are demonstrating in vivo that the PPy used offered good histocompatibility with central nervous system cells and that PPy sheets could be used as a drug delivery device implanted onto the surface of the brain parenchyma. These results confirmed those of a previous study where PPy sheets have been shown to be well tolerated by biological tissues, including brain tissues. (George, Lyckman et al. 2005, Ateh, Navsaria et al. 2006) Nevertheless, an acute short lasting inflammatory reaction leading to the formation of a fibrous capsule, around the polymer, hindering the release of drug could have been missed. The friability of the polymer did not allow explant analysis and it would limit the ability of the polymer to
be linked to any other device. For future experiments, the polymer would benefit from structural support, such as being fabricated onto a platinum surface.

As mentioned in the discussion of Chapter 6, the comparison of NeuN-IHC and GFAP-IHC scores reveals that the amount of neuronal death and gliosis was significantly less in the PPy side than the sham surgery side of the cortex implying that the application of PPy could protect the CNS tissue after surgery. PPy substrates have been used as nerve guidance channels to bridge the gap between severed nerve ends and as injectable scaffold to repair ischemic myocardium after myocardial infarction.\(^{(Schmidt, Shastri et al. 1997, Wang, Roberge et al. 2004)}\) The combination of a “neuro-protector” and an AED has the potential to help reduce the incidence of complications such as seizures following craniotomies, brain resections and brain trauma. In those cases, the isolation of the brain has already been compromised and the benefits resulting from the implantation of AED/PPy polymer could be superior to the risks. Further studies would need to be performed to confirm such hypothesis.

### 7.3 What are the challenges in the development of an implantable drug delivery device for the treatment of epilepsy?

The challenges in the development of an implantable drug delivery device for the treatment of epilepsy are numerous and complex. They include but are not limited to:

- **The nature of the materials used**

  As mentioned in the introduction (table 1.2), multiple kinds of polymers have been used to investigate the delivery of antiepileptic drugs. PLGA polymers were chosen because of their known biocompatibility, their efficacy in drug loading and delivery, and the experience in its fabrication that the collaborators working on this thesis had. Other polymers could have been used but it is beyond the scope of the thesis to compare the characteristic of all kinds of polymer available.

- **The ability to control/vary/tune drug release profiles**

  One major limiting factor in the use of PLGA polymers is the inability to adjust the release of AEDs. PPy was investigated to enable drug delivery on demand. The PPy drug release will need to be tested and the connection of the PPy polymer to electrodes
may prove to be challenging. Because of its friable nature, the PPy polymers may need to be grown onto a platinum plate to secure such connection.

- The effect of the drug on the event and population of animal used in studies

Because seizures can have multiple underlying pathophysiologies one drug/polymer combination may not have consistent effects. Even if we prove that lacosamide loaded PPy polymers alleviate seizure in GAERS, there will remain uncertainty to whether or not such polymers will be efficacious to treat seizure in human. The AED loaded polymers will likely need to be tested in multiple animal models and still risk having narrow therapeutic indications.

- The location and type of implant

Because the diffusion of AEDs from the polymer remains localised (a few mms of tissue penetration) the polymer needs to be implanted near the location of the origin of the seizure. The type of implant (i.e. mats versus injectable) may then depend of the localisation of the origin of the seizure. Implantable polymers may be preferable for seizure foci close to the periphery of the cortex and injectable polymers for more in depth seizure foci.

7.4 What is next?

Coming from a different background and as a PhD candidate, one thing that this journey taught me is how little is known about the brain and its function, and consequently about its pathophysiology and treatments. Major initiatives focusing on revolutionizing the understanding of the brain such as the “White House Neuroscience Initiative” are primordial for the development of effective therapies for neurological conditions such as epilepsy. Without significant advancements in our understanding of the brain, the development of anti-epileptic treatments such as polymer based implantable drug delivery devices will remain trial and error based. Furthermore, a wide range of professionals needs to be involved: neurologists for the identification of the patient’s need, material scientists for the development of the polymer and refinement of the drug loading and unloading ability, engineers for the integration of the polymer within an interactive device, neuroscientists and veterinarians for the trials of such devices in animal models and neurosurgeon to facilitate the transition to human clinical trials.
After demonstrating biocompatibility, the next steps in the development of the PPy implantable delivery device would be to identify an appropriate AED that can be loaded into the PPy, study the ideal conditions of polymerisation for the maximal drug loading while still optimizing drug release characteristics for long term efficient drug delivery. The delivery of AEDs will ideally be triggered by a device predicting the likelihood of seizure occurrence. Automatic drug administration would then occur only when most needed decreasing side effects and greatly improving patients’ quality of life.

Once appropriate in vitro release data have been obtained, the anti-epileptic effects of the device can be tested in different in vivo animal models of epilepsy. In addition to long term histocompatibility studies, the development of intracranial implantable devices will require functional studies for biocompatibility to be performed. Cognitive function, motor functions, memory, and other cortical and limbic functions must not be significantly compromised by PPy implantation.

The neuro-protective effect of PPy should also be investigated in a brain trauma model. For that particular application, PPy can be loaded with AEDs but could also be loaded with other drugs such as anti-inflammatories (e.g. dexamethasone).(Jiang, Sun et al. 2013) While the development of an antiepileptic device is still years away, the development of an implantable device to be used to decrease post-craniotomy brain tissue inflammation would certainly be quicker to develop.
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Appendices

Appendix 1– Updated Table 1.2 and Table 1.3

Table 1.2 and 1.3 of chapter 1 were updated to take into account the results presented in chapter 4 and 5.
Updated table 1.2 Anti-epileptic drugs delivered using polymer-based devices (in vitro experiments).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Polymer</th>
<th>Device type</th>
<th>In vitro release time</th>
<th>Release profile</th>
<th>Paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetazolamide</td>
<td>Eudragit</td>
<td>Injectable</td>
<td>10 hours (EOE) however most within 4 hours</td>
<td>Biphasic</td>
<td>(Duarte, Roy et al. 2007)</td>
</tr>
<tr>
<td>Acetazolamide</td>
<td>Eudragit</td>
<td>Injectable</td>
<td>7 hrs (EOE)</td>
<td>Linear</td>
<td>(Hanzedar and Dortunc 2004)</td>
</tr>
<tr>
<td>Adenosine</td>
<td>EVA</td>
<td>Implantable</td>
<td>17 days (EOE) however most within 4 days</td>
<td>Triphasic</td>
<td>(Boison, Scheurer et al. 1999)</td>
</tr>
<tr>
<td>Adenosine</td>
<td>Silk</td>
<td>Implantable</td>
<td>14 days (EOE)</td>
<td>Biphasic</td>
<td>(Wilz, Pritchard et al. 2008)</td>
</tr>
<tr>
<td>Adenosine</td>
<td>Silk</td>
<td>Implantable</td>
<td>14 days (EOE)</td>
<td>Linear</td>
<td>(Pritchard, Szybala et al. 2010)</td>
</tr>
<tr>
<td>Adenosine</td>
<td>Silk</td>
<td>Implantable</td>
<td>21 days (EOE)</td>
<td>Triphasic</td>
<td>(Szybala, Pritchard et al. 2009)</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Chitosan</td>
<td>Injectable</td>
<td>Between 30 and 60 minutes</td>
<td>Linear</td>
<td>(Dong, Mancent et al. 2007)</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Eudragit</td>
<td>Injectable</td>
<td>5 hours (EOE)</td>
<td>Biphasic</td>
<td>(Filipovic-Grcic, Perissutti et al. 2003)</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Gelucire</td>
<td>Injectable</td>
<td>10 minutes (EOE)</td>
<td>Linear</td>
<td>(Fresta, Cavallaro et al. 1996)</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>PECA</td>
<td>Injectable</td>
<td>4 hours (EOE)</td>
<td>Biphasic</td>
<td>(Cho, Han et al. 1999)</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>PGLA</td>
<td>Injectable</td>
<td>24 hours (EOE)</td>
<td>Biphasic</td>
<td>(Barakat and Radwan 2006)</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Precifac</td>
<td>Injectable</td>
<td>12 hrs (EOE)</td>
<td>Linear</td>
<td>(Barakat and Yassin 2006)</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>PVP/PVA</td>
<td>Injectable</td>
<td>1 hr (EOE)</td>
<td>Linear</td>
<td>(Patterson, James et al. 2008)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>PCL/PEG</td>
<td>Injectable</td>
<td>Up to 250 days (EOE)</td>
<td>Biphasic</td>
<td>(Benelli, Conti et al. 1998)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>PBLG/PEO</td>
<td>Injectable</td>
<td>8 days (EOE)</td>
<td>Linear</td>
<td>(Jeong, Cheon et al. 1998)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>CEC</td>
<td>Injectable</td>
<td>100 hours (EOE)</td>
<td>NA</td>
<td>(Ryu, Jeong et al. 2000)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>PLAG</td>
<td>Injectable</td>
<td>24 hrs (EOE)</td>
<td>Linear</td>
<td>(Montanari, Cirillo et al. 2001)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>PLAG</td>
<td>Injectable</td>
<td>8 to 9 days (EOE)</td>
<td>Biphasic</td>
<td>(Benelli, Conti et al. 1998)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>PLAG</td>
<td>Injectable</td>
<td>7 days (EOE)</td>
<td>Linear</td>
<td>(Nah, Paek et al. 1998)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>PLGA (microspheres)</td>
<td>Injectable</td>
<td>70 days</td>
<td>Biphasic</td>
<td>(Nkansah, Tzeng et al. 2008)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>PLGA (nanospheres)</td>
<td>Injectable</td>
<td>Most after 14 days</td>
<td>Triphasic</td>
<td>(Nkansah, Tzeng et al. 2008)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>EVA</td>
<td>Implantable</td>
<td>21 days (EOE)</td>
<td>Linear</td>
<td>(Haik-Creguer, Dunbar et al. 1998)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>PHBV (microspheres)</td>
<td>Injectable</td>
<td>30 days (EOE)</td>
<td>Trifasic</td>
<td>(Chen and Davis 2002)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>(Diazepam/Gelatine in PHBV microspheres)</td>
<td>Injectable</td>
<td>30 days (EOE)</td>
<td>Biphasic</td>
<td>(Chen and Davis 2002)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>PHBV/Gelatine (microcapsules)</td>
<td>Injectable</td>
<td>30 days (EOE)</td>
<td>Linear</td>
<td>(Chen and Davis 2002)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>PLA</td>
<td>Injectable</td>
<td>NA</td>
<td>NA</td>
<td>(Bodemeyer and McGinity 1987)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>PDLLA</td>
<td>Injectable</td>
<td>8 days, most after 1 day</td>
<td>Biphasic</td>
<td>(Giunchedi, Conti et al. 1998)</td>
</tr>
<tr>
<td>Diazepam</td>
<td>PLGA</td>
<td>Injectable</td>
<td>Most within 10 h</td>
<td>Linear</td>
<td>(Bohrey, Chourasysa et al. 2016)</td>
</tr>
<tr>
<td>Ethosuxamid</td>
<td>PECA</td>
<td>Injectable</td>
<td>4 hrs (EOE), most after 3 hours</td>
<td>Linear</td>
<td>(Fresta, Cavallaro et al. 1996)</td>
</tr>
<tr>
<td>Ethosuxamid</td>
<td>PLGA</td>
<td>Injectable</td>
<td>24 hrs (EOE)</td>
<td>Linear</td>
<td>(Montanari, Cirillo et al. 2001)</td>
</tr>
<tr>
<td>GABA</td>
<td>EVA</td>
<td>Implantable</td>
<td>24 to 72 hours</td>
<td>Biphasic</td>
<td>(Kokaa, Aebischer et al. 1994)</td>
</tr>
<tr>
<td>GABA</td>
<td>PNP</td>
<td>Injectable</td>
<td>6 hrs (EOE)</td>
<td>Linear</td>
<td>(Yurdas Kirinlioglu, Menceloglu et al. 2016)</td>
</tr>
<tr>
<td>Gabapentine</td>
<td>Albumine nanoparticles coated with polysorbate</td>
<td>Injectable</td>
<td>24 hours (EOE)</td>
<td>Biphasic</td>
<td>(Wilson, Lavanya et al. 2014)</td>
</tr>
</tbody>
</table>
Updated table 1.2. Anti-epileptic drugs delivered using polymer-based devices (in vitro experiments) (cont’d).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Polymer</th>
<th>Device type</th>
<th>In vitro release time</th>
<th>Release profile</th>
<th>Paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lacosamide</td>
<td>PLGA</td>
<td>Injectable/</td>
<td>Up to 250 hours (EOE)</td>
<td>NA</td>
<td>(Chen, Gu et al. 2017)</td>
</tr>
<tr>
<td>Lacosamide</td>
<td>PLGA</td>
<td>Implantable</td>
<td>100 days (EOE) however most within 60 days</td>
<td>Multiphasic</td>
<td>(Bauquier, Jiang et al. 2016)</td>
</tr>
<tr>
<td>Levetiracetam</td>
<td>PLGA</td>
<td>Implantable</td>
<td>Most within 3 days</td>
<td>NA</td>
<td>(Halliday, Campbell et al. 2013)</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>PLGA</td>
<td>Injectable</td>
<td>24 hours (EOE)</td>
<td>Biphasic</td>
<td>(Sharma, Maheshwari et al. 2014)</td>
</tr>
<tr>
<td>MK-801</td>
<td>EVA</td>
<td>Implantable</td>
<td>60 days (EOE) however most within 30 days</td>
<td>Biphasic</td>
<td>(Smith, Cordery et al. 1995)</td>
</tr>
<tr>
<td>Muscimol</td>
<td>LPSPs</td>
<td>Injectable</td>
<td>5 days however most within 20 hours</td>
<td>Biphasic</td>
<td>(Kohane, Holmes et al. 2002)</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>EVA</td>
<td>Implantable</td>
<td>Within 24 hours</td>
<td>Biphasic</td>
<td>(Kokaa, Aebischer et al. 1994)</td>
</tr>
<tr>
<td>Oxcarbazepine</td>
<td>Chitosan</td>
<td>Injectable</td>
<td>NA</td>
<td>NA</td>
<td>(Rane, Mashru et al. 2007)</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>PLGA</td>
<td>Injectable</td>
<td>NA</td>
<td>NA</td>
<td>(Barichello, Morishita et al. 1999)</td>
</tr>
<tr>
<td>Phenobarbitone</td>
<td>PCL</td>
<td>Injectable</td>
<td>NA</td>
<td>NA</td>
<td>(Berrabah, André et al. 1994)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>EVA</td>
<td>Implantable</td>
<td>105 days (EOE)</td>
<td>Biphasic</td>
<td>(Tamargo, Rossell et al. 2002)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>PCL</td>
<td>Injectable</td>
<td>22 days (EOE) however mostly within 12 days</td>
<td>Triphasic</td>
<td>(Li, Li et al. 2007)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>PECA</td>
<td>Injectable</td>
<td>4 hrs (EOE)</td>
<td>Biphasic</td>
<td>(Fresta, Cavalaro et al. 1996)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>PCL</td>
<td>Injectable</td>
<td>30 days (EOE) however mostly within 4 days</td>
<td>Triphasic</td>
<td>(Jiang, Yue et al. 2015)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>PLGA</td>
<td>Implantable</td>
<td>20 days (EOE)</td>
<td>Linear</td>
<td>(Bauquier, PhD thesis)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>ANG-ERHNPs</td>
<td>Injectable</td>
<td>24 hours (EOE)</td>
<td>Triphasic</td>
<td>(Ying, Wang et al. 2014)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>ANG-ERHNP</td>
<td>Injectable</td>
<td>4 hours (EOE) (with electrical field stimulation)</td>
<td>NA</td>
<td>(Wang, Ying et al. 2016)</td>
</tr>
<tr>
<td>Pregabalin</td>
<td>Alginate/HPMC</td>
<td>Injectable</td>
<td>12 hours (EOE)</td>
<td>Biphasic</td>
<td>(Madar, Adokar et al. 2015)</td>
</tr>
<tr>
<td>Primidone</td>
<td>PCL</td>
<td>Injectable</td>
<td>10 hours (EOE) however most within 1 hours</td>
<td>Triphasic</td>
<td>(Ferranti, Marchais et al. 1999)</td>
</tr>
<tr>
<td>Valproate</td>
<td>Hexadecanol</td>
<td>Injectable</td>
<td>8 hours</td>
<td>Biphasic</td>
<td>(Giannola, De Carlo et al. 1993)</td>
</tr>
<tr>
<td>Valproate</td>
<td>PLGA</td>
<td>Injectable</td>
<td>NA</td>
<td>NA</td>
<td>(Barichello, Morishita et al. 1999)</td>
</tr>
</tbody>
</table>

ANG, Angiopep; CEC, Triblock copolymers synthesized by non-catalyzed ring opening polymerization of caprolactone in the presence of PEG; CNF, Ciliary neurotrophic factor; EOE, End of experiment; ERHNPs, Electro-responsive hydrogel nanoparticles; EVA, Ethylene vinylacetate; GABA, Gamma amino butyric acid; HPMC, Hydroxypropyl methylcellulose; LPSPs, Lipid protein sugar particles; MK-801, Methyl-IO,II-dihydro-SH-dibenzo[a,d]cyclohepten-5,10-imine maleate; NA, Not available; PBLG, Polybenzy1 l-glutamate; PCL, polycaprolactone; PDLLA, Poly(D,L-Lactide); PECA, Polyethylcyanoacrylate; PEO, Poly(ethylene oxide); PEG, Polyethyleneglycol; PHBV, Poly(3-hydroxybutyrate-co-hydroxyvalerate); PLA, Poly(dl-lactide); PLGA, Poly-lactide-co-glycolide; PNP, dimethylacrylamide-based pegylated polymeric nanoparticles; PVA, Polyvinyl acetate ; PVP, Polyvinyl pyrrolidone ; SA, Sebacic acid.
Updated Table 1. 3 Anti-epileptic drugs delivered using polymer-based devices (in vivo experiments).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Polymer</th>
<th>Device type</th>
<th>Animal model</th>
<th>Delivery</th>
<th>In vivo results</th>
<th>Paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine</td>
<td>EVA</td>
<td>Implantable</td>
<td>Kindled rats</td>
<td>Rod, unilateral implantation in ventricle</td>
<td>1 week seizure reduction</td>
<td>(Boison, Scheurer et al. 1999)</td>
</tr>
<tr>
<td>Adenosine</td>
<td>Silk</td>
<td>Implantable</td>
<td>Kindled rats</td>
<td>Rod, unilateral implantation in the infrahippocampal cleft</td>
<td>Dose-dependent delay in kindling acquisition for 11 days, and reduce after discharge duration</td>
<td>(Wilz, Pritchard et al. 2008)</td>
</tr>
<tr>
<td>Adenosine</td>
<td>Silk</td>
<td>Implantable</td>
<td>Kindled rats</td>
<td>Rod, unilateral implantation in the infrahippocampal cleft</td>
<td>Protection from generalized seizures over a period of 10 days of suppressed seizures, delay in epileptogenesis</td>
<td>(Szybala, Pritchard et al. 2009)</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Chitosan</td>
<td>Injectable</td>
<td>Sheep</td>
<td>Powder Intranasal</td>
<td>Three hours increased drug concentration in the serum when compared to the nasal administration of the pure drug</td>
<td>(Gavini, Hegge et al. 2006)</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Eudragit, HPMC</td>
<td>Injectable</td>
<td>Rabbits</td>
<td>Oral (powder in capsules)</td>
<td>Ten hours increased plasma levels</td>
<td>(Dong, Maincent et al. 2007)</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>PCL/PEG</td>
<td>Implantable</td>
<td>Rabbits</td>
<td>IP pellet</td>
<td>Constant plasma concentration of clonazepam for 45 days after initial burst effect</td>
<td>(Cho, Han et al. 1999)</td>
</tr>
<tr>
<td>GABA</td>
<td>EVA</td>
<td>Implantable</td>
<td>Kindled rats</td>
<td>Bilateral implantation into the mesencephalon dorsal to the substantia nigra</td>
<td>At 48 h, generalized convulsions were prevented</td>
<td>(Kokaia, Aebischer et al. 1994)</td>
</tr>
<tr>
<td>GABA</td>
<td>PNP</td>
<td>Injectable</td>
<td>Rats, PTZ-induced acute seizure model</td>
<td>IP nanoparticles</td>
<td>Shorter seizure and decrease in mortality</td>
<td>(Yurttas Kirimlioglu, Menceloglu et al. 2016)</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>Albumin nanoparticles coated with polysorbate</td>
<td>Injectable</td>
<td>Rats, PTZ and electrocortic induced convulsion</td>
<td>IP nanoparticles</td>
<td>Increase gabapentin brain concentration, decrease duration of all phases of convulsion</td>
<td>(Wilson, Lavanya et al. 2014)</td>
</tr>
<tr>
<td>Lacosamide</td>
<td>PLGA</td>
<td>Implantable</td>
<td>GAERS</td>
<td>Bilateral implantation above the motor cortex</td>
<td>Decrease in seizure activity for up to 7 weeks</td>
<td>(Bassauer, Jiang et al. 2016)</td>
</tr>
<tr>
<td>Levetiracetam</td>
<td>PLGA</td>
<td>Implantable</td>
<td>Rats, hippocampal tetanus toxin model of mesial temporal lobe epilepsy</td>
<td>Bilateral implantation above motor and somatosensory cortices</td>
<td>Trend towards a reduction in seizure frequency</td>
<td>(Halliday, Campbell et al. 2013)</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>PLGA</td>
<td>Injectable</td>
<td>Rats</td>
<td>Intranasal</td>
<td>Increased brain concentration for up to 8 hours</td>
<td>(Sharma, Maheshwari et al. 2014)</td>
</tr>
<tr>
<td>Lorazepam</td>
<td>PLGA</td>
<td>Injectable</td>
<td>Sheep nasal mucosa</td>
<td>Ex vivo, biphasic controlled release 58% (24 hours)</td>
<td></td>
<td>(Sharma, Maheshwari et al. 2014)</td>
</tr>
<tr>
<td>MK-801</td>
<td>EVA</td>
<td>Implantable</td>
<td>-</td>
<td>Implanted over brain area for 28 to 65 days followed by in vitro release studies</td>
<td>Still releasing drug after 65 days in vivo (EOE). The in vitro release profiles appears to predict accurately the in vivo release.</td>
<td>(Smith, Cordery et al. 1995)</td>
</tr>
</tbody>
</table>
Updated table 1.3 Anti-epileptic drugs delivered using polymer-based devices (*in vivo* experiments) (cont’d).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Polymer</th>
<th>Device type</th>
<th>Animal model</th>
<th>Delivery</th>
<th>In vivo results</th>
<th>Paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscimol</td>
<td>LPSPs</td>
<td>Injectable</td>
<td>Rats, hippocampal injection of pilocarpine</td>
<td>Unilateral injection into the hippocampus (i.e. seizure focus)</td>
<td>Prevents seizures formation for up to 120 min (EOE)</td>
<td>(Kohane, Holmes et al. 2002)</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>EVA</td>
<td>Implantable</td>
<td>Rats, interventricular treatment with 6-hydroxydopamine</td>
<td>Bilateral implantation of matrices and electrode in the hippocampus</td>
<td>No change in kindling acquisition</td>
<td>(Kokaia, Aebischer et al. 1994)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>EVA</td>
<td>Implantable</td>
<td>Cobalt-induced rat model of epilepsy</td>
<td>Unilateral implantation in cortical seizure focus</td>
<td>Reduction in seizure activity for 6 days (EOE)</td>
<td>(Tamargo, Rossell et al. 2002)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>EVA</td>
<td>Implantable</td>
<td>Rat</td>
<td>Two weeks in vitro release study following 365 days intracortical implantation</td>
<td>NA</td>
<td>(Tamargo, Rossell et al. 2002)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>PCL</td>
<td>Injectable</td>
<td>Rats, hippocampal injection of tetanus toxin</td>
<td>Unilateral implantation into cortical seizure focus</td>
<td>Significant reduction in epileptic events for 3 days with no observed clinical side effects.</td>
<td>(Jiang, Yue et al. 2015)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>PLGA</td>
<td>Implantable</td>
<td>GAERS</td>
<td>Bilateral implantation above the motor cortex</td>
<td>No change compare to blank polymer group</td>
<td>(Bauquier, PhD Thesis)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>ANG-ERHNP</td>
<td>Injectable</td>
<td>Amygdala-kindled seizures in rats</td>
<td>IP nanoparticles</td>
<td>PHT high concentrations in the hippo-campus, amygdala, cerebellum, and brainstem regions, decrease in the kindling-stimulation-induced seizures</td>
<td>(Ying, Wang et al. 2014)</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>ANG-ERHNP</td>
<td>Injectable</td>
<td>Mices, electrical- (maximal electrical shock) and chemical-induced (pentylenetetrazole and pilocarpine) seizure models</td>
<td>IP nanoparticles</td>
<td>Lowered the effective therapeutic doses of PHT and demonstrated the improved anti-seizure effects</td>
<td>(Wang, Ying et al. 2016)</td>
</tr>
<tr>
<td>Piperine</td>
<td>ChitosanSTPP NPs</td>
<td>Injectable</td>
<td>Mices, PTZ-induced kindling model</td>
<td>IP nanoparticles</td>
<td>Enhance the neuroprotection and ameliorate the astrocytes activation</td>
<td>(Anisian, Ghasemi-Kasman et al. 2017)</td>
</tr>
<tr>
<td>TRH</td>
<td>FAD/SA</td>
<td>Implantable</td>
<td>Kindled rats</td>
<td>Unilateral implantation in amygdala</td>
<td>Reduced seizure duration for 50-60 days (EOE)</td>
<td>(Kubek, Liang et al. 1998)</td>
</tr>
<tr>
<td>Valproate</td>
<td>PCL</td>
<td>Implantable</td>
<td>Rats, necocortical injected tetanus toxin model</td>
<td>Implanted above the cortical seizure focus</td>
<td>Decrease in epileptiform potential however the model failed to induce consistent seizures.</td>
<td>(Rassner, Hebel et al. 2015)</td>
</tr>
</tbody>
</table>

ANG, Angiopep; EOE, End of experiment; ERHNP, Electro-responsive hydrogel nanoparticles; EVA, Ethylene vinylacetate; FAD, Fatty acid dimer; GABA, Gamma amino butyric acid; GAERS, Genetic absence epilepsy rats from Strasbourg; HPMC, Hydroxypropyl methylcellulose; IP, Intraperitoneal; LPSPs, Lipid protein sugar particles; MK-801, Methyl-IO,Il-dihydro-SH-dibenzo[a,d]cyclohepten-5,10-imine maleate; NA, Not available; NPs, Nanoparticles; PCL, polycaprolactone; PEG, Polyethylene glycol; PLGA, Poly-lactide-co-glycolide; PNP, dimethylacrylamide-based pegylated polymeric nanoparticles; PTZ, Pentylenetetrazole; SA, Sebacic acid; STTP, Sodium tripolyphosphate; TRH, Thyrotropin-releasing hormone.
Appendix 2- In vivo evaluation of the anti-epileptic effects of laser cut lacosamide loaded polymers implanted sub-durally in GAERS rats

Contribution

The work described in Appendix 2 was performed at the Centre for Clinical Neurosciences and Neurological Research, (St. Vincent’s Hospital Melbourne, P.O. Box 2900, Fitzroy, Victoria 3065, Australia). The polymers were provided by the University of Wollongong and the in vivo experiments were performed by Sébastien Bauquier.

Introduction

In chapter 5, the authors demonstrated a decrease in seizure activity as a result of the subdural implantation of lacosamide loaded PLGA in GAERS. Though, the decrease was associated with a significant debilitation of the rats during the post-operative period.

Lacosamide-laden polymer mats were produced using a coaxial electrospinning method with the core of the polymer composed of lacosamide 75:25 PLGA (lactide/glycolide = 75:25), and the shell composed of 85:15 PLGA (lactide/glycolide = 85:15). The shell was designed to slow the release of the lacosamide from the core of the polymer. Nevertheless, the PLGA implants were cut with scissors from the PLGA mats, potentially cutting through the PLGA fibres leaving the core of the polymer open to leakage. It was speculated that the high debilitation scores encountered could have been the result of a significant initial leakage of lacosamide from the polymer.

We are now investigating the efficacy of a laser cut lacosamide loaded PLGA mats in reducing seizure activity in GAERS. The hypothesis is that by laser cutting the polymer and consequently sealing the cut fibres of the polymer implant, the more porous core of the polymer would not be able to release as much lacosamide immediately after polymer implantation.
Material and methods:

There was no *in vitro* study performed. The *in vivo* experiment followed the same *in vivo* experimental protocol than the one described in Chapter 5 except from the fact that the polymer implants were cut using a laser (CO2 laser, Universal Laser System with power output of 4.3 W). In brief, in this prospective randomized masked experiments GAERS underwent surgery for implantation of skull electrodes (control group, n=6), skull electrodes and blank polymers (blank polymer group, n=6), or skull electrodes and lacosamide loaded polymers (lacosamide polymer group, n=6). Thirty-minute electroencephalogram (EEG) recordings were started at day 7 post-surgery and continued for eight weeks. EEGs were analysed using an automated detection algorithm. The number of SWDs, mean duration of one SWD and cumulative duration of SWD were compared week-by-week between the lacosamide polymer group and both the control and blank polymer groups using Kruskal-Wallis tests and when significant values were found, post-hoc pairwise comparisons were conducted using the Mann-Whitney U test. Significance level was set at 5%. In addition, for a minimum of three days post-surgery and until full recovery from the surgery, the rats were monitored for weight loss once a day, and for mobility and grooming twice a day. Those observations were given scores (Table 5.1).

Results

The number of SWDs, the mean duration of SWDs and the total duration of SWDs were not significantly different except during week one when they were all significantly lower in the lacosamide polymer group compared to the control group ($p=0.01$) (Figure Appendix 2.1). All rats were not affected or middle affected by the surgery during the recovery period except for one rat from group blank polymer which was moderately affected at day 3.

Brief discussion

As it has been demonstrated in chapter 4, the craniotomy itself can be responsible for a decrease in seizure activity for a couple of weeks following the surgery. In consequence the decrease in seizure activity seen during the first week is not considered a positive effect resulting from the administration of lacosamide.
Figure Appendix 2.1. Comparison of the epileptic activity monitoring results

Comparison of the measurements obtained to evaluate the epileptic activity of groups control (n=6), blank polymer (n=6) and lacosamide polymer (n=6). SWD: Spike and wave discharge; IQR: Interquartile range; Results are reported as median. * represents the time point at which the results from the lacosamide polymer group were significantly different to the control group (p<0.01).
The absence of significant decrease in seizure activity in the lacosamide group and of the post-operative side effects encountered in chapter 5 questions the assumption that appropriate lacosamide drug release occurred. Consequently, the author believe that the high debilitation scores encountered in Chapter 5 could have been the result of a significant initial leakage of lacosamide from the polymer although additional experiments (such has *in vitro* release profile and scanning electron micrographs of the edges of the implants) would need to be performed to confirm this.
Author/s:
Bauquier, Sébastien Hyacinthe

Title:
Initial development of an implantable drug delivery device for the treatment of epilepsy

Date:
2018

Persistent Link:
http://hdl.handle.net/11343/214755

File Description:
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