

LUND UNIVERSITY

Advancing gene therapy for epilepsy. Translational pre-clinical studies with neuropeptide Y and glial cell-line derived neurotrophic factor.

Melin, Esbjörn

2020

Document Version: Publisher's PDF, also known as Version of record

Link to publication

Citation for published version (APA):

Melin, E. (2020). Advancing gene therapy for epilepsy. Translational pre-clinical studies with neuropeptide Y and glial cell-line derived neurotrophic factor. [Doctoral Thesis (compilation), Department of Clinical Sciences, Lund]. Lund University, Faculty of Medicine.

Total number of authors: 1

General rights

Unless other specific re-use rights are stated the following general rights apply:

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights. • Users may download and print one copy of any publication from the public portal for the purpose of private study

or research.

- You may not further distribute the material or use it for any profit-making activity or commercial gain
 You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: https://creativecommons.org/licenses/

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117 221 00 Lund +46 46-222 00 00

Advancing gene therapy for epilepsy

Translational pre-clinical studies with neuropeptide Y and glial cell-line derived neurotrophic factor

ESBJÖRN MELIN

EPILEPSY CENTRE | DEPARTMENT OF CLINICAL SCIENCES, LUND | LUND UNIVERSITY



Advancing gene therapy for epilepsy

Translational pre-clinical studies with neuropeptide Y and glial cell-line derived neurotrophic factor

Esbjörn Melin



DOCTORAL DISSERTATION

by due permission of the Faculty of Medicine, Lund University, Sweden. To be defended at Segerfalksalen, BMC A10, on Friday 27^{th} of Mars 2020 at 13.15

Faculty opponent Professor **David Henshall**, PhD Royal College of Surgeons in Ireland

	Document name	ATION	
Eaculty of Medicine	Date of issue	Date of issue	
Department of clinical sciences. Lund	2020-03-27		
Epilepsy Centre			
Author	Sponsoring organization	n	
Esbjörn Melin			
Title and subtitle	·		
Advancing gene therapy for epilepsy: Translational pre-clinical studies with neuropeptide Y and glial cell-line derived neurotrophic factor			
Abstract			
Epilepsy is a neurological disorder, affecting approximately 1% of the population. The high rate of drug-resistance in epilepsy in general (30%), and temporal lobe epilepsy (TLE) in particular, pose a serious clinical problem. Thus, novel treatments for drug-resistant epilepsy is highly warranted.			
Gene therapy has been suggested to be a promising approach to target drug-resistant focal epilepsy since it offers the opportunity of delivering therapeutic agents directly into the seizure focus, thereby possibly being more effective in suppressing seizures and causing less adverse effects than conventional anti-seizure drugs.			
Two endogenous proteins that have been shown to be able to modulate seizures when delivered by gene therapy are neuropeptide Y (NPY) through its interaction with the Y2 receptor and glial cell-line derived neurotrophic factor (GDNF). In this thesis, the seizure supressing potential of these two theraputic strategies was investigated in an animal model of TLE. In the case of combinatorial NPY and Y2 receptor, overexpression was achived via adeno- associated viral (AAV) vector mediated in-vivo gene therapy. GDNF levels were increased by ex-vivo gene therapy via encapsulated cell biodelivery (ECB).			
The efficacy studies were performed in a manner that is suggested to increase the translational value of the results. Thus, both strategies were evaluated by continous video-EEG monitoring in the intrahippocampal kainic acid (KA) rat model of post status epilepticus spontanous recurrent seizures (SRSs), which is considered to share pathopysiology with human TLE. Moreover, the treatments were individualized by MRI to target the hippocampal seizure focus, ipsilateral to the injection of KA.			
The results indicate that both combinatorial NPY and Y2 receptor overexpression and ECB of GDNF were capable of reducing the frequency of SRSs when administered unilaterally in the seizure focus, with a responder rate of 31.3% respective 50%. Moreover, AAV derived overexpression of the NPY and Y2 receptor moduated SRS clustering patterns by increasing the latency between individual SRSs, but also between SRS clusters. These findings further strengthes the translational potential for these gene therapy strategies of treating TLE.			
Finally, the insights from the work with the model contributed to an attemt at harmonizing experimental procedures and standardising data collection within the field of pre-clinical epilepsy research. The implementation of common data elements (CDEs) might serve as support for future multicenter studies, which would improve reproducibility and hopefully enhance translation of preclinical findings to the clinic.			
Key words Enilonsy gene therapy, Neuropentide X, V2 recentor, GDNE, encapsulated cell biodelivery, esizure, in vivo			
Classification system and/or index terms (if any)			
	,		
Supplementary bibliographical information		Language English	
ISSN and key title 1652-8220		ISBN 978-91-7619-884-1	
Recipient's notes	Number of pages 100	Price	
	Security classification		

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature

Jsbjörn Helin

Date 2020-02-03

Advancing gene therapy for epilepsy

Translational pre-clinical studies with neuropeptide Y and glial cell-line derived neurotrophic factor

Esbjörn Melin



Cover artwork by Ragnvi Melin, profile photo by Olle Dahlbäck

Copyright pp 1-100 Esbjörn Melin Paper 1 © 2015 Elsevier Inc.. Paper 2 © 2020 by the Authors (Manuscript unpublished) Paper 3 © 2019 The Authors. This is an open access article under the CC BY license Paper 4 © 2018 Springer Nature Limited Paper 5 © 2016 Elsevier B.V.

Faculty of medicine Department of Clinical Sciences, Lund

ISBN 978-91-7619-884-1 ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University Lund 2020



Media-Tryck is a Nordic Swan Ecolabel certified provider of printed material. Read more about our environmental work at www.mediatryck.lu.se MADE IN SWEDEN

Till Amanda

The cure for anything is saltwater – sweat, tears, or the sea

Isak Dinesen (Karen Blixen)

Table of Contents

Summary8
Populärvetenskaplig sammanfattning10
Original papers and manuscripts12
Abbreviations13
Introduction15
Epilepsy and seizures15
The hippocampal formation19
Temporal lobe epilepsy21
Animal models of seizures and epilepsy24
Gene Therapy27
Challenges with translation
Aims of the thesis
Experimental procedures
Ethics
Animals
Viral vectors40
Encapsulated cell biodelivery
Induction of epileptic seizures41
Video-EEG monitoring
Electrophysiology
Treatment intervention
Magnetic resonance imaging
Histology
Research electronic data capture (REDCap)50
Statistical methods

Results
Development of a translational study framework (Paper I)53
Screening of AAV serotype and gene orientations for combinatorial NPY and Y2 receptor gene therapy (Paper II)55
Disease modification by single vector NPY and Y2 receptor gene therapy (Paper III)
Encapsulated cell biodelivery of GDNF suppresses SRSs (Paper IV)60
Harmonization of experimental procedures and development of a common database for pre-clinical data (Paper V)61
General Discussion
Predictive value and limitations of the i.h. KA rat model of TLE66
Screening of vector candidates and dose-response study
Seizure suppressing effects of combinatorial NPY and Y2 receptor gene therapy69
Effect of NPY and Y2 receptor overexpression on SRS clustering70
Seizure supressing effects of encapsulated cell biodelivery of GDNF71
Harmonization and standardization in preclinical studies73
Conclusions and future perspectives75
Acknowledgements77
References

Summary

Epilepsy is a common chronic neurological disorder that affects approximately 1% of the population. The disease is characterized by an enduring predisposition of the brain to generate seizures: episodes of aberrant neuronal hyperactivity and synchronization. Despite extensive research and development of new drugs, still 30% of patients with epilepsy are drug-resistant. Moreover, current treatments only address the symptoms of the disorder rather than targeting the underlying mechanisms. Thus, novel treatments for epilepsy is highly warranted.

Gene therapy is suggested to be a promising novel approach to target drug-resistant epilepsy, especially in the common case of refractory temporal lobe epilepsy (TLE), where the seizures start in a defined focus located in the temporal lobe. Targeted gene therapy offers the opportunity of delivering therapeutic agents, that would be unsuitable for systemic administration directly into the seizure focus, thereby possibly being more effective in suppressing seizures and causing less adverse effects than conventional anti-seizure drugs. Two such therapeutic agents, that are proposed to inhibit seizures in epilepsy, are neuropeptide Y (NPY) and glial cell-line derived neurotrophic factor (GDNF).

NPY has been extensively studied for its inhibitory role in epilepsy. Accumulating evidence suggest that NPY interaction with presynaptic Y2 receptors decreases Ca²⁺ influx through voltage gated calcium channels, thereby inhibiting glutamate release, which is a critical component in seizure activity. Indeed, *in-vivo* gene therapy with NPY mediated by adeno-associated virus (AAV) vectors has been shown to decrease the frequency of spontaneous recurrent seizures (SRSs). In line with this, it is hypothesized that a simultaneous overexpression of both the ligand and the receptor could have a synergistic effect on seizure suppression.

To address this question, first a proof-of-principle study was performed in the intrahippocampal (i.h.) kainic acid (KA) rat model of post *status epilepticus* (SE) SRSs. This model of epilepsy is suggested to share pathophysiological similarities with human TLE, e.g., the occurrence of SRSs and hippocampal sclerosis. Two separate vectors were administered unilaterally in the presumed seizure focus, aided by magnetic resonance imaging performed before the surgery. The stochastically occurring SRSs were examined by continuous video-electroencephalogram (EEG) monitoring. which showed that combinatorial NPY and Y2 receptor gene therapy overexpressed by two viral vectors resulted in a suppressing effect on seizures, reducing the frequency in the majority of animals.

To further investigate these findings and to increase the translational potential of the combinatorial gene therapy, a following efficacy study was performed with a single vector carrying the genetic sequence for both NPY and the Y2 receptor. This study was preceded with a screening of AAV vector candidates and a dose-response study. Because

of its high potential in inhibiting acute KA induced seizures, an AAV1-NPY-IRES-Y2 construct administered in a concentration of 10^{12} genomic particles (gp) / ml was selected to be investigated in the efficacy study.

Single vector combinatorial NPY and Y2 receptor had an inhibiting effect on SRSs when administered to the hippocampus ipsilateral to the KA injection. Specifically, the responder rate (>50% reduction of SRS frequency) was 31.3%, whereas no animals in the control group had this level of reduction. Moreover, a modulation of seizure clustering was observed, indicating that both the latency between SRSs and clusters of SRSs were prolonged in the animals that received combinatorial NPY and Y2 receptor gene therapy.

GDNF is another biomolecule demonstrated to modulate seizures in multiple models of epilepsy. Bilateral *ex-vivo* gene therapy, based on encapsulated cell biodelivery (ECB) of GDNF has been shown to ameliorate the pathology in the pilocarpine model. However, whether unilateral implantation of an ECB device into the seizure focus, which is a clinically likely scenario, is sufficient to reduce SRS frequency is unclear.

To address this question, another efficacy study was performed in the i.h. KA post-SE rat model. Unilateral ECB of GDNF reduced the occurrence of SRSs compared to control, with a responder rate of 50%. In addition, 5 out of 6 animals displayed a general reduction in SRS frequency, while the control group had the reverse relationship, with 6 out of 7 rats exhibiting disease progression. This indicates that the unilateral ECB of GDNF in the seizure focus can result in disease modification.

Finally, reasons for the lack of progress in developing novel treatments for drug resistant epilepsy has been attributed to issues with the pathophysiological relevance of models, lack of reproducibility and statistical power. To attempt to remedy these shortcomings in the field of pre-clinical epilepsy research, the European Union funded project: Targets and biomarkers for epileptogenesis (EPITARGET) developed common data elements (CDEs) with the purpose of harmonizing experimental procedures and promote standardisation in reporting. Also, the CDEs were implemented as an online data dictionary to enhance shareability. The experience gained from the abovementioned efficacy studies contributed to this process.

Taken together, the work presented in this thesis have added to the translational value of gene therapy for TLE, and thus, may have advanced gene therapy for epilepsy closer to the clinic.

Populärvetenskaplig sammanfattning

Epilepsi är en neurologisk sjukdom som drabbar ungefär 1% av befolkningen. Svenska Epilepsisällskapet har uppskattat att det idag finns ca. 81 000 personer med diagnosen i Sverige. Sjukdomen manifesterar med epileptiska anfall i varierande svårighetsgrad och orsakas av en överaktivitet av nervsignaler i hjärnan. Beroende på vilken region i hjärnan som är involverad i överaktiviteten får epilepsianfallen olika karaktär. I värsta fall utvecklas de till episoder med kramper och medvetslöshet.

av Trots årtionden forskning och utveckling av anti-epileptiska nya behandlingsmetoder har ca. en tredjedel av patienterna idag en bristande anfallskontroll. En majoritet av dessa (2/3) har fokal epilepsi, d.v.s, en typ av epilepsi där anfallen utgår från en specifik region i hjärnan, ofta hippocampus. Fokal epilepsi behandlas med anti-epileptiska mediciner som vid höga doser kan ge biverkningar. Medicinerna minskar retbarheten och överaktiviteten i det område där epilepsianfallen startar, men påverkar även hjärnans normala funktion och leder därför till dåsighet och inlärningssvårigheter. Behovet av nya behandlingsformer för fokal epilepsi är således stort.

Det har länge diskuterats om genterapi skulle kunna utgöra en möjlig behandling av fokal epilepsi. Genterapi är ett begrepp som omfattar metoder att påverka genuttrycket i kroppens celler. Genom att tillföra nya gener med t.ex. virala vektorer kan man få celler i hjärnan att producera ämnen som har en hämmande effekt på den neurala överaktiviteten. En av fördelarna med genterapi är att administration av behandlingen kan ske lokalt i den region som orsakar epilepsianfallen. I och med att cellerna själva producerar det terapeutiska ämnet och att det sker i ett avgränsat område, finns en möjlighet att denna metod är effektivare i att minska mängden epilepsianfall och leder till färre biverkningar.

Neuropeptid Y (NPY) är ett ämne som förekommer normalt i hjärnan. I vävnad från patienter med epilepsi har man kunnat se att produktionen av NPY ökar, och dessutom har man i experimentella försök kunnat visa att NPY minskar retbarheten som leder till epilepsianfall i hjärnvävnad. Mycket tyder därför på att denna peptid är ett kroppseget försvar mot epilepsianfall. Med genterapi kan man förstärka produktionen av NPY och även dess receptor Y2 och därigenom eventuellt utnyttja detta för behandling av fokal epilepsi.

I denna avhandling beskrivs arbetet med att utvärdera den anfallshämmande effekten av simultant överuttryck av NPY och Y2 receptorn genom genterapibehandling med virala vektorer. En viral vektor för in nya gener i kroppens celler, som sedan kan börja producera ämnet som generna kodar för. Studierna inleds med att undersöka om en djurmodell av epilepsi återspeglar sjukdomsförloppet i människa, och det konstateras att råttorna får spontana epilepsianfall med ökande frekvens i likhet med det humana tillståndet.

Ibland flera kandidater väljs en viral vektor av serotyp 1, med en gensekvens som placerar NPY före Y2 i produktionsordningen, baserat dess förmåga att hämma akuta kramper. Denna vektor används därefter i en långtidsstudie, där administrationen av behandlingen individualiserades efter varje råttas unika förutsättning med hjälp av magnetröntgen, liksom behandlingen är tänkt att användas i människa. Resultaten visade på en anfallshämmande effekt av vektorn som överuttryckte NPY och Y2 receptor, med en minskning av spontana epilepsianfall som var större än 50% i ungefär en tredjedel av de behandlade djuren.

En annan typ av genterapi är baserad på modifierade celler inkapslade i ett semipermeabelt membran. Celler modifieras med genterapi till att överproducera ett terapeutiskt ämne och placeras i en kapsel som kan implanteras i vävnaden som genererar epileptiska anfall. Det semipermeabla membranet tillåter näringsämnen och terapeutiska ämnen att passera, men håller de modifierade cellerna avskilda från patientens vävnad. I denna avhandling beskrivs även arbetet med att undersöka om cellkapslar som utsöndrar en tillväxtfaktor som kallas *glial cell-line derived neurotrophic factor* (GDNF) har en anfallhämmande effekt. Erfarenheterna från karakteriseringen av epilepsimodellen som beskrevs ovan användes även här för att utforma studien på ett sätt som ska öka möjligheterna till att resultaten kan överföras till människa. Från resultaten drogs slutsatsen att cellkapslar som utsöndrar GDNF minskar mängden epilepsianfall i modellen.

Att utvecklingen av nya behandlingsmetoder går långsamt sägs bero på flera samverkande faktorer. En av faktorerna har varit svårigheter att överföra vetenskapliga framsteg till fungerande behandlingar i sjukvården. Modellerna och de experimentella studierna man har genomfört i djur har saknat likhet med sjukdomen i människa, och resultaten har varit svåra att replikera.

En del av arbetet i denna avhandling har handlat om att harmonisera de experimentella protokollen mellan olika forskningsgrupper. Målet med med denna harmonisering är att resultat som tagits fram i en forskningsgrupp lätt ska kunna jämföras med resultat från en annan grupp. Förhoppningen är att detta arbete ska underlätta framtida multicenterstudier, där flera forskningsgrupper samarbetar för att göra forskningen starkare.

Sammanfattningsvis visar resultaten i denna avhandling på tydliga anfallshämmande effekter av både kombinatorisk NPY och Y2 receptor genterapi och cellkapsel behandling med GDNF. Resultaten är speciellt intressanta eftersom studierna är genomförda på ett sätt som ska öka sannolikheten att fynden kan översättas till behandling i människa.

Original papers and manuscripts

- I. Litsa Nikitidou Ledri, Esbjörn Melin, Søren H. Christiansen, Casper R. Gøtzsche, Alessandra Cifra, David P.D. Woldbye, Merab Kokaia. Translational approach for gene therapy in epilepsy: Model system and unilateral overexpression of neuropeptide Y and Y2 receptors. *Neurobiology of disease*, 86:52-61 (2016)
- II. Esbjörn Melin, Casper R. Gøtzsche, Jenny Wickham, Arnie Boender, Søren H. Christiansen, My Andersson, David P. D. Woldbye, Merab Kokaia. Effects of adeno-associated viral vector serotypes and gene orientation by combined neuropeptide Y and Y2 receptor gene therapy on induced seizures: a comparative analysis. *Manuscript*
- III. Esbjörn Melin, Avtandil Nanobashvili, Una Avdic, Casper R. Gøtzsche, My Andersson, David P.D. Woldbye, Merab Kokaia. Disease Modification by Combinatorial Single Vector Gene Therapy: A Preclinical Translational Study in Epilepsy. *Molecular Therapy - Methods & Clinical Development*, 15:179-193 (2019)
- IV. Avtandil Nanobashvili*, Esbjörn Melin*, Dwaine Emerich, Jens Tornøe, Michele Simonato, Lars Wahlberg, Merab Kokaia. Unilateral ex vivo gene therapy by GDNF in epileptic rats. *Gene Therapy*, 26(3-4):65-74 (2019)
- V. Niina Lapinlampi, Esbjörn Melin, Eleonora Aronica, Jens P. Bankstahl, Albert Becker, Cristophe Bernard, Jan A. Gorter, Olli Gröhn, Anu Lipsanen, Katarzyna Lukasiuk, Wolfgang Löscher, Jussi Paananen, Teresa Ravizza, Paolo Roncon, Michele Simonato, Annamaria Vezzani, Merab Kokaia, Asla Pitkänen. Common data elements and data management: Remedy to cure underpowered preclinical studies. *Epilepsy Research*, 129:87-90 (2017)

Abbreviations

AAV	adeno-associated virus
BBB	blood brain barrier
BDNF	brain-derived neurotrophic factor
CA	cornus ammonis
CAG	CMV-enhanced/chicken β -actin
CDE	common data element
CMV	cytomegalovirus
CNS	central nervous system
CNTF	ciliary neurotrophic factor
CPON	c-terminal peptide of NPY
DG	dentate gyrus
ECB	encapsulated cell biodelivery
EEG	electroencephalogram
ELISA	enzyme linked immunosorbent assay
EPITARGET	Targets and biomarkers for epileptogenesis
ETSP	Epilepsy therapy screening program
fEPSP	field excitatory post-synaptic potential
GABA	gamma-aminoburytic acid
GAERS	genetic absence epilepsy rat from Strasbourg
GDNF	glial cell-line derived neurotrophic factor
GFRa1	GDNF family receptor alpha 1
GLP	good laboratory practice
GMP	good manufacturing practice
gp	genomic particles
i.c.v.	intracerebroventricular
i.h.	intrahippocampal
ILAE	international league against epilepsy

IRES	internal ribosome entry site
KA	kainic acid
KAR	kainic acid receptor
LTP	long-term potentiation
MRI	magnetic resonance imaging
NCAM	neural cell adhesion molecule
NGF	nerve growth factor
NINDS	national institute of neurological disorders and stroke
NPY	neuropeptide Y
NMDA	N-Methyl-D-aspartic acid
NSE	neuron-specific enolase
PET	polyethylene terephthalate
PP	pancreatic polypeptide
PTX	picrotoxin
РҮҮ	peptide YY
ROI	region of interest
s.c.	subcutaneous
SCI	spinal cord injury
SE	status epilepticus
SRS	spontaneous recurrent seizure
SUDEP	sudden unexplained death in epilepsy
TBI	traumatic brain injury
TLE	temporal lobe epilepsy
WHO	world health organization
WPRE	woodchuck post-transcriptional regulatory element

Introduction

Epilepsy and seizures

Epilepsy has accompanied humanity throughout history, the first descriptions of seizures dating back to Mesopotamia and ancient Egypt. It was believed that the disease was related to spirits or daemons that took possession of the sufferer, probably due to the dramatic features of the seizures. Indeed, the name *epilepsy* is derived from the Greek verb *"to take hold of"*. The ancient Greeks considered the disease to be connected to the gods and called it "the sacred disease" (Magiorkinis et al., 2010). However, the notion that epilepsy was of divine origin did not go unchallenged. The father of medicine, Hippocrates (c. 460 - 370 BC), wrote in his book *"On the Sacred Disease"* that:

It is not, in my opinion, any more divine or sacred than other diseases, but has a natural cause, and its supposed divine origin is due to men's inexperience, and to their wonder at its peculiar character.

(Hippocrates and Jones, 1967)

It is important to point out, that despite that two and a half millennium has passed between Hippocrates and our days, people that suffer from epilepsy still experience stigma and encounter prejudice related to the disease. Thus, Hippocrates proved to be astonishingly perspicacious in his text on epilepsy. He addressed the origin of the disease to be the brain, but also suggested fasting as a treatment of the early stage of the disease. Parallels can be drawn to the modern ketogenic diet that has been shown to ameliorate the disease in some cases (Magiorkinis et al., 2014). Moreover, the natural cause that Hippocrates referred to is still not fully explained.

Epilepsy is present all over the world and is represented in all age-categories which makes it the third largest neurological disorder globally in terms of burden of disease, second only to Alzheimer's disease and headache disorders (GBD 2017 DALYs and HALE Collaborators, 2018). The world health organization (WHO) approximates the total number of people suffering from epilepsy in the world to be 50 million, which correlates well with a recent systematic assessment (Beghi et al., 2019; WHO, 2006). The prevalence is approximated to be 0.6 - 0.7 % by a large meta-analysis study that investigated 197 prevalence studies (Fiest et al., 2017).

People with epilepsy experience lower quality of life, not only because of the disease itself and the stigma that is attached to it, but also from the adverse effects of the drugs used to counteract the seizures. The patient's relationship with the anti-seizure drugs is often difficult, and there may be a conflict between seizure control and cognitive function (Eddy et al., 2011). It is reported that the top two most important adverse effects of anti-seizure drugs are negative impact on overall energy levels and school performance (Fisher et al., 2000). Epilepsy is also associated with higher mortality than in reference populations. Both the risk of sudden unexplained death in epilepsy (SUDEP) and suicide is elevated in individuals with epilepsy (Jallon P., 2004).

Importantly, despite decades of research and development of drugs counteracting epilepsy, still about one third of the patients experience seizures (Brodie et al., 2012; Picot et al., 2008). Thus, there is an urgent need for novel treatments, which is the one of the subjects in this thesis.

The definition of seizures and epilepsy

Epilepsy is a very heterogenous disease and should not be considered an entity, but rather a variety of disorders, characterised by underlying pathologies that make the brain prone to generating epileptic seizures (Engel Jr., 1995; Fisher et al., 2005). The epileptic seizure is the main symptom of epilepsy and was conceptionally defined by the international league against epilepsy (ILAE) in 2005:

An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain.

(Fisher et al., 2005)

Thus, epilepsy is defined around seizures:

Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures, and by the neurobiologic, cognitive, psychological, and social consequences of this condition. The definition of epilepsy requires the occurrence of at least one epileptic seizure.

(Fisher et al., 2005)

This definition has been interpreted as, for someone to have epilepsy, they must have two unprovoked seizures separated by more than 24 hours. However, to further refine the definition of epilepsy and adopt it to the clinical situation, more criteria were added in 2014. Focus was shifted to the probability of the patient having an epileptic seizure. As a consequence, a patient can currently be diagnosed with epilepsy after one seizure, given that the clinician evaluates the probability for second recurrent seizure to be high (> 60% over the next 10 years) (Fisher et al., 2014).

This latest refinement of the definition emphasises the enduring state of the disorder. With epilepsy follows a lasting propensity to generate seizures, which implies that the disease is chronic and that there are pathological alterations in the brain, of any nature, that make it different from a healthy one. Hence, it is important to make a distinction between epilepsy and seizures that occur secondary to another cause in a healthy brain, e.g., severe exhaustion, fever, alcohol withdrawal or in direct association with a head trauma (Fisher et al., 2014). Indeed, 10% of the population will experience at least one epileptic seizure in their life, however only a minority will be diagnosed with epilepsy (Pohlmann-Eden et al., 2006).

Classification of seizures and epilepsy

There are multiple types of epilepsy, with vastly different semiology and etiology. Treatment differ depending on the specific type, and also the prognosis of the outcome varies (Duncan et al., 2006). Some mild types are readily treatable with low doses of anti-seizure drugs, while other types, e.g. the Lennox-Gastaut syndrome is very severe, leaving the patient with few effective treatment options and a poor prognosis (Arzimanoglou et al., 2009). Because of the heterogeneity of the disease, it is in this context important to clarify how the epileptic disorders are classified and what types of epilepsy that is intended to be targeted with the experimental therapies described in this thesis.

Recent work by a commission issued by ILAE has updated the classification of seizures and epilepsy with the purpose of supplying clinicians with a common vocabulary (Fisher et al., 2017; Scheffer et al., 2017). This is suggested to improve communication, teaching and research within the field, but also supply the general public with a well-defined description of the disease (Fisher et al., 2017, 2005).

Seizures are classified in accordance to whether the initial features of the seizure represent a focal, generalized or unknown onset (Figure 1). The term focal describes a seizure which start in a limited part of the brain, differing from a generalized seizure, where the both hemispheres participates in the activity at the onset. The unknown category is reserved to seizures that cannot be classified to the other two due to lack of information (Fisher et al., 2017). Importantly, the category representing the seizures with focal onset holds a specific subdivision. The description *focal to bilateral tonic-clonic*, represents a specific seizure type which is very common in temporal lobe epilepsy (TLE): the epileptic subjected to the experimental treatments of this work. This specific type of seizure usually starts in the hippocampus, and then propagates to involve the whole brain which causes convulsive (tonic-clonic) seizures.

ILAE 2017 Classification of Seizure Types Expanded Version¹



Figure 1. Classification of seizures according to ILAE.

The classification is based on the onset of the seizures. Supplementary information of for example motor components is then added to further specify the seizure type. The figure is adapted from (Fisher et al., 2017).

The classification of the *epileptic disorders* is based upon a multilevel diagnostic scheme, incorporating the seizure classification described earlier. Thus, the first level of the classification defines the kind of seizure the patient is exhibiting. The second level describes the epilepsy type, whether it is focal, generalized or a combination of both. Also, here is a "Unknown" category available if sufficient clinical information is lacking. The epilepsy type diagnosis is made on clinical ground, supported by diagnostic tools such as electroencephalogram (EEG) and imaging techniques. The final level of diagnosis relates to epilepsy syndrome.

A detailed diagnosis often contributes to better management and prognosis of the disease (Engel Jr., 1995). However, lack of diagnostic tools might prevent a full diagnosis where recourses are limited. Therefore, the classification may stop at any level (Scheffer et al., 2017). Moreover, at all three levels of classification, the etiology of the disease should be considered. A structural etiology is a common finding in epilepsy. It may be acquired in the form of a stroke, traumatic brain injury (TBI) or an infection, but can also be a malformation originating from of a genetic mutation (Scheffer et al.,

2017). An example of a structural etiology is hippocampal sclerosis which is frequently found in patients with TLE (Blümcke et al., 2013).

The hippocampal formation

The hippocampal formation is a brain structure located within the medial temporal lobe (Duvernoy, 2005). It has a curved banana-like shape, present with a mirrored conformation in both hemispheres. Functionally, the hippocampal formation is associated with declarative memory formation and spatial orientation, and plays a major role in sensory processing (Lavenex and Amaral, 2000). Because of its special structure, the hippocampus has been intensively studied and has contributed to our understanding of basic neuronal mechanisms, such as long-term potentiation (LTP) (Whitlock et al., 2006).

The anatomy of the hippocampal formation is divided into subregions, including dentate gyrus (DG), the cornus ammonis (CA) regions CA1, CA2, CA3 and CA4, entorhinal cortex and subiculum. Traditionally, DG and the CA regions are often referred to as hippocampus proper. The subregions are interconnected by a main excitatory pathway, where the primary cells of the hippocampus relay activity in a circuit (Figure 2). These primary cells are excitatory glutamatergic granule cells in the DG and pyramidal cells (named so after their shape) in the CA region and SUB (Amaral and Lavenex, 2007).



Figure 2. Shematic representation of hippocampal basic connectivity. In the main hippocampal circuit, the entorhinal cortex (EC) relays sensory information to the dentate gyrus (DG) granule cells via the performant path. In turn, the granule cell's axons, called mossy fibers, enter the hilus (the region in the middle of DG) and synapse on pyramidal cells in CA3, which then relay the signal to CA1 via Shaffer collaterals. The circuit is closed when the pyramidal cells project via the subiculum (SUB) back into the deep layers of entorhinal cortex. Adopted from (Li et al., 2009).

The hippocampal formation is also populated by gamma-aminoburytic acid (GABA)ergic interneurons, modulating the activity of the primary cells (Freund and Buzsaki, 1996). These interneurons regulate network oscillations and synchronization, which is the foundation of normal hippocampal function, but can also contribute to the pathology in TLE (Isomura et al., 2008; Jefferys et al., 2013; Shuman et al., 2020). Alterations in excitatory and inhibitory balance can initiate self-sustaining activity, leading to seizures. Thus, the selective loss of interneurons observed in TLE, specifically of somatostatin and parvalbumin positive cells, has been suggested to increase the hyperexcitability in the hippocampus (Dudek and Sutula, 2007; Gorter et al., 2001). Moreover, excess of glutamatergic activity is being tightly associated with the disease, leading to alterations in neural signaling and network connectivity. Thus, targeting glutamate in epilepsy is suggested to be a promising approach to counteract seizure activity (Barker-Haliski and Steve White, 2015).

Temporal lobe epilepsy

One of the most common forms of epilepsy is TLE, characterized by seizures originating from the hippocampus, (de Lanerolle et al., 2003; Margerison and Corsellis, 1966; O'Dell et al., 2012; Sharma et al., 2007). The seizures in TLE are progressive, often starting with an aura, predominately with an abdominal feeling followed by altered consciousness, automatisms, and depending on the seizure propagation, clonic motor symptoms (Blair, 2012; Wieser and ILAE Commission on Neurosurgery of Epilepsy, 2004).

TLE predominately develops as a consequence of a precipitating event that initiates epileptogenesis, the process where the healthy brain develops into an epileptic brain. Such events that may cause TLE are stroke, TBI, central nervous system (CNS) infections, febrile convulsions and *status epilepticus* (SE) (French et al., 1993; Pitkänen and Sutula, 2002; Sharma et al., 2008). Only a fraction of the patients presenting with these brain insults will develop epilepsy. In those cases however, the epileptogenic event is followed by a latency period that can range in duration from 5-10 years before the person experience the first unprovoked seizure (O'Dell et al., 2012; Sharma et al., 2007).

Epileptogenesis in TLE

There are multiple structural and biochemical changes involved in the process of epileptogenesis in TLE. Findings from both animal models and resected hippocampal tissue from patients have shown astroglial scarring and astrogliosis, neuronal cell death, inflammation and aberrant mossy fibre sprouting (O'Dell et al., 2012; Pitkänen and Sutula, 2002; Ravizza et al., 2011). The specific mechanism underlying the disease is still not fully understood, but it is believed that together, or individually, these changes contribute to the development and progression of TLE (O'Dell et al., 2012) (Figure 3). At present, it is widely accepted that the term epileptogenesis does not only involve pathological changes taking place during the latency phase, but should also contain underlying processes in established epilepsy, contributing to the progression of the disease (Pitkänen, 2010).



Figure 3. The ongoing pathological processes in epileptogenesis.

Following an insult to the brain such as stroke or SE, multiple changes uccur that contribute to an enduring propensity of the brain to generate spontaneus seizures. Adopted from (Pitkänen and Sutula, 2002).

Current treatments of TLE

Currently, the primary choice of treatment for TLE is anti-seizure drugs. Typically, these drugs decrease hyperexcitability by enhancing inhibitory GABAergic signalling or by reducing excitatory transmission through modification of sodium and calcium channels (Löscher and Schmidt, 2011; O'Dell et al., 2012). As the drugs are

administered systemically, and affect brain regions that are not contributing to the pathology, they also give rise to adverse effects, such as drowsiness and nausea (Eddy et al., 2011; Fisher et al., 2000).

Despite decades of research and development of new therapies, still about one third of the patients with epilepsy have insufficient seizure control (Brodie et al., 2012; Duncan et al., 2006). This is highly problematic, since it has been shown that seizure freedom contributes significantly to quality of life (Birbeck et al., 2002). The majority of the refractory patients (2/3) have focal epilepsy, and especially in TLE, hippocampal sclerosis is a strong predictor of drug-resistance (Kwan et al., 2011; Picot et al., 2008).

Drug-resistant epilepsy is defined as a failure to achieve seizure freedom using two tolerated, appropriately chosen and appropriately used drugs, given either as monotherapy or as combination (Kwan et al., 2011; Tang et al., 2017). Available treatments today, e.g., anti-seizure drugs, target the symptoms (seizures) rather than the epileptogenesis (Walker MC, White HS, 2002). They are in many cases only transiently effective and do not reverse the underlying propensity to develop seizures (Linard et al., 2010; O'Dell et al., 2012; Sharma et al., 2007). Better would be to achieve disease modification, where the epileptogenesis is reversed, resulting in an enduring reduction in seizure frequency or seizure freedom (Pitkänen and Engel, 2014).

The lack of treatment alternatives for drug-resistant epilepsy pose a big clinical problem, and the need for novel ways of treating the refractory patients is urgent (Kullmann et al., 2014; Perucca et al., 2007; Schachter, 2007). Surgical resection of the epileptic focus is an alternative to AEDs, associated with a high rate of seizure freedom (West et al., 2015). However, despite its advantages, it is only applicable to a minority of the pharmacoresistant patients (Duncan et al., 2006; Engel et al., 2003). Also, one study have reported that only 50% of patients that had undergone surgery were seizure free after 5 years (De Tisi et al., 2011).

In addition to surgery and pharmacotherapy, there are alternative strategies to ameliorate drug resistant epilepsy. Switching to ketogenic diet has been shown to decrease seizure frequency in children, but is unpleasant and hard to maintain (Kwan et al., 2011). Moreover, vagal nerve stimulation or responsive neurostimulation is efficient in some patients, however does not produce seizure freedom in all (Ben-Menachem, 2002).

Multiple hypotheses have been formed to explain the mechanisms of drug-resistant epilepsy. One suggests that loss of drug targets, as a result form, e.g., differential expression of receptor subunits, could render the drugs ineffective. An alternative mechanism could be that overexpression of efflux transporters prevents the drugs to reach their targets in the seizure focus (Kwan et al., 2011; Tang et al., 2017). Gene therapy offers a possibility to circumvent some of these proposed problems, e.g., by

delivering a therapeutic compound "behind" the blood brain barrier (BBB). This strategy is investigated in this thesis, utilizing an animal model of TLE.

Animal models of seizures and epilepsy

As discussed previously, epilepsy is a multifactorial disease, with different factors of the pathology contributing to hyperexcitability and seizures (Löscher, 2002). Despite the multifaceted nature of epilepsy, focus has long been solemnly on seizures as the main feature taken into consideration when modelling the disease. Acute seizure models in rodents, such as the maximal electroshock (MES), pentylenetetrazol (PTZ) seizure test and the electrical kindling model has long been used for screening of new anti-seizure drugs (Löscher, 2017). It is now speculated that shortcomings of these models are one of the reasons for lack of progress in treating refractory epilepsy (Löscher and Schmidt, 2011). A good model of TLE needs to reflect as many features of the human conditions as possible, including the fact that it is a progressive disease with spontaneous occurrence of seizures and epileptiform events. Moreover, it should show resistance to anti-seizure drugs (Simonato et al., 2012). Thus, careful consideration is needed when choosing among the abundance of animal models (Kandratavicius et al., 2014; Simonato et al., 2014).

Translational pre-clinical models of TLE

There are a plethora of epilepsy models, mimicking different aspects of the disease (Pitkänen et al., 2017). Traditionally, repetitive electrical stimulations in hippocampus or amygdala that cause afterdischarges have been used to model TLE, a phenomenon called kindling (Goddard et al., 1969; Sato, 1982). This model has led to the understanding of many mechanisms underlying epilepsy. However, the etiological relevance and the predictive value of the model has been criticized (Löscher, 2017; Wilcox et al., 2019).

An important category is the post SE models of epilepsy, where a stimulus (either electrical or chemoconvulsive) give rise to SE, which triggers epileptogenesis. Similarly to what is observed in humans, the initial insult is followed by a latency phase where no seizures occur. After a couple of weeks, the animals develop spontaneous recurrent seizures (SRSs) along with pathological hallmarks of the disease, e.g. hippocampal sclerosis (Kandratavicius et al., 2014). In this context, two chemoconvulsants are used extensively, delivered by either systemic or intracerebral injection (Raedt et al., 2009). *Pilocarpine* produces SE presumably by activating the muscarinic subtype receptor M1 (Curia et al., 2008). The other chemoconvulsant, *kainic acid* (KA), is a glutamate analogue which triggers excitotoxicity by activating the KA receptor (see below). An

important difference between the two is that pilocarpine gives rise to damages also in neocortex, while KA predominantly affects the hippocampus (Sharma et al., 2007).

Another etiologically important model is the TBI model, where a mechanical damage is used to trigger the disease development (Thompson et al., 2005). Moreover, genetic models have been used to investigate various mechanisms involved in the disease development, e.g., the Genetic Absence Epilepsy Rat from Strasbourg (GAERS) which displays features of absence epilepsy (Marescaux et al., 1992).

Given the multitude of models available to mimic epilepsy, there is an ongoing discussion on which models to use in the efforts of finding novel treatments for pharmacoresistant epilepsy and epileptogenesis (Löscher, 2016). Currently, models of post SE are considered to have a predictive value in finding treatments for pharmacoresistant epilepsy. Specifically, the systemic KA post SE induced rat model of SRSs is used in the differentiation phase in the Epilepsy Therapy Screening Program (ETSP). One of the main arguments for the claim of the accuracy of these chronic models is the fact that experiments are performed in an epileptic brain rather than in a healthy brain that is subjected to a seizure provoking insult. The chronic disposition to generate seizures is considered to increase the translational value of the model (Kehne et al., 2017; Wilcox et al., 2019).

Although models featuring SRSs are advantageous when it comes to predictability, they are not suited for screening of treatment alternatives and high throughput. Monitoring of SRSs is laborious and expensive and require extensive experimental setups to yield decisive results (Kandratavicius et al., 2014). Therefore, there is a need for a preceding model, which lacks the high predictive value, but still with reasonable ability can evaluate treatment effect in a highly efficient manner, limiting the amount of candidates to be investigated in chronic models (Löscher, 2017).

In this work, two animal models have been utilised in the screening and subsequent efficacy testing of direct and indirect gene therapy for reasons discussed below. The subcutaneous (s.c.) KA model of acute induced seizures was used for screening of viral vector candidates, and the i.h. KA post SE rat model of TLE was used for efficacy studies in the chronic phase of the disease.

Intrahippocampal kainic acid (Chronic seizures)

KA administration in animals was first proposed as a model of TLE in the 80s (Ben-Ari, 1985). The compound is a L-glutamate analogue that interacts with KA receptors (KARs), which are highly expressed in the CA3 region of the hippocampus and have a high affinity for glutamate. This renders this region susceptible to excitotoxicity due to excessive KA induced neuronal signalling (Bloss and Hunter, 2010; Vincent and Mulle, 2009). An intrahippocampal (i.h.) microinjection of 0.4 μ g KA results in initiation of SE, characterized by a progressive increase in EEG amplitude, followed by episodes of discharge complexes with high frequency synchronized spiking. The initial insult lasts between 3.7 - 20.5 hours if not stopped by administration of drugs, and is followed by a latency phase that range from 5 - 30 days before SRSs emerge (Lévesque and Avoli, 2013; Rattka et al., 2013). The rate of animals developing SRSs has been reported to be around 80% (Bragin et al., 2005; Raedt et al., 2009; Rattka et al., 2013). Though, there is evidence of that the use of anaesthesia acts as an insult modifier, resulting in fewer animals developing SRSs (Bar-Klein et al., 2016; Rattka et al., 2013). As 41% of patients with SE develops epilepsy, one can argue that this is an etiologically relevant model (Walker MC, White HS, 2002). However, the initiation of SE with KA is of course artificial and more resembles domoic acid poisoning (Cendes et al., 1995).

Interestingly, the neuropathology of rats injected with KA is very similar to the human condition of TLE (Kandratavicius et al., 2014; Rattka et al., 2013). The ipsilateral hippocampus to the KA injection demonstrates gliosis, neural loss and mossy fibre sprouting (Raedt et al., 2009). The damage is mostly present in the CA3 region and the dentate hilus, while the CA1 region is preserved.

The frequency of SRSs varies between animals and over time, which highlights the importance of continuous video-EEG monitoring to accurately investigate seizure occurrence. However, no correlation has been found between SE severity and subsequent frequency of SRSs (Rattka et al., 2013). Disease progression, characterized by a gradual increase in SRS frequency, along with clustering of seizures strengthens the similarity between the model and the human condition (Lévesque and Avoli, 2013; Rattka et al., 2013). Clustering of seizures is a common feature in human epilepsy and is associated with refractoriness (Haut et al., 2005; Sillanpää and Schmidt, 2008). Interestingly, seizure clustering has been suggested to be a result of accumulation of post-ictal refractoriness attributed e.g. neuropeptides such as neuropeptide Y (NPY) (Löscher and Köhling, 2010).

Continuous video-EEG is usually applied to investigate the SRSs in the model. Depth and cortical electrodes record the local field potentials generated by synchronized neural depolarizations and subsequent transmembrane ion flow (Buzsáki et al., 2012). Video synchronized with the EEG gives additional information of any behavioral component. By reviewing the data, SRSs can be identified and characterized (Bertram et al., 1997).

The advantage of focal (i.h.) administration of KA, compared to systemic administration, is that it results in a regionally restricted focus (Rattka et al., 2013). Rats injected with i.h. KA display mainly convulsive seizures starting in the ipsilateral hippocampus, and propagate to contralateral amygdala, hippocampus and frontal cortex (Leite et al., 1996; Lévesque and Avoli, 2013; Rattka et al., 2013; Riban et al., 2002). This feature is important when investigating experimental focal treatments, such as the studies presented in this work.

Sub-cutaneous kainic acid (Acute seizures)

Sub-cutaneous (s.c.) injection of 10 mg / kg of KA in rats induces acute excitotoxicity, resulting in seizures that gradually develop into SE, as described above. The behavioural seizures can be counted and characterized as a measure of excitability, and be used to screen for anti-seizure effects of an experimental treatment (Woldbye et al., 2010). Scoring of seizure severity is done according to the Racine scale. The scale ranges from 1 to 5, where (1) is a seizure involving mouth and facial movements, (2) head nodding, (3) Forelimb clonus, (4) rearing, (5) rearing and falling (Racine, 1972).

Similar to the i.h. injection procedure, the acute seizures start in the hippocampus, making it suitable for investigation of any seizure inhibiting effects of focal experimental treatments (Lothman et al., 1981), such as gene therapy.

Gene Therapy

Given the unmet need of alternative treatments in focal drug-resistant epilepsy, there has been a priority to develop novel approaches to counteract the disease. Due to the nature of focal epilepsies, and TLE in particular, several focal targeting strategies are under development. Cell grafting into the hippocampus aims at repairing damaged structures or releasing modulating substances that inhibit hyperexcitability (Raedt et al., 2007), deep brain stimulation has been shown to reduce seizers in TLE patients (Boon et al., 2007) and specific modulation of miRNAs by antagomirs have been demonstrated to exert a potent anti-seizure effect in multiple animal models when administered i.c.v. (Reschke et al., 2017).

The most frequently mentioned novel approach to target TLE is by gene therapy. The principle of gene therapy is to introduce new genes into cells, modulating them into producing, e.g., a therapeutic agent. This approach can be divided into two categories, *ex-vivo* gene therapy and *in-vivo* gene therapy. In *ex-vivo* gene therapy, cells are engineered to express a transgene *in-vitro* and then implanted into tissue, while *in-vivo* gene therapy is based on direct gene transfer by viral or non-viral vectors.

Both cases offer advantages over conventional pharmacotherapy. Many therapeutic agents have limited BBB penetrance and are interacting with targets outside CNS (Simonato et al., 2013). This rules out systemic administration, as it would result in poor bioavailability at the target site and cause adverse effects. By delivering the therapeutic agent via gene therapy directly into the epileptic focus or in a seizure propagation pathway, these hurdles are potentially overcome (Löscher et al., 2008). Such focal treatment of the epileptic tissue could possibly also decrease any adverse effects, that are often associated with anti-seizure drugs (Riban et al., 2009; Simonato, 2014).

The potential of gene therapy in epilepsy has been demonstrated in numerous preclinical studies (Kullmann et al., 2014; McCown, 2016). Specifically, overexpression of endogenous neuropeptides and neurotrophic factors has been shown to increase seizure threshold and decrease the number of seizures. Recently, the development of novel gene editing techniques and molecular tools has enabled advanced strategies for seizure therapy-based suppression. Methods gene involving optogenetics, chemogenetics and engineered potassium channels have all showed potential in seizure suppression (Krook-Magnuson et al., 2013; McCown, 2016; Snowball et al., 2019). In the context of this thesis, gene therapy based on NPY and glial-cell line derived neurotropic factor (GDNF) overexpression is highlighted later.

The main method of delivering genes directly *in-vivo* is by utilizing viral vectors. This technique takes advantage of the natural ability of a virus to transfer genetic material to cells. However, in order to safely utilize this capacity, the viruses are made replication deficient, meaning that they are unable to proliferate. There are three main classes of viral vectors that are considered in CNS gene therapy, lentivirus, herpes simplex virus and adeno-associated virus (AAV). They differ in packaging capability, transduction efficacy and method of transgene integration (Ingusci et al., 2019; Simonato, 2014). As, AAV based vectors are considered safe, non-pathogenic and are capable of sustained gene expression, these have been commonly used in clinical trials (Choudhury et al., 2016).

Adeno-associated viral vectors

AAV is a small (25 nm) non-enveloped single-strand DNA parvovirus capable of infecting both dividing and non-dividing cells (Zincarelli et al., 2008). It has a packaging capability of 4.7 kb, which makes it suitable for delivering simple molecules such as neuropeptides, however it has limited use for long expression constructs or regulatory sequences (Kullmann et al., 2014). AAVs are considered a good choice in gene therapy, since they are classified as dependoviruses, uncapable of replication by its own. Also, the delivered genetic material remains episomal, without integrating into the chromosome, which reduces the risk of mutagenesis (Hitti et al., 2019). AAVs display low immunogenicity and are capable of sustained long lasting expression (J McCown, 2011; Weinberg et al., 2013). However, as humans are naturally exposed to AAVs, there might be a risk of some individuals carrying neutralizing antibodies towards a specific type (Sun et al., 2003).

There are numerous serotypes identified that differ in tropism depending on the composition of capsid proteins. The differences in tropism might be explained by the recent identification of an AAV receptor (Pillay et al., 2016). Co-receptors and other factors could influence the endocytosis of a specific serotype (Colella et al., 2018). Serotype AAV1, AAV2, AAV5 and AAV8 display neuronal tropism, while AAV9 has

been shown to be able to cross the BBB (Burger et al., 2004; Klein et al., 2008). Thus, the choice of serotype is important to achieve adequate expression in the target tissue (Weinberg et al., 2013).

It is possible to further modulate the expression of the transgene with cell-type or region-specific promoters. The promoter is also important for stable long-term expression, as it has been shown that e.g. the ubiquitous cytomegalovirus (CMV) promoter is downregulated over time (Fitzsimons et al., 2002). However, the neuron-specific enolase (NSE) promoter is capable of sustain expression, and also, the synthetic CMV-enhanced/chicken β -actin (CAG) promoter drives long-term strong expression (Klein et al., 2002).

For focal epilepsy, gene therapy is suggested to be a promising approach, and particularly overexpression of neuropeptide Y has been investigated for seizure supressing effects, which is discussed next (Kovac and Walker, 2013; Kullmann et al., 2014; Simonato, 2014).

In-vivo gene therapy based on Neuropeptide Y and its receptors

Neuropeptide Y (NPY) is a member of the neuropeptide Y family of biologically active peptides (PP-fold peptides), which contains also pancreatic polypeptide (PP) and peptide YY (PYY) (Holzer et al., 2012). It is named because of its 5 tyrosine (Y) residues in its primary structure, and was first discovered almost 4 decades ago (Tatemoto et al., 1982). The peptides in the family are all 36 amino acids long and share structural motifs (Hökfelt et al., 1998). Interestingly, NPY is also highly conserved throughout evolution (Larhammar et al., 2001), and its amino acid sequence is identical between, e.g., human and rat (O'Hare et al., 1988; Thorsell and Mathé, 2017). While all the peptides in the NPY-family are produced and released by endocrine cells from the gut, NPY is also widely distributed in the mammalian brain (Hendry et al., 1984; Holzer et al., 2012). Indeed, NPY is the one of the most abundant neuropeptides in the brain, and high levels are found in the arcuate- and paraventricular nuclei of the hypothalamus, amygdala, periaqueductal grey, locus coeruleus, septum and the hippocampal formation (Kask et al., 2002; Thorsell and Mathé, 2017).

In the brain, NPY is produced predominantly by interneurons where it is stored in large dense core vesicles at synaptic terminals (de Quidt and Emson, 1986). The synthesis takes place in cell soma in the form of the precursor protein preproNPY, which is then packed in the Golgi apparatus and transferred to the synaptic terminal via anterograde transport (Hökfelt et al., 2000; Vezzani and Sperk, 2004). The precursor protein is truncated, resulting in its active form NPY1-36 and a c-terminal peptide of NPY (CPON) (Thorsell and Mathé, 2017). NPY1-36 can then be further processed into NPY2-36 and NPY3-36, which affects receptor binding affinities (Soud et al., 2018).

As with most neuropeptides, a sustained high frequency activity of the neuron is required for the release of NPY (Hökfelt, 1991; Vezzani et al., 1999). Once released, the neuropeptide can be active over longer duration, since deactivation is slow due to peptidase degradation, in contrast to the fast removal of classical neurotransmitters in the synaptic cleft (Hökfelt et al., 2000). This also enables NPY to act as a volume transmitter, interacting with targets far away from the release site (Hökfelt, 1991; Sajdyk et al., 2004; Sørensen et al., 2008b).

Being one of the most abundant neuropeptides in the brain, NPY has been shown to be involved in many physiological functions, such as memory formation, food intake, stress, blood pressure, angiogenesis, neurogenesis and circadian rhythm (Benarroch, 2009; Decressac and Barker, 2012; Gøtzsche and Woldbye, 2016; Hökfelt et al., 1998). Moreover, NPY plays a part in various pathological conditions, including neurodegenerative diseases such as Alzheimer's, Huntington's and Parkinson's disease, stress, depression, anxiety post-traumatic stress disorder (PTSD), alcoholism and epilepsy (Decressac and Barker, 2012; Heilig, 2004; Reichmann and Holzer, 2016; Sajdyk et al., 2004; Thorsell and Mathé, 2017; Vezzani et al., 1999).

Five NPY receptors have been identified (Y1, Y2, Y4, Y5, y6), all of which are G-protein coupled receptors (Michel et al., 1998). However, the y6 receptor has been shown to be functionally expressed only in mouse and rabbit (Redrobe et al., 1999). The Y1, Y2 and Y5 receptors are the most abundant in the brain, present at high to moderate levels in the hippocampus, while the Y4 receptor is hardly detected (Redrobe et al., 1999; Silva et al., 2005).

Changes in the Neuropeptide Y system in epilepsy

Levels of NPY (and other neuropeptides) have been shown to increase after sustained seizure activity, such as KA or electrical stimulation induced SE in rats (Marksteiner et al., 1990b; Vezzani and Sperk, 2004; Zachrisson et al., 1995). Similarly, repetitive shorter seizures induced by electrical or chemical kindling also induce NPY upregulation (Bendotti et al., 1991; Marksteiner et al., 1990a). In the chronic phase, where rats subjected to KA induced SE exhibit SRSs, NPY is still upregulated (Marksteiner et al., 1990b). This overexpression of NPY is suggested to be a consequence of the seizure activity, as it can be blocked either by administration of antiseizure drugs after SE or disappears 1-2 weeks after kindling (Marksteiner et al., 1989; Schwarzer et al., 1996). In contrast to NPY expression in the normal hippocampus, ectopic immunoreactivity of NPY is detected in mossy fibres and is markedly increased in hilar interneurons after KA induced SE. Also, a transient expression is found in the CA1 10 hours after SE in rats (Vezzani and Sperk, 2004).

In addition to the changes in NPY expression, the receptor distribution is altered in epileptic rats. Binding of the Y2 receptor has been found to increase in the Schaffer collateral terminals and mossy fibres (Röder et al., 1996; Schwarzer et al., 1998). Y1

receptors are on the other hand downregulated in the molecular layer of the DG (Kofler et al., 1997). The situation is reported to differ in humans. In tissue resected from human patients with TLE, significant NPY overexpression is only detected in interneurons, which also display axonal sprouting, resulting in increased NPY and GABAergic innervation of mossy fibres. Also, the Y2 receptor is upregulated in the DG, and the Y1 receptor is downregulated in the molecular layer in DG compared to autopsy controls (Furtinger et al., 2001). The Y1 and Y2 receptor have been shown to have diverse, counteracting effects on hyperexcitability. Thus, these adaptive changes in expression of NPY and the Y1 and Y2 receptor have been suggested to constitute a endogenous protective mechanism against seizures (Benmaamar et al., 2003; Vezzani and Sperk, 2004).

The inhibitory role of NPY in seizures

There is a large body of evidence for the seizure supressing properties of NPY (Baraban, 2004; Benarroch, 2009; Vezzani and Sperk, 2004). Exogenous application of NPY via a cannula to the lateral ventricle inhibits acute KA induced seizures and supress epileptiform activity when added to mouse hippocampal slices *in-vitro* (Baraban, 2002; Woldbye et al., 1997). In line with these findings, transgenic knock out mice, lacking NPY, had a higher susceptibility to picrotoxin (PTX) or KA induced acute seizures (Baraban et al., 1997; Erickson et al., 1996). Also, transgenic overexpression of NPY in rats rendered the animals more resistant to kindling (Vezzani et al., 2002).

There is much pointing in the direction that the inhibitory mechanism is due to the NPY interaction with its Y2 receptor (El Bahh et al., 2005). NPY inhibits glutamatergic excitatory transmission in the hippocampal formation (Colmers and Bleakman, 1994). This inhibition has been attributed to the Y2 receptor, mainly located pre-synaptically, which inhibits the release of glutamate by reducing the influx of Ca²⁺ via voltage dependent calcium channels (Colmers et al., 1988; Qian et al., 1997; Stanić et al., 2011). Experiments with receptor specific antagonists and receptor knockout mice have concluded that, indeed, also the supressing effect on seizures is mediated by the Y2 receptor (El Bahh et al., 2005; Klapstein and Colmers, 1997). On the other hand, the Y1 receptor has been shown to mediate a weak excitatory effect, as antagonism of the Y1 receptor inhibits the development of KA induced seizures and delays epileptogenesis in kindled rats and aggravates KA induced acute seizures (Benmaamar et al., 2003; Gariboldi et al., 1998; Olesen et al., 2012).

Due to the interesting properties of the NPY system in epilepsy, it has been suggested as a promising target for a focal anti-epilepsy treatment (Kovac and Walker, 2013). Indeed, NPY has been shown to decrease synaptic transmission by activating the Y2 receptor in human resected tissue (Ledri et al., 2015; Patrylo et al., 1999). Moreover, NPY application to in-vitro preparations of resected epileptic hippocampal tissue was also recently shown to inhibit epileptiform activity (Wickham et al., 2019). These findings indicate that focal overexpression of NPY could be applicable in humans for treating TLE.

Gene therapy utilizing the NPY system

There have been multiple pre-clinical studies investigating the effects of NPY overexpression in epilepsy using gene therapy. In 2004, it was shown that a bilateral injection with an AAV vector overexpressing NPY reduced i.h. KA induced acute seizures and significantly delayed kindling (Richichi et al., 2004). These findings were later confirmed, when AAV derived overexpression of both NPY and NPY13-36 (a Y2 preferring agonist) reduced grade 3 and 4 seizures induced by systemic administration of KA (Foti et al., 2007). As these initial studies were done in models of induced seizures, there was a question of whether focal AAV derived NPY overexpression would also supress SRSs. Indeed, in 2008, it was demonstrated that spontaneous seizures, following electrically induced SE, could be supressed by bilateral overexpression of NPY. Moreover, the rate of progressive animals (defined in this case as individuals exhibiting an increase in seizure frequency by 50%) was reduced in the treated group compared to the control (Noè et al., 2008). With these encouraging findings, the potential of translation to the clinic increased. However, it was not clear whether overexpression of NPY would have adverse effects on cognitive function. NPY overexpression was shown to cause a delay in hippocampal based learning, accompanied by an attenuation of LTP in the CA1 area (Sørensen et al., 2008a). Though, the effect did not exacerbate the memory deficits already present in epileptic animals (Sørensen et al., 2009). In contrast, no behavioural deficits were reported when injecting naïve rats with either AAV1-NPY or the chimeric AAV1/2-NPY (Noe et al., 2010; Noè et al., 2008). To explain these conflicting findings, it is argued that the location of NPY overexpression in the hippocampus could affect the cognitive outcome (Noe et al., 2012).

As the seizure supressing effect of NPY is predominately mediated by the activation of the Y2 receptor, it was hypothesised that overexpression of this receptor would have a seizure supressing effect. Indeed, it was shown that Y2 overexpression attenuated KA induced acute seizures and retarded kindling development. Importantly, when the Y2 receptor was overexpressed in concert with NPY, the after-discharge threshold was significantly elevated both compared to Y2 alone and empty control. This indicated that a combinatorial overexpression of NPY and the Y2 receptor could have a synergistic effect on seizures (Woldbye et al., 2010). Also, the Y5 receptor has been shown to strengthen the effect of NPY on acute KA induced seizures, however failed to alone exhibit a supressing effect (Gøtzsche et al., 2012).

AAV mediated gene therapy overexpressing NPY has also been proposed to inhibit absence seizures. A recent study demonstrated a significant reduction of spontaneous seizures in GAERS. Viral vector derived overexpression was targeted to thalamus or somatosensory cortex, which resulted in a reduction of seizure frequency and duration (Powell et al., 2018).

This body of accumulating evidence for the seizure supressing potential of AAV mediated NPY overexpression talks in favour for translation to the clinic. Indeed, a clinical grade, Good Manufacturing Practice (GMP) vector, based on the AAV1 serotype, was recently produced (Patrício et al., 2018). However, neither of the described studies have investigated whether focal overexpression in the seizure focus (e.g. unilateral to a KA injection) attenuates SRSs. Moreover, intervention with a single vector, driving both NPY and the Y2 receptor, has never been described. Both of these questions are addressed in this thesis.

Encapsulated cell biodelivery

Although gene therapy based on viral vectors has shown great promise in targeting CNS disorders, there are still questions about safety, as administration of a viral vector is irreversible. Also, *ex-vivo* gene therapy, modulating cells *in-vivo* for subsequent grafting is hard to cancel, and migration of cells outside the therapeutic area might lead to adverse effects (Lindvall and Wahlberg, 2008).

Encapsulated cell biodelivery (ECB) was developed to increase safety of *ex-vivo* gene therapy. The technique is based on cells, engineered to overexpress a therapeutic agent, that are encapsulated in a semipermeable membrane, allowing oxygen and nutrients to reach the cells, and preventing them from dispersing into the tissue and coming in contact with host immune system (Figure 4). This offers some advantages compared to both viral vector-based gene therapy and cell therapy. As the cells are confined in a device, they can be explanted if there are concerns of safety, or if unable to be ameliorate the disorder (Lindvall and Wahlberg, 2008; Paolone et al., 2019).



Figure 4. A shematic illustration of a ECB device

The engineered cells are encapsulated in a supporting matrix composed of polyethylene terephthalate (PET) yarn. This is surrounded by a semipermeable membrane, allowing for transport of nutrients and theraputic agent, however separating the cells from the host tissue. Adopted from (Eyjolfsdottir et al., 2016).
The approach of ECB of therapeutic agents has been investigated for neurodegenerative disorders. Delivery of nerve growth factor (NGF) to patients with Alzheimer's disease has indicated positive therapeutic effect (Eyjolfsdottir et al., 2016). Also, a clinical study performed in Huntington's disease, delivering ciliary neurotrophic factor (CNTF), has provided evidence of safety and tolerability (Bloch et al., 2004).

The efficacy of the ECB in epilepsy is indicated by pre-clinical studies, where e.g., adenosine release from ECB devices inhibited kindling induced behavioural seizures and after-discharges (Güttinger et al., 2005). More recently, both GDNF (see below) and brain-derived neurotropic factor (BDNF) have demonstrated an inhibitory effect on seizures (Falcicchia et al., 2018; Paolone et al., 2019).

Ex-vivo gene therapy based on GDNF

Neurotropic factors are biomolecules, crucial for the development and survival of neurons. There are several subgroups, one of the most studied being neurotrophins, which includes BDNF and NGF (Hefti et al., 1993). Another subgroup is the GDNF-family ligands (GFLs), including GDNF, which was isolated from a pig glial cell line (Lin et al., 1993). In addition to GDNF, there are three other ligands in the GFLs: Neurturin, Artemin and Persephin (Ibáñez and Andressoo, 2017). The GFLs each bind to an individual GDNF family receptor (GFR α 1-4) with some low affinity overlap. Specifically, GDNF bind to GFR α 1 and form a ligand receptor complex that in turn can activate the receptor tyrosine kinase RET. In addition to GFR α 1 and RET, GDNF can also signal by interacting with the neural cell adhesion molecule (NCAM) and Syndecan-3 (Airaksinen and Saarma, 2002; Ibáñez and Andressoo, 2017).

Because of its ability to promote midbrain dopaminergic neuron survival, GDNF has been extensively studied in Parkinson's disease. However, a recent double-blind clinical study, delivering GDNF to the putamen, have failed to prove a positive effect over control (Kirkeby and Barker, 2019). In the context of epilepsy, GDNF has gained an increasing amount of attention due to its ability to counteract seizures (Paolone et al., 2019).

GDNF in epilepsy

GDNF is suggested to be linked to epilepsy by the finding that KA or pilocarpine induced seizures increase the levels of both GDNF mRNA and protein levels in the hippocampus (Humpel et al., 1994; Kokaia et al., 1999; Schmidt-Kastner et al., 1994). Interestingly, unilateral administration of KA led to a bilateral increase in GDNF immunoreactivity in granule cells, lasting for 4 days (Mikuni et al., 1999). The GDNF receptors are also regulated by seizures. Both electrical stimulation and systemic administration of KA has been shown to induce an upregulation of hippocampal GFR α 1 (Kokaia et al., 1999; Nanobashvili et al., 2003; Reeben et al., 1998).

Interestingly, GFR α 2-deficient mice showed a resistance to kindling, raising the question of whether GFR α 1 and GFR α 2 have opposing effects on neural excitability (Ibáñez and Andressoo, 2017; Nanobashvili et al., 2000).

A more direct evidence for the seizure inhibiting potential of GDNF comes from studies showing that intracerebroventricular (i.c.v) administration of GDNF inhibits KA induced seizures and retards behavioral progression of kindling (Li et al., 2002; Martin et al., 1995). These findings have brought about studies trying to suppress seizures by focal delivery of GDNF.

Gene therapy utilizing the GDNF system

Both ECB and viral vector delivery of GDNF into the hippocampus have been shown to decrease various aspects of seizures. Unilateral adenoviral mediated expression of GDNF in the hippocampus inhibited KA induced acute seizures along with rescuing glutamic acid decarboxylase (GAD) expression, which is involved in GABAergic signalling (Yoo et al., 2006). Similarly, AAV vector driven GDNF overexpression decreased the frequency of generalized seizures in SE induced by electrical stimulation and increased seizure induction threshold in pre-kindled rats. However, no significant effect was observed on the progression of seizure severity during kindling (Kanter-Schlifke et al., 2007). Interestingly, ECB of GDNF indicated a dose dependent relationship between re-kindling susceptibility and released amount of GDNF from the implants. The animals implanted with a device that showed a low post-explantation release of GDNF exhibited shorter after-discharge duration and milder seizures at the 5^{th} day of rekindling than animals with implanted with devises that released higher amounts. It was argued that high levels of overexpression might lead to downregulation of the signalling pathway downstream the GFRa1 receptor, as the levels of the receptor was not changed when compared to either the high release specimens and the control (Kanter-Schlifke et al., 2009).

The above described studies were based on either acute induced seizures or the kindling model. As discussed previously, the predictive properties of these models have been criticised as they lack, e.g., the development of SRSs. Recently, however, a comprehensive study was published, utilizing the systemic pilocarpine model with SRSs and bilateral implantation of ECB devices 20 days after SE. Motor and non-motor SRS frequency were reported to be reduced, both in a short-term an a long-term (90 days) efficacy experiment. Moreover, a persistent effect was found after device removal, which indicates that the intervention had a disease modifying effect (Paolone et al., 2019). Though, the question remains, if whether unilateral implantation of an ECB device releasing GDNF into the seizure focus, which is a likely clinical scenario, would be sufficient to inhibit SRSs. This question is addressed in this thesis.

Challenges with translation

As discussed previously, lack of progress in the development of novel therapeutic approaches in drug-resistant epilepsy have been related to use of models with limited predictive value, low statistical power and poor reproducibility between different laboratories (Simonato et al., 2014; Steward et al., 2012). Specifically, small sample size have led to overestimation of effects, which consequently are hard to reproduce (Button et al., 2013). One reason for these deficits have been attributed to miscommunication between research groups and lack of standardisation (Landis et al., 2012).

Multicentre studies have been suggested to remedy reproducibility and increase the translational value of pre-clinical studies (O'Brien et al., 2013; Simonato et al., 2014). However, in order to be feasible, standardisation and harmonization of practices in pre-clinical studies is required.

Efforts have been made in the field of spinal cord injury (SCI) and TBI, to standardize reporting and harmonizing experimental procedures (Lemmon et al., 2014; Smith et al., 2015). Specifically, the experimental data was suggested to be arranged in common data elements (CDEs), similar to how the National institute of neurological disorders and stroke (NINDS) have done for clinical studies (Stone, 2010). A CDE can be described as a basic data unit that is common across study objectives, and holds information about e.g. animals' species, or type of model used.

To meet the need for standardisation and harmonization in the field of pre-clinical epilepsy research, the European Union funded project, Targets and biomarkers for epileptogenesis (EPITARGET), started the development of CDEs and an online data dictionary, which would allow for sharing and comparing data between research groups. This work is described in this thesis.

Aims of the thesis

Despite decades of research and development of anti-seizure drugs, still approximately one third of epilepsy patients do not respond satisfactory to treatment. The majority of these refractory patients have focal epilepsy and gene therapy has been suggested to be a promising approach to treat these patients. The overall aim of this thesis has been to investigate the seizure suppressing effects of such gene therapies by utilizing a translational pre-clinical study framework.

Specific aims of the thesis were:

- To characterize the i.h. KA rat model of chronic epilepsy in terms of disease severity and progression (Paper I).
- To evaluate the effect of AAV serotype and gene orientation, exemplified by combinatorial NPY and Y2 gene therapy, on acute KA induced seizures (Paper II).
- To explore the supressing effect of focal combinatorial NPY and Y2 receptor gene therapy on spontaneous recurrent seizures in the i.h. KA rat model of chronic epilepsy (Paper I and III).
- To investigate whether focally released GDNF by ECB supresses spontaneous recurrent seizures in the i.h. KA rat model of chronic epilepsy (Paper IV).
- To contribute to harmonization of experimental procedures and the development of a data sharing platform to support multicentre pre-clinical studies. (Paper V)

Experimental procedures

Ethics

The experimental procedures performed in Sweden (Paper I-IV) were approved by the local ethical committee for experimental animals (Ethical permit no. M190-09 and M49-15) and conducted in agreement with the Swedish Animal Welfare Agency regulations. The dose-response study (Paper III) were performed in Israel in compliance with The Israel Board for Animal Welfare Act and was approved by the Israel Board for Animal Experiments (Approval no. IL-16-09-359).

Animals

All animals used in this work were male Wistar rats (Charles River, Germany) weighing 200-230 g at the arrival to the facility. They were housed in pairs or in groups of three unless under video-EEG monitoring, during which they were kept singly. The animals had *ad libitum* access to food and water, but in the long study (> 10 months) described in paper I, where they had a daily forage of 15-20 g. The animal facility had a set 12 h light / dark cycle, and the animals were checked daily for any signs of stress and morbidity.

Animals were randomly assigned to treatment groups (Paper I-IV). Specifically, in the case of the long-term efficacy study of combinatorial single vector NPY and Y2 receptor gene therapy (Paper III), the animals were randomly added to the treatment groups according to the SRS frequency exhibited before intervention. Thus, new animals were matched in pairs of similar seizure frequency and then randomly divided to receive either treatment or control, resulting in two groups with an equal average pre-intervention SRS frequency (Figure 5). All data was analysed by an operator blind to the treatment conditions.



Figure 5. Pre-intervention SRS frequency in rats Stratification of the animals in paper III resulted in two groups with equal pre-treatment SRS frequency (p = 0.93, Mann-Whitney test).

Viral vectors

All recombinant viral vectors used in this work were produced by GeneDetect (Auckland, New Zealand) as previously described (During et al., 2003). In the study where two viral vectors were used to overexpress NPY and the Y2 receptor (Paper I), a mixture of serotype 1 and 2 was utilized. Human pre-proNPY and mouse Y2 receptor were driven by the rat NSE promoter. Also, the expression cassette contained the woodchuck post-transcriptional regulatory element (WPRE) and the bovine growth hormone polyA signal to enhance expression (Woldbye et al., 2010). The control empty vector was composed of the same expression cassette, however lacking the transgene.

The single vectors containing both NPY and the Y2 receptor (Paper II and III) had the same expression enhancing components as in paper I, but differed in Y2 receptor origin and promoter. Moreover, they were not mixtures of serotypes but were composed only of either AAV1, AAV2 or AAV8 capsids. The Y2 receptor was the human gene and the promoter was CAG (chicken beta-actin promoter hybridized with the cytomega virus [CMV] immediate enhancer sequence)(Hitoshi et al., 1991). To ensure splicing of the ligand and the receptor, an internal ribosome entry site (IRES) was placed between the transgenes.

The administrated titers of the vectors were 10^{12} genomic particles (gp) / ml at all occasions but in the dose-response study, where a range of 10^9 to 10^{13} gp / ml were injected (Table 1).

Table 1. Viral vectors and the titers used in the thesis.

The conentration of 1.0 x 10^{12} gp / ml was used at all times, but in the dose-response study, where a range of 1.0 x 10^{9} – 1.0 x 10^{13} gp / ml was administered.

Viral vector	Study	Titer
Mixture AAV1/2 NPY, AAV 1/2 Y2	Paper I	1.0 x 10 ¹² gp / ml
Mixture AAV1/2 Empty	Paper I	1.0 x 10 ¹² gp / ml
	Paper II	1.0 x 10 ¹² gp / ml
AAV1-NPY-IRES-Y2	Paper III (Dose-responce)	1.0 x 10 ⁹ – 1.0 x 10 ¹³ gp / ml
	Paper III (Efficasy study)	1.0 x 10 ¹² gp / ml
AAV1-Y2-IRES-NPY	Paper II	1.0 x 10 ¹² gp / ml
	Paper II	1.0 x 10 ¹² gp / ml
AAV1-Empty	Paper III (Dose-responce)	1.0 x 10 ¹³ gp / ml
	Paper III (Efficasy study)	1.0 x 10 ¹² gp / ml
AAV2-NPY-IRES-Y2	Paper II	1.0 x 10 ¹² gp / ml
AAV2-Y2-IRES-NPY	Paper II	1.0 x 10 ¹² gp / ml
AAV2-Empty	Paper II	1.0 x 10 ¹² gp / ml
AAV8-NPY-IRES-Y2	Paper II	1.0 x 10 ¹² gp / ml
AAV8-Y2-IRES-NPY	Paper II	1.0 x 10 ¹² gp / ml
AAV8-Empty	Paper II	1.0 x 10 ¹² gp / ml

Encapsulated cell biodelivery

The ECB devises were provided by NsGene A/S (Denmark). They consisted of 7 mm long cylinders of 725 µm diameter, with an outer membrane of polyethersulfone and internally fitted with 72 filaments of polyethylene terephthalate yarn for cell adhesion. The devises were filled with 60,000 human retinal pigment epithelial cells (ARPE-19), genetically modified to produce and release GDNF. For the control group, similar devices were produced, but lacking the ability to release GDNF. The release of GDNF was measured by enzyme linked immunosorbent array (ELISA) one day before implantation to be 435.9 ng/ml/24h.

Induction of epileptic seizures

Two different methods of modelling seizures where used in this thesis. 1) The induction of acute seizures by s.c. KA served as screening method, due to high throughput and still reasonably high prediction of efficacy. In this case, the viral vector preceded the induction of seizures (Paper II). 2) In the chronic studies (I, III and IV), where any supressing effects on SRSs were investigated to increase the translational value of the results, the KA was injected unilaterally into the hippocampus of the rats to initiate SE. This epileptogenic insult triggered the development of SRSs, which were subsequently monitored. In these studies, the treatment intervention was introduced after the development of SRSs, to investigate any seizure supressing or disease modifying effects (Simonato, 2014). The two methods are described in detailed below.

Kainic acid induced acute seizures

Kainic acid (ab120100, Sigma Aldrich) was prepared fresh at the day of the experiment. 50 mg of KA was diluted to a final concentration of 10 mg / ml in sterile isotonic 0.9% saline, and pH was adjusted with 1M NaOH (Sigma Aldrich) to 7.4 to reduce any pain response when injected. The pH was continuously monitored with a pH meter (FiveGo F2, Mettler Toledo) during calibration to prevent overshoot.

The administration of KA in rats were done by injecting a dose of 10 mg / kg s.c. in the neck region. The animals were subsequently placed in transparent Plexiglass boxes with the dimensions 30x19x29 cm. This allowed for video monitoring of seizure activity for 2h, before the animals were sacrificed. The video was then reviewed and the seizures scored according to a modified scale of Racine (Gøtzsche et al., 2012; Racine, 1972). In addition, the time until 1st motor seizure, number of motor seizures, latency to SE and number of seizures were measured.

Post status epilepticus spontaneous recurrent seizures

To induce SE, KA was administered to the medial or ventral part of the right hippocampus by stereotaxic surgery. The excitotoxic compound was prepared by diluting KA to a final concentration of 1 mg / ml and adjusting pH to 7.4 by addition of 1M NaOH.

At the beginning of the procedure, the rat was placed in an anaesthesia induction box containing 4% isoflurane / air mixture (450 ml/min, Intervet, Sweden). Once the animal was asleep, it was transferred and fixed to a stereotax (Kopf instruments, Tujuga, USA) while decreasing the anaesthesia to approximately 2%. The head was cleaned with chlorhexidine, and 0.4 ml of local s.c. anaesthesia Marcain (AstraZeneca, Sweden) was applied. A longitudinal incision was made, exposing the scull, and a burr hole of 1 mm in diameter was drilled above the injection point. The *dura mater* was removed by a sterile needle tip, and then a glass capillary, preloaded with KA was lowered to the predefined depth (Table 2). 0.4 µl was subsequently slowly injected (0.2 µl/min), where after the capillary was left in place for 2 - 5 minutes to prevent backflow. The wound was closed using staples immediately after capillary retraction.

Two different sets of coordinates, based on a stereotaxic atlas of the rat brain, were used to induce SE by injecting KA into the right hippocampus (Table 2) (Paxinos and Watson, 2007). In the case of ECB implantation (Paper IV), the same burr hole was used for both KA administration and device implantation. Bregma was used as

reference for the anterior/posterior coordinate, and midline for the medial/lateral coordinate. The dorsal/ventral coordinate was measured from *dura mater*.

Study	Coordinates (mm)
Paper I	AP: -5.3 ML: 4.5 DV: -3.2
Paper III	AP: -5.3 ML: 4.5 DV: -3.2
Paper IV	AP: -4.8 ML: 5.2 DV: -6.3

Table 2. Injection coordinates for KA administration.

The coordinated targeted the medial hippocampus (Paper I and III) and ventral hippocampus (Paper IV).

Video-EEG monitoring

The rats were continuously video-EEG monitored to ensure accurate measurement of SRS frequency. Seizure clustering and individuals exhibiting a low seizure frequency might lead to missed seizures when intermittent monitoring is utilized (Rattka et al., 2013). Video-EEG monitoring by telemetry was preferred for long-term monitoring of SRSs, because it enabled the animals to move freely, as well as decreased the strain on the implant.

Continuous video-EEG monitoring in this work was done by utilizing the F40-EET transmitter system (Data Sciences International, USA), capable of measuring two EEG channels while also indicating the temperature of the animal. The data is transmitted to a receiver plate, positioned under the cage, which relays the signal to a computer for storage and analysis (Figure 6). As the transmitter is battery powered, it can be turn on and off by a magnet to preserve its service life.

Four network cameras (Axis Communication, Sweden) were used to video monitor the animals in order to assist in SRS detection and analysis of the seizures. The EEG data was synchronized with video of the animal's behavior, resulting in a file that could be analyzed with the Neuroscore software (Data Sciences International, USA).



Figure 6. Telemetric monitoring of SRSs in epileptic rats.

The rats were continously monitored by video-EEG. The F40-EET implant transmitted two EEG singals to a receiver plate, which relayed the signal to a computer. This data was then synchronised with video to facilitate SRS detection. The implantation of the F40-EET transmitter was performed at different stages of the studies depending on the prerequisites of the particular experimental setup, e.g., magnetic resonance imaging (MRI) is incompatible with the implant, or the scientific question in mind. In study I, when characterizing the model, the implantation was performed in the same surgical session as the SE induction to allow for direct monitoring of the seizure progression after the epileptogenic insult. Contrary to this, in study III, the implantation was done after the MRI session, 6 weeks after SE, so that it would not interfere with imaging. Finally, in study IV, implantation of the transmitter was performed 1 week before the injection of KA to enable monitoring of SE but shorten the KA injection procedure.

All the video-EEG monitoring was done with one electrode placed ipsilateral to the KA injection on motor cortex, and one depth electrode placed in the hippocampus contralateral to the KA injection, as described below.

Implantation of telemetric device for EEG monitoring

The F40-EET transmitter was prepared by soldering a stainless-steel electrode (Plastics One, USA) to one of the four wires. The soldering was insulated by a short strip of shrink-tube to ensure stability. At the time of transmitter implantation, the rats were anaesthetised with a 4% isoflurane / air mixture and placed in a stereotax as described for the injection of KA. The transmitter was positioned in a s.c. pocket on the back of the rats with the wires guided to the scull. A drill was used to prepare a burr hole for the hippocampal depth electrode contralateral to the KA injection at coordinates: AP: -4.8, ML: -5.2 and DV: -6.3 (-6.2 in paper III). The coordinates were measured in relation to bregma, midline and *dura mater* as reference points. The second electrode was placed on *dura mater*, ipsilateral to the KA injection, above motor area M2/M1, and the reference electrodes were placed on *dura mater*, caudal to the lambdoid suture. Once the electrodes were in position, dental cement was added to cover and attach the implant to the scull (Figure 7).



Figure 7. The disposition of electrodes on the rat skull used in study II.

(A) Disposition of electrodes on the rat skull as seen from above. The cortical electrode (a) was placed on *dura mater* above motor area M1/M2, and the hippocampal depth electrode (b) contralateral to the KA injection (s). Reference electrodes (d1, d2) were place on top of *dura mater*, caudal to the lamboid structure. The shaded area was covered by dental cement, and a region (e) was left clear to allow for individualized viral vector administration. Two screws were used to increase stability. (B) Illustration of the electrodes in the rat brain.

Monitoring of spontaneous recurrent seizures

At the time for SRS monitoring, a magnet was put in the vicinity of the implanted transmitter to turn it on. The timing of the monitoring differed depending on the posed scientific question. The initial characterization of the model (Paper I) required monitoring to commence immediately after SE. However, for investigation of the anti-seizure effect of an experimental treatment, two monitoring periods were used, one before and one after treatment. When exploring the seizure supressing effects of combinatorial NPY and Y2 receptor gene therapy (Paper III), the monitoring periods were 3 weeks each. In the case of the of the ECB of GDNF (Paper IV), 5 weeks of pre-treatment monitoring was followed by 2 week long post-treatment monitoring.

The video-EEG was analysed by an operator blind to the treatment condition. The continuous EEG was visually inspected for high amplitude fast-spiking activity (Figure 8), and the synchronized video aided the analysis where there was uncertainty of whether an EEG pattern was indeed caused by a SRS.



Figure 8. An example of a generalized SRS found in a rat injected with KA.

The SRS is characterized by high amplitude fast spiking activity. The upper trace (blue) represents the hippocampal depth electrode, and the lower (black) is the cortical electrode ipsilateral to the KA injection. The SRS starts in the hippocampus and propagates to the cortex (generalization).

Electrophysiology

In paper II, *ex-vivo* electrophysiological recordings were performed in animals injected with combinatorial NPY and Y2 receptor gene therapy. Field recordings of excitatory post synaptic potentials (fEPSPs) were conducted in the CA1 *stratum radiatum* while stimulating the Schaffer collaterals with square constant current pulses of 0.1 µs. At the beginning of the recording, the input/output relationship between the presynaptic fiber volley and the fEPSP was measured, and only slices capable of generating fEPSP greater than 1 mV was included. For stimulation of the slices, a current that elicited a 30-50% response of maximal fEPSP was used throughout the recording.

The short-term plasticity of the fEPSP were determined by paired-pulse stimulation. Different interstimulus intervals were used (25, 50, 100, 200 ms) separated by 30 seconds. The paired-pulse ratio was measured by determining the initial slope of the second and dividing it by the slope of the first fEPSP.

To investigate the effect of transgene NPY expression on excitatory neurotransmission, high frequency stimulations trains of 25 Hz (50 stimulations) were given to one synaptic pathway followed by a paired-pulse stimulation in a neighbouring independent pathway. This paradigm was repeated 8 times separated by 5 minutes. To prevent induction of LTP, a N-Methyl-D-aspartic acid (NMDA) receptor antagonist, D(2)-2-Amino-5-phosphopentanoic acid (D-AP5) was applied to the aCSF. NPY Y2-receptor antagonist BIIE0246 (0.6 μ M) was added to the aCSF to evaluate the contribution of NPY Y2-receptors in the effect of transgene NPY on synaptic transmission.

Treatment intervention

The administration of either *in-vivo* (viral vectors) or *ex-vivo* (ECB devices) gene therapy into the hippocampus was done by stereotaxic surgery. The rats were anaesthetised with and isoflurane / air mixture and placed in a stereotaxic frame (Kopf instruments, USA), as described for the injection of KA, to allow for high precision local treatment. The coordinates used bregma as reference for anterior/posterior, midline for the medial / lateral and *dura mater* for the dorsal / ventral coordinate (Paxinos and Watson, 2007).

Injection of viral vectors

The injection of viral vectors was performed by first drilling burr holes in the skull bone, and thereafter lowering a glass capillary attached to a 5 μ l Hamilton syringe to

the desired depth. The viral vector suspension was slowly injected (0.2 μ l / 60 sec) and the capillary was left in place for 10 minutes to prevent backflow.

Pre-insult injections (Paper II) was performed using well established coordinates since no hippocampal lesion had to be considered. However, in chronic epileptic animals, MRI was used to determine the injection coordinates. For the first long-term study (Paper I), where two separate vectors were administered, a set of coordinates that would achieve good targeting in all animals was used (Table 3). Though, in the chronic efficacy study (Paper III) individualized targeting of the hippocampus was performed to achieve optimal administration (Figure 9). All injection coordinates aimed at achieving expression in the entire hippocampus ipsilateral to the injection of KA.

Table 3. Injection coordinates and volume of viral vector administered in paper I and paper II.

In paper I, MRI was used to find a set of coordinates that would suit all animals in the study. This was not required in study II since the viral injections were performed before the KA injection and no morphological alterations of the hippocampus was exprected.

Study	Dorsal coordinate (mm)	Ventral coordinate (mm)	Volume per injection site
Paper I (MRI-guided universal coordinates)	AP: -4.0	AP: -6.3	1 μl rAAV-NPY
	ML: 1.5	ML: 4.8,	1.5 μl rAAV-Y2
	DV: -3.6	DV: -4.8, -5.8	
	AP: -3.3	AP: -4.8	1 µl
Paper II (Pre insuit injection of viral vectors)	ML: ±1.8	ML: ±5.2	
	DV: -2.6	DV: -3.8, -6.4	



Figure 9. Individualised viral vector coordinates used in study III.

The viral vector inection coordinates in paper III were individualized for each rat based on MRI. The experiment was performed in three batches (green, red and blue), the yellow dot in origo marks bregma. The average dorsal coordinates was: AP: 4.07 ± 0.26 mm, ML: 2.17 ± 0.27 mm, DV: -2.46 ± 0.16 mm. The average ventral coordinates were: AP: -5.71 ± 0.40 mm, ML: 4.86 ± 0.20 mm, DV1: -4.53 ± 0.54 mm, DV2: -6.05 ± 0.30 mm (mean \pm SD).

ECB implantation

The ECB devices were implanted through the same injection tract where KA was administered. With the rat attached to the stereotaxic frame as described before, a stainless-steel cannula was used to lower the device to coordinates; AP: -4.8, ML: + 5.2 and DV: -7.0 mm (Figure 10). At the termination of the experiment, the rats were perfused with 0.9% saline through the ascending aorta, which allowed for explantation of the ECB device and subsequent measurement of GDNF release.



Figure 10. Illustration of the ECB implantation in the rat brain. The ECB implant was positioned in the right hippocampus using the same burr hole as the KA injection.

Magnetic resonance imaging

MRI was used to find the optimal injection coordinates for viral vector administration. Also, in paper I, the data obtained was used to investigate the extent of tissue damage following i.h. KA and subsequent SE. The rats were anesthetised with isoflurane during the procedure and any remaining staples were removed before insertion into an animal holder and positioning in the machine. The MRI machine was designed for imaging in small animals with a field strength of 9.4 T. T2 weighted images were obtained using the settings described in table 4.

The MRI images were analysed with the OsiriX software (Geneva, Switzerland) and compared to a stereotaxic atlas (Paxinos and Watson, 2007), for alignment and to determine the injection coordinates.

Table 4. Properties	and settings of the I	IRI used in paper	and paper III.
Tuble 4.1 Topoldo	and ootango of the f	ma acca m paper	ana papor mi

Study	Machine	Coil	No. Of slices	Thickness of slices	Echo time	Repetietion time
Paper I	9.4 T 400 MHz Aglient Technologies	205 / 120 HD gradient coil	45	400µm	39.39 ms	4000 ms
Paper III	9.4 T Bruker	4 element RF coil	22	500µm	41 ms	3000 ms

Histology

At the termination of each animal experiment, brain tissue was collected for analysis. Depending on the planned methods, the tissue was treated differently. Functional binding of the Y2 receptor required the tissue not to be fixed with paraformaldehyde. Thus, in those experiments the brains were snap frozen in dry ice after decapitation. If functional binding was not planned, the rats were first transcardially perfused with cold 0.9% saline and then by 4% paraformaldehyde. The brains were subsequently removed from the skull to be processed for histology.

Immunohistochemistry

Snap frozen tissue was cut with a cryostat in 16 μ m thick slices which were mounted on glass slips. Before immunohistochemical staining, the tissue was fixed on the glass with 4% paraformaldehyde. Tissue from transcardinally perfused rats was cut by a microtome to 30 μ m thick slices. Subsequent staining proceeded with incubation with the primary antibody in 4° C over night, and the secondary antibody conjugated with a fluorophore for 2h at room temperature the next day (Table 5). Each staining included specimens that had the primary antibody excluded from the protocol to control for unspecific binding of the secondary antibody. After staining, the tissue was coverslipped with a glycerol-based mounting media (DABCO, Sigma Aldrich).

Antigen	Host	Dilution	Source and catalouge	
ED1	Mouse	1:200	AbD Serotech, MCA341	
NPY	Rabbit	1:500	Sigma Aldrich, N9528	
GFAP	Mouse	1:500	Sigma Aldrich, G3893	
NeuN	Mouse	1:100	Millipore, MAP377	

Primary antibodies for immunohistochemistry

Table 5.

Functional and basal binding of the Y2 receptor

For visualization of the Y2 receptor (Paper I-III), functional binding was applied as previously described (Christensen et al., 2006; Sim et al., 1995; Woldbye et al., 2010). The slices were treated with [35S]-GTP γ S in the presence of synthetic NPY (Schafer-N, Denmark) while blocking Y1 and Y5 receptors with antagonists BIBP3226 (Y1 antagonist, Bachem, Switzerland) and L-152,804 (Y5 antagonist, Tocris, UK). Basal binding was determined by incubation without NPY and the receptor antagonists. After exposure on Kodac BioMax films and development, the images were analysed in Fiji (image processing software(Schindelin et al., 2012)). To compare functional binding, two regions of interest (ROIs) were defined by two equal rectangles, ipsi- and contralateral to the viral vector injection. A ratio was formed from the optical density of these regions.

Enzyme linked immunosorbent assay

The release of GDNF from the ECB devices (Paper IV) were measured by ELISA 24h before implantation and the day after explantation. A commercially available kit (DuoSet for human GDNF, R&D Systems, USA) was used according to the manufacturer's instructions. The media, in which the sample was incubated, was sampled after 4h.

Microscopy

Digitalized images were acquired by an Olympus BX61 microscope (Olympus, Japan) in concert with the CellLens software. For analysis of the immunoreactivity, all images were acquired using the same exposure settings. Subsequently, the same paradigm described for the functional binding was applied for calculating a ratio between the optical density, ipsi- and contralateral to the viral vector administration.

For analysis of the tropism of the viral vector (Paper III), a scanning laser confocal microscope (A1, Nikon, Japan) was used to take images of tissue stained for NPY plus a cellular marker of neurons (NeuN) or astrocytes (GFAP).

Research electronic data capture (REDCap)

In paper V, CDEs were developed for the field of pre-clinical epilepsy research. To further enhance the use of the harmonized procedures and standardisation, the CDEs were implemented as an online resource based on the REDCap system. REDCap is a software solution that enables data collection and management in projects that can be

shared between research groups (Harris et al., 2009). Data from individual animals is collected via a web-based user interface and stored with a unique entry number. This creates a searchable data dictionary, that can be accessed online by authorized users.

Statistical methods

Distributions passing a normality test were compared with a Student's t-test (similar variance) or Welch test (not similar variance). In the cases where normal distributions could not be assumed, Mann-Whitney (two comparisons) or Kruskal-Wallis (three comparisons) were used for between-group comparisons. For multiple comparisons in normally distributed data, the one-way ANOVA (one independent variable) or the two-way repeated measures ANOVA (two independent variables, repeated measures) were used with appropriate post hoc tests.

The Chi-square or the Fisher's test was utilized to conclude if observed occurrence of a defined outcome in one group differed from the occurrence of the same outcome in another group. Correlations between variables were tested with the Pearson's correlation test. Finally, the Kolmogorov-Smirnov test was used to compare non-normal distributions with a large sample size. The Prism software (GraphPad, USA) was used to perform the analysis.

Results

Development of a translational study framework (Paper I)

A study was conducted to explore a study framework reflecting a clinical trial for gene therapy in epilepsy. Two principal questions were investigated: (i) whether the i.h. KA induced SE model of chronic epilepsy is a clinically relevant model of chronic epilepsy, and (ii) if unilateral combinatorial NPY and Y2 receptor gene therapy can decrease seizures in this model of SRS if administered ipsilateral to the KA injection.

The questions were answered in two sets of experiments. The first part investigated the disease progression in the model, focusing on the incidence and the development of SRS frequency over approximately 4 months following SE. The second part explored the seizure supressing effect of combinatorial NPY and Y2 gene therapy 10 months after SE.

Characterization of the i.h. KA model of post-SE chronic epilepsy

In the initial characterization of the model, all rats were implanted with telemetry in the same surgical session as KA was administered. Hence, the initial insult (SE) and the following seizure activity could be followed by continuous video-EEG monitoring. This paradigm resulted in 38% of the animals developing SRSs with a latency of 5.8 to 60.2 days (median 12.3 days). The EEG during a SRS was characterized by high amplitude fast-spiking activity, easily distinguishable from that during normal behaviour. The seizure frequency was highly variable in those rats that developed SRSs, with an average of 6.33 ± 3.03 SRS per the 3^{rd} week after the 1^{st} seizure. Some individuals showed a clear progressive development of seizures over time, while others had a more stable seizure frequency. An overall trend of disease progression was observed.

Effects of unilateral combinatorial NPY and Y2 receptor gene therapy

In the second part of the study, rats were injected with KA and then held for 10 months to ensure that the animals demonstrated the chronic phase of the disease. MRI was conducted to characterize the lesion that is associated with KA induced SE, and to find

suitable coordinates for unilateral vector delivery. A damage was observed in all animals with an average volume of $32.7 \pm 4.7 \text{ mm}^3$, covering the region between the dorsal and ventro-caudal hippocampus ipsilateral to the KA injection. No difference was observed between animals with or without SRSs and the contralateral hemisphere showed no observable damage by MRI. A common set of coordinates were found to target the hippocampus ipsilateral to the KA injection.

Following the MRI characterization, the rats were implanted with two electrodes targeting the contralateral hippocampus and the ipsilateral neocortex relative to the KA injection. 54% of the animals showed SRSs during the 2 week long pre-treatment monitoring, with an average seizure count of 28.4 ± 10 seizures. No correlation between the number of SRSs and the volume of hippocampal damage was found. As the animals were divided into two treatment groups, a post-hoc analysis of the SRS frequency revealed no difference in inter-group frequency.

The treatment group was injected with mixed serotype 1 and 2 AAV vectors encoding full-length cDNA preproNPY and the mouse Y2 receptor driven by the NSE promoter, while the control group received the same expression cassette but lacked the transgene. To ensure maximal plateau transgene expression, the rats were left to recover for 4 weeks, thereafter the continuous video-EEG monitoring was reinitiated for 2 weeks.

During the post-treatment monitoring, the control groups exhibited disease progression reflected by an average increase in SRS frequency with $167.4 \pm 23\%$ and an increase in time spent in seizure. In contrast, the NPY and Y2 treated group showed a maintained SRS frequency (98.0 ± 43.2%) and had a reduction in total time spent in seizure, which was significant compared to the control group. Moreover, 4 out of 5 animals in the treated group had a reduction in SRS frequency, while all animals (5) in the control group exhibited disease progression.

NPY and Y2 receptor overexpression in vector treated animals

An increased NPY overexpression was confirmed with immunohistochemistry in animals treated with NPY and Y2 receptor gene therapy compared to empty controls. Expression was located to neuronal fibres in the hippocampus.

The Y2 receptor overexpression and functionality was investigated with Y2 functional binding. The binding was increased in NPY and Y2 receptor treated animals compared to empty control and the signal was blocked with application of an Y2 antagonist, confirming the increased abundance and functionality of the Y2 receptor after treatment.

Importantly, the inflammatory response in the hippocampus was compared between animals treated with NPY and Y2 receptor gene therapy and empty control. No difference was found in activated microglia investigated by Iba1 and ED1 immunoreactivity, indicating that a change in inflammation did not contribute to the results.

Taken together, this study shows the clinical relevance of the i.h. KA model as it resembles TLE in humans. Important common features are: (i) occurrence of SRSs, which has previously been described to originate from the KA injected hippocampus (Bragin et al., 2005), (ii) a proportion of animals develop SRSs while some do not, (iii) the animals show a large variation in seizure frequency, (iv) the disease progress over time resulting a gradual increase in seizure frequency.

Moreover, the model successfully demonstrated the therapeutic effect of combinatorial NPY and Y2 receptor gene therapy when delivered unilaterally with two separate vectors. The effect was shown by prevention of the disease progression compared to the empty control animals.

Screening of AAV serotype and gene orientations for combinatorial NPY and Y2 receptor gene therapy (Paper II)

The previous section described the proof of principal of the seizure supressing effects of combinatorial NPY and Y2 receptor gene therapy in the i.h. KA model of chronic epilepsy using two separate vectors. However, for the translational process, a single vector carrying both the NPY and Y2 receptor is more favourable due to regulatory reasons. Use of such single vector requires the separation of the transgenes by an element that ensures splicing of the products during translation. This is achievable with an IRES sequence; however, it is known that element of the expression cassette can reduce translation of the downstream gene (Mizuguchi et al., 2000). Moreover, it is not known how viral vector serotype influence the therapeutic effect of combinatorial NPY and Y2 receptor gene therapy, as AAV serotypes have been shown to result in different transfection of hippocampal regions (Burger et al., 2004). To answer these questions a screening study was performed, investigating three different serotypes (AAV1, AAV2 and AAV8) along with two transgene orientations (NPY-IRES-Y2 and Y2-IRES-NPY).

For screening purposes, the i.h. KA model of chronic epilepsy is less suitable due to multiple reasons. Compared to a model of induced acute seizures, e.g., s.c. KA, the chronic models have a lower incidence of seizures, low throughput and high variability. It needs to be emphasized, though, that they have a higher translational value than the models of acute seizures. Given the number of vectors to be evaluated, and the reasons stated above, the s.c. KA rat model of induced seizures was utilized in the screening for the most efficient vector.

The AAV1 serotype with expression cassette NPY-IRES-Y2 performed better in seizure suppression

To investigate if serotype and gene orientation affected the seizure suppressing effect of combinatorial NPY and Y2 receptor gene therapy, the rats were injected with the vectors bilaterally in the hippocampus. Three weeks later, they received a dose of 10 mg / kg KA to induce seizures. The animal's behaviour was video monitored and their seizures were assessed during 2 hours in regard to four parameters: latency to 1st motor seizure, time spent in motor seizure, number of seizures and latency to SE. Each vector was compared to its serotype control, comprised of a similar construct but with an empty expression cassette.

The experiment resulted in three significant differences. The AAV1-NPY-IRES-Y2 group spent less time in seizure and had a longer latency to SE than the AAV1-Empty. Also, the AAV8-Y2-IRES-NPY had a shorter latency to develop the 1st motor seizure compared the AAV-Empty. Taken together, the AAV1-NPY-IRES-Y2 construct was the one to show the best seizure supressing potential.

Comparison of NPY immunoreactivity and Y2 receptor functional binding

Given these results, we were curious to investigate whether the expression of NPY and the Y2 receptor could explain our findings. NPY immunohistochemistry was performed, and the immunoreactivity was assessed by an optical density measurement. Consistent with the better performance of AAV1-NPY-IRES-Y2 in seizure suppression, NPY immunoreactivity was significantly higher (2.7 ± 0.77 a.u., n = 8) compared to the empty control (1.04 ± 0.03 a.u., n = 8). Also, AAV1-Y2-IRES-NPY and AAV8-NPY-IRES-Y2 had significantly higher NPY reactivity than respective control.

Similar result was obtained from Y2 receptor functional binding. Both orientations carried by the AAV1 serotype had significantly higher functional binding of the Y2 receptor than control, and AAV1-Y2-IRES-NPY scored slightly higher than the AAV1-NPY-IRES-Y2. A similar pattern was found in the AAV8 group, where both vectors carrying transgenes resulted in a higher radiolabelling than the AAV-Empty. Once again, the vector with the gene coding for the Y2 receptor placed upstream the IRES site scored slightly higher than the reverse alternative.

AVV1-NPY-IRES-Y2 enhances NPY release beyond endogenous levels

As AAV1-NPY-IRES-Y2 showed potential in decreasing KA induced seizures, we investigated whether the overexpression using this vector would alter the synaptic release probability of glutamate, which is suggested to be the consequence of the presynaptic mechanism of NPY and Y2 receptor interaction (Qian et al., 1997). High-

frequent train stimulation caused an Y2-receptor dependent depression in neighbouring glutamatergic synapses in the AAV1-NPY-IRES-Y2-group, in line with the proposed mechanism of action. These findings show that overexpression of AAV1-NPY-IRES-Y2 increase expression of NPY, leading to increased NPY-release, and increased expression of functional Y2-receptors.

Disease modification by single vector NPY and Y2 receptor gene therapy (Paper III)

Based on the proof of principal results with NPY and Y2 combinatorial gene therapy (Paper I), utilizing two separate vectors for NPY and Y2 receptor overexpression, the hypothesis that a *single vector* would exert a seizure suppressing effect in a chronic model of epilepsy when administered unilaterally was investigated. This study was formulated from the insights of the characterization of the i.h. KA model of chronic epilepsy in rats and the establishment of the translational study framework described above. Moreover, the results from the screening of vector serotypes and orientations (Paper II) were used, by choosing the AAV1-NPY-IRES-Y2 vector for investigation in this study.

A second objective of this study was to investigate the optimal dose of viral vector administration for a therapeutic effect.

Dose-response

For the purpose of investigating the optimal dose for seizure suppression, the s.c. KA rat model of induced seizures was used. 5 groups of rats were injected with an increasing titer of 10⁹ to 10¹³ gp / ml of AAV1-NPY-IRES-Y2 and one control group received 10¹³ gp / ml of AAV1-Empty. The induced seizures were evaluated for latency to 1st motor seizure, latency to SE, time spent in seizure and number of seizures. Moreover, the seizures were scored according to a modified scale of Racine (Gøtzsche et al., 2012; Racine, 1972). It was concluded that the titer of 10^{12} gp / ml resulted in a significant reduction in seizure score, along with an increased latency to 1st motor seizure and SE compared to the control group. Also, the time spent in seizures were significantly reduced. The titer of 10^{13} gp /ml also supressed seizures reflected by a decreased latency to 1st motor seizure and a decreased number of seizures during the monitoring period. However, the higher titer did not contribute to a higher seizure suppressant effect than the 10^{12} gp / ml titer, which led to the decision to choose the next highest titer for administration in the chronic study. This decision was based on the better performance, but also taking safety into account, seeking the lowest effective titer possible for translation.

Disease modification by single vector combinatorial NPY and Y2 receptor gene therapy

A total of 34 rats received either combinatorial NPY and Y2 receptor gene therapy (n = 16) or empty control (n = 18). Comparing 3 weeks of video-EEG monitoring before and after intervention showed a disease modifying effect in the treated animals. 5 of 16 animals in the NPY / Y2 treated group had a reduction of SRS frequency that was larger than 50%, resulting in a responder rate of 31.3%. None of the control animals had a reduction of SRS frequency that exceeded 50%, which results in a significant difference between the groups.

As shown previously (Paper I), the disease progression in i.h. KA injected rats results in a gradual increase in the group average seizure frequency over time. The median increase in the control group during the monitoring period was 21.1%, which correlates to a disease progression rate of 1.221. This value was used to define non-progressive animals as individuals with a disease progression rate lower than 1.211.

Comparing nonprogressive animals from both groups, the NPY / Y2 treated animals exhibited a statistically larger reduction in SRS frequency ($57 \pm 33\%$) than the control group ($17 \pm 22\%$). This seizure suppressing effect was also reflected in the time spent in seizure, where the NPY / Y2 group showed a significant reduction ($64 \pm 28\%$), compared to the control ($28 \pm 16\%$).

NPY and Y2 receptor gene therapy alters inter SRS intervals and SRS clustering

Seizure clustering is a common feature in human epilepsy patients, and it is associated with drug-resistance (Haut et al., 2005; Sillanpää and Schmidt, 2008). To investigate whether seizure clustering was affected by NPY and Y2 receptor gene therapy, we performed an analysis focusing on the inter SRS intervals (the latency between seizures). Two distributions were formed including all inter SRS intervals in the two respective groups. From the distributions, it was clear that most intervals were represented in the spectrum of 0-10 hours, but there were also two peaks of inter SRS intervals in the range of 10-35 hours and 35-55 hours (Figure 11). The interpretation of this pattern would be that clusters of SRSs with short inter seizure intervals are separated with periods of refractoriness between clusters (long seizure intervals).



Figure 11. Distribution of inter SRS intervals.

The distribution of SRS intervals show that most intervals are in the range of 0-10 hours. However, there are also two peaks of inter SRS intervals in the range of 10-35 hours and 35-55 hours, which indicates that the SRS occur in clusters. Moreover, the NPY and Y2 receptor animals (magenta) had longer intervals between clusters than the control group (black), resulting in a right shift of the distribution.

The distribution of inter SRS intervals in the NPY and Y2 receptor treated group was significantly shifted towards longer intervals compared to the control group, indicating that, overall, the latency between SRSs are longer after NPY and Y2 receptor gene therapy. Similarly, focusing on the two peaks representing the latency between clusters, a significant shift towards longer latencies was observed in both cases. Thus, also the inter cluster interval was longer after NPY and Y2 receptor gene therapy.

Increased levels of both NPY and the Y2 receptor in the hippocampus after single vector NPY and Y2 receptor gene therapy

Immunohistochemical analysis of NPY expression revealed increased levels of NPY in the CA1 and CA3 region of the hippocampus in animals injected with AAV1-NPY-IRES-Y2 compared to the empty control. Confocal microscopy of slices stained for NPY and cellular markers for astrocytes (GFAP) and neurons (NeuN) showed that expression was localised to neuronal fibres rather than astrocytes. Moreover, functional binding of the Y2 receptor also showed increased levels of binding in the CA1 and CA3 region of the hippocampus. However, no correlation between expression levels and either NPY or Y2 receptor expression could be statistically found.

Encapsulated cell biodelivery of GDNF suppresses SRSs (Paper IV)

An alternative approach to deliver a therapeutic agent into the parenchyma is by exvivo ECB gene therapy. In order to enhance safety and overcome problems with immune rejection, cells can be enclosed in a semipermeable capsule, allowing for nutrients to enter and the therapeutic agent produced by the cell to escape the implant.

Such ECB is suggested to be a viable option to deliver GDNF into the epileptic hippocampus, which has been shown to ameliorate the disease condition when administered by direct i.c.v. infusion or by viral vector overexpression in animal models of epilepsy.

To investigate the suppressant effects of such approach on SRSs, ECB devices were implanted unilaterally into the hippocampus of chronic epileptic rats. Any effect on SRS suppression was monitored by continuous video-EEG.

Production of GDNF before and after implant

To investigate the viability of the ECB devices, GDNF levels were measured by ELISA after explantation. After 21 days in the brain, the implants were still releasing GDNF (232.5 \pm 17.7 ng/ml/24h) although the average level had declined from the pre-implant release of 435.9 \pm 11.5 ng/ml/24h.

Seizure suppressant effects of unilateral encapsulated cell biodelivery of GDNF

GDNF treatment with ECB did not change the duration of SRSs when compared to animals implanted with non GDNF-releasing cell capsules. On the other hand, SRS frequency decreased in 5 out of 6 rats in the GDNF treated group compared to control group, where only 1 out of 7 had a reduction in SRS frequency. The difference in the distribution of an ameliorated disease state was statistically different between the groups.

While the control group exhibited a median increase in SRS frequency by 50%, the GDNF treated group had a median reduction of 51%, which was statistically different. Thus, unilateral GDNF treatment with ECB can reduce the frequency of SRSs and reverse the progression of the disease in the i.h. KA model of chronic epilepsy.

Inflammatory response after ECB implantation

It has been suggested that the seizure suppressant effect exerted by GDNF might be due to inhibition of neuroinflammation (Rocha et al., 2012). To assess the activation of microglia, immunohistochemical staining of ED1 was performed, however no difference was detected by optical density measurement of the immunoreactivity.

Harmonization of experimental procedures and development of a common database for pre-clinical data (Paper V)

The field of neuroscience in general, and epilepsy in particular, has suffered from failures in translating pre-clinical findings, and multiple setbacks in drug development have made the pharmaceutical companies less interested in developing treatments for CNS disorders. The problems have been attributed to a set of factors related to how the pre-clinical work is conducted and communicated. Specifically, some animal models have been identified to lack predictive power for efficacy in humans. Other factors involve lack of statistical power and reproducibility of results.

In an attempt to address the problem, the EPITARGET consortium developed CDEs to facilitate reproducibility and multicentre studies. The work involved harmonization of models and experimental procedures, as well as establishing a common platform to collect and share data in a standardized manner. In this study, the process of establishing a database containing pre-clinical data from harmonized studies is described.

Common data elements and harmonization of experimental procedures

Over the course of one year, the EPITARGET consortium discussed experimental procedures in work groups with the purpose of harmonizing experimental protocols and standardizing data collection. Guidelines were written for animal models of epileptogenesis, imaging, blood sampling, etc. Moreover, it was agreed upon which variables should be collected and how. The result was a set of CDEs (>1000) representing different aspects of the experiment with corresponding guidelines on operation.

The CDEs were logically organized in eight modules (Figure 12). The first and mandatory module contained core animal characteristics, which describes essential elements, e.g., age of an experimental animal at the beginning of the experiment. Then followed modules related to the epilepsy model, monitoring, treatment, laboratory tests, pathology, imaging and assessment of functional outcome. The EPITARGET CDEs are available to the public at the EPITARGET website: www.epitarget.eu/cdes/.





Development of a platform for data collection and sharing

The CDEs were used to form an online data dictionary in REDCap, which is accessible to all consortium members and managed from Lund University. As the REDCap database was not able to handle the variable data collected from experiments, e.g., images or miRNomics, metadata was collected instead. Thus, the data dictionary holds information about how the experiments was performed and what data is available from the member that performed the experiment.

General Discussion

The overall aim of this thesis has been to investigate the effect of gene therapy on SRSs, utilizing a translational pre-clinical study framework. This framework was based on an initial characterization of the i.h. KA post-SE rat model, which shares many features with the human condition, e.g. hippocampal sclerosis and the progressive occurrence of SRSs. Based on the insights of the disease progression in the model, translationally inspired studies were performed, individualizing the treatment of each animal and timing the intervention to resemble a treatment in established epilepsy in patients. Two different gene therapy interventions were investigated, combinatorial NPY and Y2 receptor *in-vivo* gene therapy and ECB ex-vivo gene therapy with GDNF, both administered to the presumed seizure focus. To further enhance the translational value of the results, the efficacy study investigating the seizure supressing effects of combinatorial NPY and Y2 receptor gene therapy was preceded by a screening of vector candidates and a dose-response study. These preparatory studies concluded that administration of 1012 gp/ml of AAV1-NPY-IRES-Y2 resulted in the best inhibitory effect on acute induced seizures. Thus, this vector and concentration was administered in the chronic efficacy study.

Both combinatorial NPY and Y2 receptor *in-vivo* gene therapy and *ex-vivo* gene therapy based on ECB of GDNF successfully inhibited SRSs in the chronic stage of the disease when administered unilaterally in the vicinity of the KA injection site. The findings add to the cumulating evidence of the seizure suppressing and translational potential of these two gene therapy approaches.

Finally, given the experience gained from the performed studies, and acknowledging the need and advantages of multi centre studies, an attempt at standardizing and harmonizing pre-clinical procedures was presented. The development of pre-clinical CDEs in epilepsy and the establishment of an online dictionary, could supply a foundation for future data sharing and collaboration.

Predictive value and limitations of the i.h. KA rat model of TLE

The i.h. KA model is by many suggested to be a good model of human TLE (Kandratavicius et al., 2014; Rattka et al., 2013). Multiple studies, including the results presented in this thesis, have indicated the progressive development of seizure frequency (Raedt et al., 2009; Rattka et al., 2013). The progression of the disease in humans is however not necessarily accompanied by an increase in seizure frequency, but rather by progression of seizure complexity (Blume, 2006; French et al., 1993).

Along with the seizures emerging from the hippocampus, histological alterations in the model also share similarities with the human condition, e.g., neuronal cell loss, and hippocampal sclerosis (Bragin et al., 2005; Lévesque and Avoli, 2013; Rattka et al., 2013). However, there are considerations that the lesion induced by the KA is more extensive than what is observed in humans, where the changes related to TLE are more subtle (Henshall, 2017; Sloviter et al., 2007). Still the similarity with how TLE develops in humans, with an epileptogenic insult followed by a latency period before spontaneous seizures emerge, makes the model relevant for translational studies (Kandratavicius et al., 2014).

The variance in SRS frequency in the rat i.h. KA model is considerable. This makes analysis of data challenging, but it also contributes to the overall translational value of the model, as patients with TLE are presenting with varying degrees of disease severity (Moran et al., 2004). This feature is important to consider when designing a study, since random assignment of animals to treatment groups, without taking into consideration SRS frequency, might result in one group representing on average a more severe disease state. Thus, stratifying the animals on the basis of pre-intervention SRS frequency, should result in a fairer comparison of treatment outcome. Another factor to take into consideration when designing efficacy studies is the progressive development of the SRS frequency. The underlying progression brings that also preventing an increase in SRS frequency (disease modification) should be considered a positive outcome of an experimental treatment.

In our hands, the i.h KA rat model of TLE exhibit almost exclusively generalized seizures, which is not the case in human TLE patients, where focal seizures are common (Wieser and ILAE Commission on Neurosurgery of Epilepsy, 2004). Other studies have reported the occurrence of focal seizures, although the majority have been reported to be grade 4/5 generalized convulsive seizures (Rattka et al., 2013). The differences in reported disease severity in the same model might be attributed to different experimental procedures, e.g. location of KA injection or use of anaesthesia when inducing SE (Bar-Klein et al., 2016).

In rats, the seizure spread from the initiating focus is rapid, and might over time induce a secondary focus (mirror focus) contralateral to the KA injection (Sobayo and Mogul, 2013). A reason for this might be extensive hippocampal commissures, demonstrated to mediate spread of electrically induced afterdischarges to homologous contralateral sites (Fernandes de Lima et al., 1990). A mirror focus would escape the unilateral gene therapy, thereby dampening the treatment effect or rendering some animals unresponsive to the intervention. In primates however, the interhemispheric hippocampal interconnectivity is less prominent (Amaral et al., 1984), which may predict unilateral administration of gene therapy being more successful in human patients.

In line with this, the electrode placement presented in these studies, with one electrode in the contralateral hippocampus and one cortical ipsilateral to the KA injection, would not be able to distinguish between seizures emerging at a contralateral mirror focus or in the vicinity of the KA injection. Therefore, these studies cannot conclude whether focal gene therapy would prevent the spreading of seizures from a secondary mirror focus to the original one. Thus, further studies, ideally utilizing bilateral hippocampal electrodes, would provide more information of the local inhibitory potential of the treatment.

Importantly, in mice, the recruitment of the contralateral hemisphere in seizure activity has been shown to be a contributing factor in the progression of seizure intensification (Berglind et al., 2018). The prevention of spread of seizures by focal gene therapy, even if the seizure emerged outside the treated hippocampus would then be beneficial in preventing disease progression, which could contribute to the disease modifying effect demonstrated in these studies.

Finally, the i.h. KA rat model may represent drug-resistant seizures (Rattka et al., 2013). However, available literature is not conclusive on this matter, as only a few studies have investigated the effects of anti-seizure drugs. In one study, phenobarbital was shown to decrease SRSs considerably, however not achieving seizure freedom in all rats (4/8) (Rattka et al., 2013). Another study administered carbamazepine to rats injected bilaterally with KA, resulting in a decrease in SRS frequency compared to control. Similarly to the previous study, complete seizure freedom was not achieved (Jia et al., 2018). However, the version of the rat model, where KA is administered systemically, is considered to be resistant to some standard anti-seizure drugs, and is currently a part of the ETSP (Grabenstatter et al., 2005; Grabenstatter and Dudek, 2019; Kehne et al., 2017). Taken together, the i.h. KA model in rats may represent drug-resistant seizures. However, further studies are required to conclude whether this is the case.

A cohort of animals that display SRSs after KA induced SE, is composed of animals exhibiting different degrees of disease severity. Thus, it is conceivable that there might be individual animals that are drug-resistant, as has been demonstrated in e.g., the mouse version of the model (Klein et al., 2015). Screening a cohort for these drug-

resistant individuals, before introducing an experimental treatment, could be a way to further enhance the translational aspect of a study. This could also provide an opportunity to investigate synergies or drug-interactions with experimental treatments.

Screening of vector candidates and dose-response study

In-vivo gene therapy based on the NPY system has long been suggested as a promising approach in treating focal epilepsy in general, and TLE in particular (Löscher et al., 2008; Vezzani and Sperk, 2004). Previous studies have indicated that simultaneous overexpression of both NPY and the Y2 receptor results in a synergistic inhibitory effect on rapid kindling (Woldbye et al., 2010). It is not known however, how the AAV vector serotype or the transgene orientation affects the seizure suppressing properties of NPY and Y2 receptor overexpression if a single vector is used to carry both the receptor and the ligand. The AAV serotypes have different tropism and also vary in the ability to spread in tissue (Burger et al., 2004). Moreover, a single bicistronic vector, carrying both NPY and the Y2 receptor require an intermediate element, separating the proteins after translation. The IRES sequence fit that purpose, but has been reported to reduce expression of the downstream gene (Mizuguchi et al., 2000).

To investigate how these factors affect seizure suppression, a screening study was performed, investigating candidates that differed in AAV serotype and transgene orientation. It was found that the AAV1-NPY-IRES-Y2 vector had a significant inhibitory effect on KA induced acute seizures, as it decreased time spent in motor seizures and increased the latency to SE. Why this particular vector performed better than the other candidates could be explained by the AAV1 serotype's ability to achieve widespread expression in the hippocampus, especially in comparison to AAV2, which was unable to elevate both NPY immunoreactivity and Y2 functional binding. Surprisingly, the AAV8 vectors did not perform well in suppressing seizures, even though it is reported to be superior to the AAV1 serotype in this aspect (Aschauer et al., 2013). Further investigation of the cell-type specific expression (tropism) of the transgenes could shed light on these observed differences.

In preparation for the efficacy study in the i.h. KA model of SRSs, a dose-response study was performed. Based on the better performance of AAV1-NPY-IRES-Y2 driven overexpression in supressing acute seizures, this vector was administered bilaterally to rats in concentration varying from 10^9 to 10^{13} gp/ml. Concentrations greater than 10^{12} gp/ml were able to supress KA induced acute seizures. However, the highest dose of 10^{13} gp/ml failed to significantly increase the effect. A similar narrow therapeutic window has been described for AAV based gene therapy in Parkinson's disease (Cederfjäll et al., 2013), indicating that further increases of the dose might not lead to better therapeutic effect, but increase the risk of adverse effects. Thus, for translational

purposes, it was decided to minimize the number of viral particles delivered. Thus, the dose of 10¹² gp/ml AAV1-NPY-IRES-Y2 was administered to chronic epileptic rats in the efficacy study.

Seizure suppressing effects of combinatorial NPY and Y2 receptor gene therapy

Few studies have investigated the seizure suppressing properties of NPY in models of SRSs (Noè et al., 2008), and particularly, no data is available on the supressing effect of combinatorial NPY and Y2 receptor gene therapy on SRSs.

Thus, for proof-of-principle, combinatorial NPY and Y2 receptor gene therapy was achieved with two separate AAV vectors in the i.h. KA rat model, administered unilaterally in the presumed seizure focus. A disease modifying effect was found in the treated rats, as they on average maintained SRS frequency. In contrast, the control group exhibited an increase in SRS frequency, illustrating the progressive features of the disease in the model. Moreover, a tendency to shorter seizures along with the reduction in SRS frequency resulted in the treated animals exhibiting a significant difference in the relative change in time spent in SRSs compared to control. A clinical interpretation of the results gives at hand a responder rate (> 50% reduction in SRS frequency) of 20% and a seizure free rate of 20%, since one animal became seizure free after treatment. No animals in the control group reached these criteria. However, the low number of animals included in this initial study limits the strength of these conclusions.

To further investigate these findings, an efficacy study was performed utilizing a single vector for transgene overexpression. The choice of vector and administered concentration was based on the preceding screening and dose-response study. AAV1-NPY-IRES-Y2 gene therapy, administered unilaterally aided by MRI resulted in a responder rate of 31.3% (5 out of 16) and a seizure free rate of 12.5% (2 out of 16), which is comparable to the outcomes of vagal nerve stimulation in humans (Ben-Menachem, 2002). Again, no animals in the control group fulfilled these criteria. Thus, the results are in line with, or even slightly better than the two vector approach, again with reservation for the low number of animals included in the proof-of-principal study.

An interesting question is to what extent the bicistronic expression of the Y2 receptor contribute to the suppression of SRSs in comparison to only NPY. A consequence of simultaneous overexpression of NPY and the Y2 receptor is the possibility that the Y2 receptor inhibits not only release of glutamate, but also modulate the release of NPY by auto-regulation (Silva et al., 2005). Studies in hypothalamic rat slices have indicated
that application of Y2 selective agonists reduce both basal and high-potassium induced NPY release (King et al., 1999). Such auto-regulation of NPY release could potentially limit the therapeutic effect of the combinatorial gene therapy approach. However, a synergistic affect has been described on the development of kindling induced afterdischarges (Woldbye et al., 2010). In addition, another study has investigated the effect of only NPY gene therapy on SRSs (Noè et al., 2008). This study utilized the model of SRSs following electrically induced SE and bilateral administration of the vector. It was reported that one animal (10%, 1 of 10) had a response to treatment that reduced the SRS frequency with more that 50%. This is significantly lower than the responder rate of 31.3% that was found after unilateral combinatorial gene therapy. This indicates that combinatorial NPY and Y2 overexpression could have a synergistic effect on SRS inhibition that is not defeated by auto-regulation of NPY release. Though, more studies are required to investigate to what extent the potential autoregulation affects transgenic NPY release.

Effect of NPY and Y2 receptor overexpression on SRS clustering

Interestingly, the continuous video-EEG monitoring revealed a non-normal temporal distribution of the SRSs following KA induced SE. Clustering of seizures seemed to be present, as periods of more intense SRS occurrence was separated by SRS free intervals. Such seizure clustering has been previously been described after both systemic and i.h. KA induced SE, pilocarpine, and in the model of perinatal hypoxia-ischemia (Dudek and Staley, 2011; Goffin et al., 2007; Grabenstatter et al., 2005; Kadam et al., 2010; Rattka et al., 2013). Most importantly, seizure clustering is a frequent finding in human epilepsy, and is often coupled to drug-resistance (Haut et al., 2005; Sillanpää and Schmidt, 2008). It has been hypothesised that the clustering of seizures might be attributed to the accumulation of refractoriness as a result of repetitive seizure activity. Successive in changes ionic-microenvironment, pH-shifts and levels of neuromodulators (such as NPY) could eventually render the epileptic focus incapable of generating further seizures. However, as the seizure events disappear, the refractoriness would gradually wear off, and the seizures re-emerge (Löscher and Köhling, 2010; Rattka et al., 2013).

AAV1-NPY-IRES-Y2 gene therapy had an overall effect on the inter-SRS intervals but also modified the clustering of the SRSs. A prolonged duration between seizures in general, but also between seizure clusters was observed compared to the control group. It is possible that the modulation of the NPY and Y2 receptor levels by AAV derived overexpression could explain this effect. The replenishment of neuropeptides is slow compared to neurotransmitters, and it has been shown that immunoreactivity of NPY decreases during 3 hours following SE in rats (Marksteiner et al., 1989; van den Pol, 2012; Vezzani and Sperk, 2004). NPY overexpression might lead to a faster refilling of the releasable pool, increasing the NPY contribution to the accumulating refractoriness, e.g. by its ability to act as a volume transmitter (Sørensen et al., 2008b). Moreover, since the Y2 receptor exhibits low internalization and desensitization (Nederpelt et al., 2016), episodic release of NPY could lead to accumulation of Y2 signalling, eventually decreasing the excitability to a level where seizures are no longer generated and terminates the seizure cluster. Enhancing this effect by also overexpressing the Y2 receptor could explain the modification of the clustering pattern (Figure 13). This hypothesis requires further investigation, possibly with an NPY radioligand and PET imaging at different intervals following a seizure.



Figure 13. A schematic illustration of the accumulation of refractoryness. Modifying the NPY and Y2 receptor levels by AAV driven overexpression might lead to larger contributions to refractoryness following a seizure, resulting in lower seizure frequency and altered clustering patterns.

Seizure supressing effects of encapsulated cell biodelivery of GDNF

Accumulating evidence suggest that GDNF is capable of modulating seizure in various models of epilepsy. Specifically, the seizure suppressing potential of GDNF released by ECB has been demonstrated to inhibit electrically induced SE, and to supress SRSs in the pilocarpine model (Kanter-Schlifke et al., 2009; Paolone et al., 2019). ECB offers some important advantages over both *in-vivo* gene therapy and direct cell therapies, as the encapsulation of cells enables explantation of the device, thus the option of cancelling the treatment if there are concerns about safety or if the treatment is ineffective (Lindvall and Wahlberg, 2008). Moreover, it offers a method of localized

delivery of the therapeutic agent directly to the parenchyma. This is important from a translational perspective, since i.c.v. administration has been associated with adverse effects, such as nausea, anorexia and vomiting, demonstrated by a double blind clinical trial in Parkinson's disease (Nutt et al., 2003).

To further strengthen the translational potential of ECB as a treatment of TLE, a study was performed to investigate whether ECB of GDNF has a supressing effect on SRSs when delivered unilaterally into the presumed seizure focus. The results indicate a significant inhibiting effect on SRSs, with a responder rate of 50% (> 50% reduction is SRS frequency). Similar to the efficacy study with NPY and the Y2 receptor, no animals in the control group had this level of reduction. Instead, 6 out of 7 animals in the control group displayed disease progression while 5 of 6 animals in the treated group had a reduction in SRS frequency. These results show that unilateral ECB of GDNF is sufficient to supress SRSs.

A question of the long-term function of ECB is valid, since we observed decreased levels of released GDNF for the devices after explantation. In-line with this, a clinical trial utilizing ECB devices for delivery of NGF in Alzheimer's patients reported that the devices released 10% of the original levels 12 months after implantation, and that this could be attributed to cell death within the implants (Eriksdotter-Jönhagen et al., 2012). However, long-term release of GDNF from explanted ECB devices has been demonstrated in naïve rats after 24 weeks post implantation. Interestingly, the released levels of GNDF were higher than the pre-implantation measurements (Paolone et al., 2019). Similarly, another clinical study with ECB of NGF in Alzheimer patients identified some devices that released elevated levels after explantation as compared to the initial values (Eyjolfsdottir et al., 2016). Further development of the encapsulation of cells might lead to better homogeneity of the release of products. Though, the longevity of the devices might not be crucial for modulating the disease with GDNF, as a supressing effect on SRSs in epileptic rats was detectable 20 days after explantation (Paolone et al., 2019). Thus, a transient increase in GDNF levels might be enough to ameliorate the disease. This makes further investigations of the anti-epileptogenic properties of GDNF highly warranted.

The mechanism behind the inhibitory effect of GDNF on SRSs is not fully understood. ECB of GDNF has been shown to partly prevent the morphological changes in hippocampal volume and the neurodegeneration that is associated with the pathology in the pilocarpine model. Furthermore it promoted the survival of GABAergic parvalbumin positive interneurons (Paolone et al., 2019). Since GABAergic dysfunction is suggested to play a crucial role in the generation of seizures (Isomura et al., 2008; Shuman et al., 2020), it is possible that the rescue of GABAergic signalling could explain the observed therapeutic effect. Interestingly, GFR α 1 knockout mice showed a significant loss of interneurons in the subventricular zone - olfactory bulb neurogenic system, indicating that GFR α 1 could be crucial for the differentiation,

migration and survival of interneuronal precursors (Marks et al., 2012). Thus, an exciting hypothesis would then be that increased levels of GDNF in the hippocampus redirects the differentiation of newborn granule cells into becoming interneurons. However, this must be investigated further, e.g. with BrdU labelling of newborn cells.

Harmonization and standardization in preclinical studies

The problems in translating preclinical treatments into the clinic, especially when it comes to CNS disorders, are jeopardising the pharmaceutical industries' interest in supporting and developing new drugs. Issues with the pathophysiological relevance of models, lack of reproducibility and statistical power are suggested to be important factors that have been contributing to the failure to meet the need of novel treatments for drug-resistant epilepsy (Simonato et al., 2014).

Recent initiatives in harmonizing experimental procedures and standardizing reporting aim at solving some of these issues and thereby enhancing the probability of pre-clinical findings reaching the clinic. In-line with this, CDEs for pre-clinical research in the fields of SCI and TBI have been developed in similarity to how NINDSs suggest to gather clinical information from neurological disorders (Lemmon et al., 2014; Smith et al., 2015). Acknowledging the need of standardisation also in field of pre-clinical epilepsy research, CDEs were developed by the EPITARGET consortium.

To further facilitate the use of these CDEs, an implementation in form of a data dictionary was generated based on the REDCap database (Harris et al., 2009). Adhering to the FAIR guiding principles, this online resource is expected to reduce miscommunication and increase data comparability between the research groups (Wilkinson et al., 2016). Good maintenance of the data dictionary will facilitate *findability*, *accessibility* and *interoperability*, but most importantly *reusability* of the data produced by the research groups.

However, there are some challenges with the implementation. During the development of the database, there were issues with the data security. Even though no clinical data is contained in the database, still unauthorized access to the files could compromise the integrity of the system, e.g. by researchers hesitating in sharing data or by the risk of data misinterpretation. This could be addressed by limiting the number of people that have access to the database and by logging user activity. Moreover, there is a challenge in motivating researchers to put in data into the dictionary. The current implementation is by some considered to be too cumbersome and hard to navigate. Further development of the user interface along with training the users might solve these issues. Taken together, the development of pre-clinical CDEs for epilepsy is expected to enhance reproducibility and may provide a foundation for multicentre studies. Hopefully, this will result in more successful translation of pre-clinical finding and in extension, better treatments for patients.

Conclusions and future perspectives

Epilepsy is a complex and diverse disorder, with multiple continuously ongoing mechanisms facilitating the disease. Still these underlying processes are not fully understood, which is a major contributing factor for the lack of progress in developing new treatments for drug-resistant epilepsy. The need of novel approaches to address these cases are urgent, as the disease is burdensome, not only for the patient, but also for the nearest caring and society in general.

The studies presented in this thesis further strengthens the translational value of both ECB of GDNF and combinatorial NPY and Y2 receptor gene therapy as treatments for TLE. Though, before these treatments are considered for clinical trials, the toxicity and safety must be investigated. Specifically, studies exploring the biodistribution of viral vector administration must confirm that transgene overexpression is located only in the target site with no adverse effects. In this context, tissue resected from epileptic patients could serve as an important bridging platform, by providing an intermediate step between animal and clinical research. In addition to the question about safety, practical issues need to be solved. Targeting of the epileptic focus in humans require upscaling, and it is possible that research on larger animals, e.g., pigs or primates, is necessary to address these challenges. Moreover, production of the larger quantities of viral vectors or ECB devices needed for administration in humans must follow good laboratory practice (GLP), as well as good manufacturing practice (GMP), with reliable assays that would address the reproducibility/homogenicity of all products.

Gene therapy is a powerful and flexible tool, and the development of cell-type specific vectors and novel molecular techniques is rapid. Both *in-vivo* gene therapy based on the NPY system, and *ex-vivo* gene therapy with GDNF takes advantage of endogenous mechanisms to supress seizures. However, this also brings the risk of off target effects. Novel inventions such as optogenetics or chemogenetics circumvent some of these issues by being activated by light or inert molecules. Further development of these techniques might enable better selectivity and specificity, and thereby resulting in potentially also better efficacy.

Finally, the studies presented in this thesis have investigated the seizure supressing effects of focal gene therapy administrated in the chronic stage of the disease. They have shown that both ECG of GDNF and overexpression of NPY and the Y2 receptor could reverse the disease progression, decreasing the frequency of SRSs. Now, an intriguing question is whether these approaches could prevent epileptogenesis, rescuing the brain form developing seizures after an insult such as stroke and head trauma. This should be investigated in accurate models with high translational value and could possibly be performed as multicentre studies.

Acknowledgements

I am thoroughly grateful for all support I have received during my years at the lab. Although You all might not be mentioned in this text, I can assure you that none of You are forgotten.

Merab, thank you for all friendship and support, both during times of struggle and tailwind. There have been occasions when I have felt that nothing is going my way, and that the only rational thing to do, would be to surrender, to give up. Full of doubt, I have come to your office for advice, and after talking to you, subsequently left with renewed hope and enthusiasm. I am grateful for you always sharing your experience, not only on the professional level, but also regarding life in general.

My, without your help, none of this would have been possible. Thank you for taking care of me, and for all our wonderful conversations. You have an extraordinary ability to lift and support people around you.

Tania, thank you for always taking your time to listen and for supplying me with new perspectives. After talking to you, it is easy to pinpoint what is important and where to put in energy. The chats we have had over coffee (or tee in you case) have been invaluable for me.

David and Casper, it's been a true pleasure to work with you. Thank you for always sharing your expertise, and for supplying me with a sort of Danish entrepreneurial enthusiasm, which I truly believe is inherited by all Danes.

Jenny, my long friend and fellow brewer. We have shared so many experiences over the years. Sharing laughs and problems with you have made everything easier. I truly admire, and try to learn from your bravery and resoluteness.

Marco, thank you for all advice, not only in the field of neuroscience but also when it comes to beer-science. Litsa, you served as a role model when I first arrived at the lab, teaching me the essentials for survival in the field of science. Together, you two continue to show the way forward.

Avto and Javan, thank you for all the amazing moments in the lab. Working with you two has been great fun, overcoming technical difficulties and surgical challenges.

Alessandra, your happy spirits guided the way when I was a newbie. Most importantly, you taught me that all problems can be solved by cursing in Italian.

Fredrik, working with you is always a learning experience. Your eye for detail and structure is incredible. Thank you for all interesting chats, about life, science and extremely nerdy old school games.

Jan, Karin och Annika, thank you for introducing me to a completely new perspective of research. Working with you have been one of the most rewarding and developing aspects of my PhD.

Nora, what would I have done without you. I can't count how many times you have saved me, or indeed the entire lab, from the abyss. You, sharing your *sisu* helped not only in accomplishing great feats in the lab, but also with challenges in the real world.

Susanne J, Susanne G and Ann-Katrin, your help has been invaluable. Thank you for always lining up for solving emergencies, and for sharing your experience with life in general.

Thank you Ana, Eliška, Apostolis and Valentina. I truly enjoy the humor and laughter that seem to surround you at all times. I wish you the best of luck with your future endeavors.

Matilda and Una, my science fellows in crime. It's has been wonderful to travel the world with you, sharing laughter and adventures. I will never forget the night / morning in Washington, when a tipsy bee lady forced us out on the streets in search of an apartment without an address.

My dear family, the comfort of knowing that I can always fall back on you for support and love is an immense luxury.

Finally, Amanda. I know you at times have put your life on hold in order for me to pursue my PhD, but never even once have you complained, but always been supportive and encouraging. I'm so grateful to have you, and I love you more than you can imagine.

References

- Airaksinen, M.S., Saarma, M., 2002. The GDNF family: Signalling, biological functions and therapeutic value. Nat. Rev. Neurosci. 3, 383–394. https://doi.org/10.1038/nrn812
- Amaral, D., Lavenex, P., 2007. Hippocampal neuroanatomy. The hippocampus book.
- Amaral, D.G., Insausti, R., Cowan, W.M., 1984. The commissural connections of the monkey hippocampal formation. J. Comp. Neurol. 224, 307–336. https://doi.org/10.1002/cne.902240302
- Arzimanoglou, A., French, J., Blume, W.T., Cross, J.H., Ernst, J.P., Feucht, M., Genton, P., Guerrini, R., Kluger, G., Pellock, J.M., Perucca, E., Wheless, J.W., 2009. Lennox-Gastaut syndrome: a consensus approach on diagnosis, assessment, management, and trial methodology. Lancet Neurol. 8, 82–93. https://doi.org/10.1016/S1474-4422(08)70292-8
- Aschauer, D.F., Kreuz, S., Rumpel, S., 2013. Analysis of Transduction Efficiency, Tropism and Axonal Transport of AAV Serotypes 1, 2, 5, 6, 8 and 9 in the Mouse Brain. PLoS One 8, 1–16. https://doi.org/10.1371/journal.pone.0076310
- Bar-Klein, G., Klee, R., Brandt, C., Bankstahl, M., Bascuñana, P., Töllner, K., Dalipaj, H., Bankstahl, J.P., Friedman, A., Löscher, W., 2016. Isoflurane prevents acquired epilepsy in rat models of temporal lobe epilepsy. Ann. Neurol. 80, 896–908. https://doi.org/10.1002/ana.24804
- Baraban, S.C., 2004. Neuropeptide Y and epilepsy: Recent progress, prospects and controversies. Neuropeptides 38, 261–265. https://doi.org/10.1016/j.npep.2004.04.006
- Baraban, S.C., 2002. Antiepileptic actions of neuropeptide Y in the mouse hippocampus require Y5 receptors. Epilepsia 43, 9–13. https://doi.org/10.1046/j.1528-1157.43.s.5.13.x
- Baraban, S.C., Hollopeter, G., Erickson, J.C., Schwartzkroin, P.A., Palmiter, R.D., 1997. Knock-out mice reveal a critical antiepileptic role for neuropeptide Y. J. Neurosci. 17, 8927–8936. https://doi.org/10.1523/jneurosci.17-23-08927.1997
- Barker-Haliski, M., Steve White, H., 2015. Glutamatergic mechanisms associated with seizures and epilepsy. Cold Spring Harb. Perspect. Med. 5, 1–15. https://doi.org/10.1101/cshperspect.a022863

- Beghi, E., Giussani, G., Abd-Allah, F., Abdela, J., Abdelalim, A., Abraha, H.N., Adib, M.G., Agrawal, S., Alahdab, F., Awasthi, A., Ayele, Y., Barboza, M.A., Belachew, A.B., Biadgo, B., Bijani, A., Bitew, H., Carvalho, F., Chaiah, Y., Daryani, A., Do, H.P., Dubey, M., Endries, A.Y.Y., Eskandarieh, S., Faro, A., Farzadfar, F., Fereshtehneiad, S.M., Fernandes, E., Fijabi, D.O., Filip, I., Fischer, F., Gebre, A.K., Tsadik, A.G., Gebremichael, T.G., Gezae, K.E., Ghasemi-Kasman, M., Weldegwergs, K.G., Degefa, M.G., Gnedovskaya, E. V., Hagos, T.B., Haj-Mirzaian, Arvin, Haj-Mirzaian, Arya, Hassen, H.Y., Hay, S.I., Jakovljevic, M., Kasaeian, A., Kassa, T.D., Khader, Y.S., Khalil, I., Khan, E.A., Khubchandani, J., Kisa, A., Krohn, K.J., Kulkarni, C., Nirayo, Y.L., Mackay, M.T., Majdan, M., Majeed, A., Manhertz, T., Mehndiratta, M.M., Mekonen, T., Meles, H.G., Mengistu, G., Mohammed, S., Naghavi, M., Mokdad, A.H., Mustafa, G., Irvani, S.S.N., Nguyen, L.H., Nichols, E., Nixon, M.R., Ogbo, F.A., Olagunju, A.T., Olagunju, T.O., Owolabi, M.O., Phillips, M.R., Pinilla-Monsalve, G.D., Oorbani, M., Radfar, A., Rafay, A., Rahimi-Movaghar, V., Reinig, N., Sachdev, P.S., Safari, H., Safari, S., Safiri, S., Sahraian, M.A., Samy, A.M., Sarvi, S., Sawhney, M., Shaikh, M.A., Sharif, M., Singh, G., Smith, M., Szoeke, C.E.I., Tabarés-Seisdedos, R., Temsah, M.H., Temsah, O., Tortajada-Girbés, M., Tran, B.X., Tsegay, A.A.T., Ullah, I., Venketasubramanian, N., Westerman, R., Winkler, A.S., Yimer, E.M., Yonemoto, N., Feigin, V.L., Vos, T., Murray, C.J.L., 2019. Global, regional, and national burden of epilepsy, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. 18, 357-375. https://doi.org/10.1016/S1474-4422(18)30454-X
- Ben-Ari, Y., 1985. Limbic seizure and brain damage produced by kainic acid: Mechanisms and relevance to human temporal lobe epilepsy. Neuroscience 14, 375–403. https://doi.org/10.1016/0306-4522(85)90299-4
- Ben-Menachem, E., 2002. Vagus-nerve stimulation for the treatment of epilepsy. Lancet Neurol. 1, 477–482. https://doi.org/https://doi.org/10.1016/S1474-4422(02)00220-X
- Benarroch, E.E., 2009. Neuropeptide Y: Its multiple effects in the CNS and potential clinical significance. Neurology 72, 1016–1020. https://doi.org/10.1212/01.wnl.0000345258.18071.54
- Bendotti, C., Vezzani, A., Serafini, R., Servadio, A., Rivolta, R., Samanin, R., 1991. Increased preproneuropeptide Y mRNA in the rat hippocampus during the development of hippocampal kindling: Comparison with the expression of preprosomatostatin mRNA. Neurosci. Lett. 132, 175–178. https://doi.org/10.1016/0304-3940(91)90295-5
- Benmaamar, R., Pham-Lê, B.T., Marescaux, C., Pedrazzini, T., Depaulis, A., 2003. Induced down-regulation of neuropeptide Y-Y1 receptors delays initiation of kindling. Eur. J. Neurosci. 18, 768–774. https://doi.org/10.1046/j.1460-9568.2003.02810.x
- Berglind, F., Andersson, M., Kokaia, M., 2018. Dynamic interaction of local and transhemispheric networks is necessary for progressive intensification of hippocampal seizures. Sci. Rep. 8, 1–15. https://doi.org/10.1038/s41598-018-23659-x

- Bertram, E.H., Williamson, J.M., Cornett, J.F., Spradlin, S., Chen, Z.F., 1997. Design and construction of a long-term continuous video-EEG monitoring unit for simultaneous recording of multiple small animals. Brain Res. Protoc. 2, 85–97. https://doi.org/10.1016/S1385-299X(97)00033-0
- Birbeck, G.L., Hays, R.D., Cui, X., Vickrey, B.G., 2002. Seizure reduction and quality of life improvements in people with epilepsy. Epilepsia 43, 535–538. https://doi.org/10.1046/j.1528-1157.2002.32201.x
- Blair, R.D.G., 2012. Temporal Lobe Epilepsy Semiology. Epilepsy Res. Treat. 2012, 1–10. https://doi.org/10.1155/2012/751510
- Bloch, J., Bachoud-Lévi, A.C., Déglon, N., Lefaucheur, J.P., Winkel, L., Palfi, S., Nguyen, J.P., Bourdet, C., Gaura, V., Remy, P., Brugières, P., Boisse, M.F., Baudic, S., Cesaro, P., Hantraye, P., Aebischer, P., Peschanski, M., 2004. Neuroprotective gene therapy for Huntington's disease, using polymer-encapsulated cells engineered to secrete human ciliary neurotrophic factor: Results of a phase I study. Hum. Gene Ther. 15, 968–975. https://doi.org/10.1089/hum.2004.15.968
- Bloss, E.B., Hunter, R.G., 2010. Hippocampal kainate receptors. Vitam. Horm. 82, 167–184. https://doi.org/10.1016/S0083-6729(10)82009-6
- Blümcke, I., Thom, M., Aronica, E., Armstrong, D.D., Bartolomei, F., Bernasconi, A.,
 Bernasconi, N., Bien, C.G., Cendes, F., Coras, R., Cross, J.H., Jacques, T.S., Kahane,
 P., Mathern, G.W., Miyata, H., Moshé, S.L., Oz, B., Özkara, Ç., Perucca, E., Sisodiya,
 S., Wiebe, S., Spreafico, R., 2013. International consensus classification of hippocampal
 sclerosis in temporal lobe epilepsy: A Task Force report from the ILAE Commission on
 Diagnostic Methods. Epilepsia 54, 1315–1329. https://doi.org/10.1111/epi.12220
- Blume, W.T., 2006. The progression of epilepsy. Epilepsia 47, 71–78. https://doi.org/10.1111/j.1528-1167.2006.00665.x
- Boon, P., Vonck, K., De Herdt, V., Van Dycke, A., Goethals, M., Goossens, L., Van Zandijcke, M., De Smedt, T., Dewaele, I., Achten, R., Wadman, W., Dewaele, F., Caemaert, J., Van Roost, D., 2007. Deep brain stimulation in patients with refractory temporal lobe epilepsy. Epilepsia 48, 1551–1560. https://doi.org/10.1111/j.1528-1167.2007.01005.x
- Bragin, A., Azizyan, A., Almajano, J., Wilson, C.L., Engel, J., 2005. Analysis of chronic seizure onsets after intrahippocampal kainic acid injection in freely moving rats. Epilepsia 46, 1592–1598. https://doi.org/10.1111/j.1528-1167.2005.00268.x
- Brodie, M.J., Barry, S.J.E., Bamagous, G.A., Norrie, J.D., Kwan, P., 2012. Patterns of treatment response in newly diagnosed epilepsy. Neurology 78, 1548 LP 1554.
- Burger, C., Gorbatyuk, O.S., Velardo, M.J., Peden, C.S., Williams, P., Zolotukhin, S., Reier, P.J., Mandel, R.J., Muzyczka, N., 2004. Recombinant AAV viral vectors pseudotyped with viral capsids from serotypes 1, 2, and 5 display differential efficiency and cell tropism after delivery to different regions of the central nervous system. Mol. Ther. 10, 302–317. https://doi.org/10.1016/j.ymthe.2004.05.024
- Button, K.S., Ioannidis, J.P.A., Mokrysz, C., Nosek, B.A., Flint, J., Robinson, E.S.J., Munafo, M.R., 2013. Power failure: Why small sample size undermines the reliability of neuroscience. Nat. Rev. Neurosci. 14, 365–376. https://doi.org/10.1038/nrn3475

- Buzsáki, G., Anastassiou, C.A., Koch, C., 2012. The origin of extracellular fields and currents-EEG, ECoG, LFP and spikes. Nat. Rev. Neurosci. 13, 407–420. https://doi.org/10.1038/nrn3241
- Cederfjäll, E., Nilsson, N., Sahin, G., Chu, Y., Nikitidou, E., Björklund, T., Kordower, J.H., Kirik, D., 2013. Continuous DOPA synthesis from a single AAV: Dosing and efficacy in models of Parkinson's disease. Sci. Rep. 3, 1–12. https://doi.org/10.1038/srep02157
- Cendes, F., Andermann, F., Carpenter, S., Zatorre, R.J., Cashman, N.R., 1995. Temporal lobe epilepsy caused by domoic acid intoxication: Evidence for glutamate receptor– mediated excitotoxicity in humans. Ann. Neurol. 37, 123–126. https://doi.org/10.1002/ana.410370125
- Choudhury, S.R., Harris, A.F., Cabral, D.J., Keeler, A.M., Sapp, E., Ferreira, J.S., Gray-Edwards, H.L., Johnson, J.A., Johnson, A.K., Su, Q., Stoica, L., DiFiglia, M., Aronin, N., Martin, D.R., Gao, G., Sena-Esteves, M., 2016. Widespread central nervous system gene transfer and silencing after systemic delivery of novel AAV-AS vector. Mol. Ther. 24, 726–735. https://doi.org/10.1038/mt.2015.231
- Christensen, D.Z., Olesen, M. V, Kristiansen, H., Mikkelsen, J.D., Woldbye, D.P.D., 2006. Unaltered neuropeptide Y (NPY)-stimulated [35S]GTPγS binding suggests a net increase in NPY signalling after repeated electroconvulsive seizures in mice. J. Neurosci. Res. 84, 1282–1291. https://doi.org/10.1002/jnr.21028
- Colella, P., Ronzitti, G., Mingozzi, F., 2018. Emerging Issues in AAV-Mediated In Vivo Gene Therapy. Mol. Ther. - Methods Clin. Dev. 8, 87–104. https://doi.org/10.1016/j.omtm.2017.11.007
- Colmers, W.F., Bleakman, D., 1994. Effects of neuropeptide Y on the electrical properties of neurons. Trends Neurosci. 17, 373–379. https://doi.org/10.1016/0166-2236(94)90046-9
- Colmers, W.F., Lukowiak, K., Pittman, Q.J., 1988. Neuropeptide Y action in the rat hippocampal slice: site and mechanism of presynaptic inhibition. J. Neurosci. 8, 3827– 3837.
- Curia, G., Longo, D., Biagini, G., Jones, R.S.G., Avoli, M., 2008. The pilocarpine model of temporal lobe epilepsy. J. Neurosci. Methods 172, 143–157. https://doi.org/10.1016/j.jneumeth.2008.04.019
- de Lanerolle, N.C., Kim, J.H., Williamson, A., Spencer, S.S., Zaveri, H.P., Eid, T., Spencer, D.D., 2003. A retrospective analysis of hippocampal pathology in human temporal lobe epilepsy: Evidence for distinctive patient subcategories. Epilepsia 44, 677–687. https://doi.org/10.1046/j.1528-1157.2003.32701.x

- de Quidt, M.E., Emson, P.C., 1986. Distribution of neuropeptide Y-like immunoreactivity in the rat central nervous system-II. Immunohistochemical analysis. Neuroscience 18, 545–618. https://doi.org/10.1016/0306-4522(86)90057-6
- De Tisi, J., Bell, G.S., Peacock, J.L., McEvoy, A.W., Harkness, W.F., Sander, J.W., Duncan, J.S., 2011. The long-term outcome of adult epilepsy surgery, patterns of seizure remission, and relapse: A cohort study. Lancet 378, 1388–1395. https://doi.org/10.1016/S0140-6736(11)60890-8
- Decressac, M., Barker, R.A., 2012. Neuropeptide Y and its role in CNS disease and repair. Exp. Neurol. 238, 265–272. https://doi.org/10.1016/j.expneurol.2012.09.004
- Dudek, F.E., Staley, K.J., 2011. The time course of acquired epilepsy: Implications for therapeutic intervention to suppress epileptogenesis. Neurosci. Lett. https://doi.org/10.1016/j.neulet.2011.03.071
- Dudek, F.E., Sutula, T.P., 2007. Epileptogenesis in the dentate gyrus: a critical perspective. Prog. Brain Res. 163, 755–773. https://doi.org/10.1016/S0079-6123(07)63041-6
- Duncan, J.S., Sander, J.W., Sisodiya, S.M., Walker, M.C., 2006. Adult epilepsy. Lancet 367, 1087–1100. https://doi.org/10.1016/S0140-6736(06)68477-8
- During, M.J., Young, D., Baer, K., Lawlor, P., Klugmann, M., 2003. Development and optimization of adeno-associated virus vector transfer into the central nervous system, in: Viral Vectors for Gene Therapy. Springer, pp. 221–236.
- Duvernoy, H.M., 2005. The human hippocampus: functional anatomy, vascularization and serial sections with MRI. Springer Science & Business Media.
- Eddy, C.M., Rickards, H.E., Cavanna, A.E., 2011. The cognitive impact of antiepileptic drugs. Ther. Adv. Neurol. Disord. 4, 385–407. https://doi.org/10.1177/1756285611417920
- El Bahh, B., Balosso, S., Hamilton, T., Herzog, H., Beck-Sickinger, A.G., Sperk, G., Gehlert, D.R., Vezzani, A., Colmers, W.F., 2005. The anti-epileptic actions of neuropeptide Y in the hippocampus are mediated by Y2 and not Y5 receptors. Eur. J. Neurosci. 22, 1417–1430. https://doi.org/10.1111/j.1460-9568.2005.04338.x
- Engel, J., Wiebe, S., French, J., Sperling, M., Williamson, P., Spencer, D., Gumnit, R., Zahn, C., Westbrook, E., Enos, B., 2003. Practice parameter: Temporal lobe and localized neocortical resections for epilepsy - Report of the quality standards subcommittee of the American Academy of Neurology, in association with the American Epilepsy Society and the American Association of Neur. Neurology 60, 538–547. https://doi.org/10.1212/01.WNL.0000055086.35806.2D
- Engel Jr., J., 1995. Concepts of Epilepsy. Epilepsia 36, 23–29. https://doi.org/10.1111/j.1528-1157.1995.tb01648.x
- Erickson, J.C., Clegg, K.E., Palmiter, R.D., 1996. Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. Nature 381, 415–418. https://doi.org/10.1038/381415a0

- Eriksdotter-Jönhagen, M., Linderoth, B., Lind, G., Aladellie, L., Almkvist, O., Andreasen, N., Blennow, K., Bogdanovic, N., Jelic, V., Kadir, A., Nordberg, A., Sundström, E., Wahlund, L.O., Wall, A., Wiberg, M., Winblad, B., Seiger, Å., Almqvist, P., Wahlberg, L., 2012. Encapsulated cell biodelivery of nerve growth factor to the basal forebrain in patients with Alzheimer's disease. Dement. Geriatr. Cogn. Disord. 33, 18–28. https://doi.org/10.1159/000336051
- Eyjolfsdottir, H., Eriksdotter, M., Linderoth, B., Lind, G., Juliusson, B., Kusk, P., Almkvist, O., Andreasen, N., Blennow, K., Ferreira, D., Westman, E., Nennesmo, I., Karami, A., Darreh-Shori, T., Kadir, A., Nordberg, A., Sundström, E., Wahlund, L.O., Wall, A., Wiberg, M., Winblad, B., Seiger, Å., Wahlberg, L., Almqvist, P., 2016. Targeted delivery of nerve growth factor to the cholinergic basal forebrain of Alzheimer's disease patients: Application of a second-generation encapsulated cell biodelivery device. Alzheimer's Res. Ther. 8, 1–11. https://doi.org/10.1186/s13195-016-0195-9
- Falcicchia, C., Paolone, G., Emerich, D.F., Lovisari, F., Bell, W.J., Fradet, T., Wahlberg, L.U., Simonato, M., 2018. Seizure-Suppressant and Neuroprotective Effects of Encapsulated BDNF-Producing Cells in a Rat Model of Temporal Lobe Epilepsy. Mol. Ther. Methods Clin. Dev. 9, 211–224. https://doi.org/10.1016/j.omtm.2018.03.001
- Fernandes de Lima, V.M., Pijn, J.P., Filipe, C.N., Lopes da Silva, F., 1990. The role of hippocampal commissures in the interhemispheric transfer of epileptiform afterdischarges in the rat: a study using linear and non-linear regression analysis. Electroencephalogr. Clin. Neurophysiol. 76, 520–539. https://doi.org/10.1016/0013-4694(90)90003-3
- Fiest, K.M., Sauro, K.M., Wiebe, S., Patten, S.B., Kwon, C.-S., Dykeman, J., Pringsheim, T., Lorenzetti, D.L., Jetté, N., 2017. Prevalence and incidence of epilepsy: A systematic review and meta-analysis of international studies. Neurology 88, 296–303. https://doi.org/10.1212/WNL.000000000003509
- Fisher, R.S., Acevedo, C., Arzimanoglou, A., Bogacz, A., Cross, J.H., Elger, C.E., Engel, J., Forsgren, L., French, J.A., Glynn, M., Hesdorffer, D.C., Lee, B.I., Mathern, G.W., Moshé, S.L., Perucca, E., Scheffer, I.E., Tomson, T., Watanabe, M., Wiebe, S., Engel Jr, J., Forsgren, L., French, J.A., Glynn, M., Hesdorffer, D.C., Lee, B.I., Mathern, G.W., Moshé, S.L., Perucca, E., Scheffer, I.E., Tomson, T., Watanabe, M., Wiebe, S., 2014. ILAE Official Report: A practical clinical definition of epilepsy. Epilepsia 55, 475–482. https://doi.org/10.1111/epi.12550
- Fisher, R.S., Boas, W. van E., Blume, W., Elger, C., Genton, P., Lee, P., Engel Jr., J., 2005. Epileptic Seizures and Epilepsy: Definitions Proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). Epilepsia 46, 470–472. https://doi.org/10.1111/j.0013-9580.2005.66104.x

- Fisher, R.S., Cross, J.H., French, J.A., Higurashi, N., Hirsch, E., Jansen, F.E., Lagae, L., Moshé, S.L., Peltola, J., Roulet Perez, E., Scheffer, I.E., Zuberi, S.M., 2017. Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE Commission for Classification and Terminology. Epilepsia 58, 522–530. https://doi.org/10.1111/epi.13670
- Fisher, R.S., Vickrey, B.G., Gibson, P., Hermann, B., Penovich, P., Scherer, A., Walker, S., 2000. The impact of epilepsy from the patient's perspective II: Views about therapy and health care. Epilepsy Res. 41, 53–61. https://doi.org/10.1016/S0920-1211(00)00128-5
- Fitzsimons, H.L., Bland, R.J., During, M.J., 2002. Promoters and regulatory elements that improve adeno-associated virus transgene expression in the brain. Methods 28, 227– 236. https://doi.org/10.1016/S1046-2023(02)00227-X
- Foti, S., Haberman, R.P., Samulski, R.J., McCown, T.J., 2007. Adeno-associated virusmediated expression and constitutive secretion of NPY or NPY13-36 suppresses seizure activity in vivo. Gene Ther. 14, 1534–1536. https://doi.org/10.1038/sj.gt.3303013
- French, J.A., Williamson, P.D., Thadani, V.M., Darcey, T.M., Mattson, R.H., Spencer, S.S., Spencer, D.D., JA, F., PD, W., VM, T., TM, D., Mattson RH, SS, S., DD, S., 1993. Characteristics of medial temporal lobe epilepsy: I. Results of history and physical examination. Ann. Neurol. Off. J. Am. Neurol. Assoc. Child Neurol. Soc. 34, 774–780.
- Freund, T.F., Buzsaki, G., 1996. Interneurons of the Hippocampus. Hippogimpus 6, 347–470.
- Furtinger, S., Pirker, S., Czech, T., Baumgartner, C., Ransmayr, G., Sperk, G., 2001. Plasticity of Y1 and Y2 receptors and neuropeptide Y fibers in patients with temporal lobe epilepsy. J. Neurosci. 21, 5804–5812. https://doi.org/21/15/5804 [pii]
- Gariboldi, M., Conti, M., Cavaleri, D., Samanin, R., Vezzani, A., 1998. Anticonvulsant properties of BIBP3226, a non-peptide selective antagonist at neuropeptide Y Y1 receptors. Eur. J. Neurosci. 10, 757–759. https://doi.org/10.1046/j.1460-9568.1998.00061.x
- GBD 2017 DALYs and HALE Collaborators, 2018. Global, regional, and national disabilityadjusted life-years (DALYs) for 359 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 392, 1859–1922. https://doi.org/10.1016/S0140-6736(18)32335-3
- Goddard, G. V., McIntyre, D.C., Leech, C.K., 1969. A permanent change in brain function resulting from daily electrical stimulation. Exp. Neurol. 25, 295–330. https://doi.org/10.1016/0014-4886(69)90128-9
- Goffin, K., Nissinen, J., Van Laere, K., Pitkänen, A., 2007. Cyclicity of spontaneous recurrent seizures in pilocarpine model of temporal lobe epilepsy in rat. Exp. Neurol. 205, 501– 505. https://doi.org/10.1016/j.expneurol.2007.03.008

- Gorter, J.A., Van Vliet, E.A., Aronica, E., Lopes Da Silva, F.H., 2001. Progression of spontaneous seizures after status epilepticus is associated with mossy fibre sprouting and extensive bilateral loss of hilar parvalbumin and somatostatin-immunoreactive neurons. Eur. J. Neurosci. 13, 657–669. https://doi.org/10.1046/j.1460-9568.2001.01428.x
- Gøtzsche, C.R., Nikitidou, L., Sørensen, A.T., Olesen, M. V., Sørensen, G., Christiansen, S.H.O., Ängehagen, M., Woldbye, D.P.D., Kokaia, M., 2012. Combined gene overexpression of neuropeptide Y and its receptor Y5 in the hippocampus suppresses seizures. Neurobiol. Dis. 45, 288–296. https://doi.org/10.1016/j.nbd.2011.08.012
- Gøtzsche, C.R., Woldbye, D.P.D., 2016. The role of NPY in learning and memory. Neuropeptides 55, 79–89. https://doi.org/10.1016/j.npep.2015.09.010
- Grabenstatter, H.L., Dudek, F.E., 2019. Effect of carbamazepine on spontaneous recurrent seizures recorded from the dentate gyrus in rats with kainate-induced epilepsy. Epilepsia 636–647. https://doi.org/10.1111/epi.14680
- Grabenstatter, H.L., Ferraro, D.J., Williams, P.A., Chapman, P.L., Dudek, F.E., 2005. Use of chronic epilepsy models in antiepileptic drug discovery: The effect of topiramate on spontaneous motor seizures in rats with kainate-induced epilepsy. Epilepsia 46, 8–14. https://doi.org/10.1111/j.0013-9580.2005.13404.x
- Güttinger, M., Padrun, V., Pralong, W.F., Boison, D., 2005. Seizure suppression and lack of adenosine A1 receptor desensitization after focal long-term delivery of adenosine by encapsulated myoblasts. Exp. Neurol. 193, 53–64. https://doi.org/10.1016/j.expneurol.2004.12.012
- Harris, P.A., Taylor, R., Thielke, R., Payne, J., Gonzalez, N., Conde, J.G., 2009. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. J. Biomed. Inform. 42, 377–381. https://doi.org/10.1016/j.jbi.2008.08.010
- Haut, S.R., Shinnar, S., Moshé, S.L., 2005. Seizure clustering: Risks and outcomes. Epilepsia 46, 146–149. https://doi.org/10.1111/j.0013-9580.2005.29004.x
- Hefti, F., Denton, T.L., Knusel, B., Lapchak, P.A., 1993. Neurotrophic factors: what are they and what are they doing. Neurotrophic factors 25–49.
- Heilig, M., 2004. The NPY system in stress, anxiety and depression. Neuropeptides 38, 213–224. https://doi.org/10.1016/j.npep.2004.05.002
- Hendry, S.H.C., Jones, E.G., Emson, P.C., 1984. Morphology, distribution, and synaptic relations of somatostatin- and neuropeptide Y-immunoreactive neurons in rat and monkey neocortex. J. Neurosci. 4, 2497–2517. https://doi.org/10.1523/jneurosci.04-10-02497.1984
- Henshall, D.C., 2017. Chapter 41 Poststatus Epilepticus Models: Focal Kainic Acid, in: Pitkänen, A., Buckmaster, P.S., Galanopoulou, A.S., Moshé, S.L.B.T.-M. of S. and E. (Second E. (Eds.), . Academic Press, pp. 611–624. https://doi.org/https://doi.org/10.1016/B978-0-12-804066-9.00042-0

- Hippocrates, Jones, W.H.S., 1967. Prognostic. Regimen in acute diseases. The sacred disease. The art. Breaths. Law. Decorum. Dentition, Loeb Classical Library. Harvard University Press.
- Hitoshi, N., Ken-ichi, Y., Jun-ichi, M., 1991. Efficient selection for high-expression transfectants with a novel eukaryotic vector. Gene 108, 193–199. https://doi.org/10.1016/0378-1119(91)90434-D
- Hitti, F.L., Gonzalez-Alegre, P., Lucas, T.H., 2019. Gene Therapy for Neurologic Disease: A Neurosurgical Review. World Neurosurg. 121, 261–273. https://doi.org/10.1016/j.wneu.2018.09.097
- Hökfelt, T., 1991. Neuropeptides in perspective: The last ten years. Neuron 7, 867–879. https://doi.org/10.1016/0896-6273(91)90333-U
- Hökfelt, T., Broberger, C., Xu, Z.Q.D., Sergeyev, V., Ubink, R., Diez, M., 2000. Neuropeptides - An overview. Neuropharmacology 39, 1337–1356. https://doi.org/10.1016/S0028-3908(00)00010-1
- Hökfelt, T., Broberger, C., Zhang, X., Diez, M., Kopp, J., Xu, Z.Q., Landry, M., Bao, L., Schalling, M., Koistinaho, J., DeArmond, S.J., Prusiner, S., Gong, J., Walsh, J.H., 1998. Neuropeptide Y: Some viewpoints on a multifaceted peptide in the normal and diseased nervous system. Brain Res. Rev. 26, 154–166. https://doi.org/10.1016/S0165-0173(97)00052-0
- Holzer, P., Reichmann, F., Farzi, A., 2012. Neuropeptide Y, peptide YY and pancreatic polypeptide in the gut-brain axis. Neuropeptides 46, 261–274. https://doi.org/10.1016/j.npep.2012.08.005
- Humpel, C., Hoffer, B., Strömberg, I., Bektesh, S., Collins, F., Olson, L., 1994. Neurons of the hippocampal formation express glial cell line-derived neurotrophic factor messenger rna in response to kainate-induced excitation. Neuroscience 59, 791–795. https://doi.org/10.1016/0306-4522(94)90284-4
- Ibáñez, C.F., Andressoo, J.O., 2017. Biology of GDNF and its receptors Relevance for disorders of the central nervous system. Neurobiol. Dis. 97, 80–89. https://doi.org/10.1016/j.nbd.2016.01.021
- Ingusci, S., Cattaneo, S., Verlengia, G., Zucchini, S., Simonato, M., 2019. A Matter of Genes: The Hurdles of Gene Therapy for Epilepsy. Epilepsy Curr. 19, 38–43. https://doi.org/10.1177/1535759718822846
- Isomura, Y., Fujiwara-Tsukamoto, Y., Takada, M., 2008. A network mechanism underlying hippocampal seizure-like synchronous oscillations. Neurosci. Res. 61, 227–233. https://doi.org/10.1016/j.neures.2008.04.002
- J McCown, T., 2011. Adeno-associated virus (AAV) vectors in the CNS. Curr. Gene Ther. 11, 181–188.
- Jallon P., 2004. Mortality in patients with epilepsy. [Review] [51 refs]. Curr. Opin. Neurol. 17, 141–146. https://doi.org/10.1097/01.wco.0000124703.48483.86
- Jefferys, J.G.R., Jiruska, P., de Curtis, M., Avoli, M., 2013. Limbic Network Synchronization and Temporal Lobe Epilepsy. Jasper's Basic Mech. Epilepsies 176–189. https://doi.org/10.1093/med/9780199746545.003.0014

- Jia, C., Han, S., Wei, L., Dang, X., Niu, Q., Chen, M., Cao, B., Liu, Y., Jiao, H., 2018. Protective effect of compound danshen (Salvia miltiorrhiza) dripping pills alone and in combination with carbamazepine on kainic acidinduced temporal lobe epilepsy and cognitive impairment in rats. Pharm. Biol. 56, 217–224. https://doi.org/10.1080/13880209.2018.1432665
- Kadam, S.D., White, A.M., Staley, K.J., Dudek, F.E., 2010. Continuous Electroencephalographic Monitoring with Radio-Telemetry in a Rat Model of Perinatal Hypoxia-Ischemia Reveals Progressive Post-Stroke Epilepsy. J. Neurosci. 30, 404–415. https://doi.org/10.1523/JNEUROSCI.4093-09.2010
- Kandratavicius, L., Balista, P., Lopes-Aguiar, C., Ruggiero, R., Umeoka, E., Garcia-Cairasco, N., Bueno-Junior, L., Leite, J., 2014. Animal models of epilepsy: use and limitations. Neuropsychiatr. Dis. Treat. 10, 1693. https://doi.org/10.2147/NDT.S50371
- Kanter-Schlifke, I., Fjord-Larsen, L., Kusk, P., Ängehagen, M., Wahlberg, L., Kokaia, M., 2009. GDNF released from encapsulated cells suppresses seizure activity in the epileptic hippocampus. Exp. Neurol. 216, 413–419. https://doi.org/10.1016/j.expneurol.2008.12.021
- Kanter-Schlifke, I., Georgievska, B., Kirik, D., Kokaia, M., 2007. Seizure suppression by GDNF gene therapy in animal models of epilepsy. Mol. Ther. 15, 1106–1113. https://doi.org/10.1038/sj.mt.6300148
- Kask, A., Harro, J., Von Hörsten, S., Redrobe, J.P., Dumont, Y., Quirion, R., 2002. The neurocircuitry and receptor subtypes mediating anxiolytic-like effects of neuropeptide Y. Neurosci. Biobehav. Rev. 26, 259–283. https://doi.org/10.1016/S0149-7634(01)00066-5
- Kehne, J.H., Klein, B.D., Raeissi, S., Sharma, S., 2017. The National Institute of Neurological Disorders and Stroke (NINDS) Epilepsy Therapy Screening Program (ETSP). Neurochem. Res. 42, 1894–1903. https://doi.org/10.1007/s11064-017-2275-z
- King, P.J., Widdowson, P.S., Doods, H.N., Williams, G., 1999. Regulation of neuropeptide Y release by neuropeptide Y receptor ligands and calcium channel antagonists in hypothalamic slices. J. Neurochem. 73, 641–646. https://doi.org/10.1046/j.1471-4159.1999.0730641.x
- Kirkeby, A., Barker, R.A., 2019. Parkinson disease and growth factors is GDNF good enough? Nat. Rev. Neurol. 15, 312–314. https://doi.org/10.1038/s41582-019-0180-6
- Klapstein, G.J., Colmers, W.F., 1997. Neuropeptide Y suppresses epileptiform activity in rat hippocampus in vitro. J. Neurophysiol. 78, 1651–1661. https://doi.org/10.1152/jn.1997.78.3.1651
- Klein, R.L., Dayton, R.D., Tatom, J.B., Henderson, K.M., Henning, P.P., 2008. AAV8, 9, Rh10, Rh43 vector gene transfer in the rat brain: Effects of serotype, promoter and purification method. Mol. Ther. 16, 89–96. https://doi.org/10.1038/sj.mt.6300331
- Klein, R.L., Hamby, M.E., Gong, Y., Hirko, A.C., Wang, S., Hughes, J.A., King, M.A., Meyer, E.M., 2002. Dose and promoter effects of adeno-associated viral vector for green fluorescent protein expression in the rat brain. Exp. Neurol. 176, 66–74. https://doi.org/10.1006/exnr.2002.7942

- Klein, S., Bankstahl, M., Löscher, W., 2015. Inter-individual variation in the effect of antiepileptic drugs in the intrahippocampal kainate model of mesial temporal lobe epilepsy in mice. Neuropharmacology 90, 53–62. https://doi.org/10.1016/j.neuropharm.2014.11.008
- Kofler, N., Kirchmair, E., Schwarzer, C., Sperk, G., 1997. Altered expression of NPY-Y1 receptors in kainic acid induced epilepsy in rats. Neurosci. Lett. 230, 129–132. https://doi.org/10.1016/S0304-3940(97)00492-8
- Kokaia, Z., Airaksinen, M.S., Nanobashvili, A., Larsson, E., Kujamaki, E., Lindvall, O., Saarma, M., 1999. GDNF family ligands and receptors are differentially regulated after brain insults in the rat. Eur. J. Neurosci. 11, 1202–1216. https://doi.org/10.1046/j.1460-9568.1999.00513.x
- Kovac, S., Walker, M.C., 2013. Neuropeptides in epilepsy. Neuropeptides 47, 467–475. https://doi.org/10.1016/j.npep.2013.10.015
- Krook-Magnuson, E., Armstrong, C., Oijala, M., Soltesz, I., 2013. On-demand optogenetic control of spontaneous seizures in temporal lobe epilepsy. Nat. Commun. 4, 1–8. https://doi.org/10.1038/ncomms2376
- Kullmann, D.M., Schorge, S., Walker, M.C., Wykes, R.C., 2014. Gene therapy in epilepsy is it time for clinical trials? Nat. Rev. Neurol. 10, 300–304. https://doi.org/10.1038/nrneurol.2014.43
- Kwan, P., Schachter, S.C., Brodie, M.J., 2011. Drug-resistant epilepsy. N. Engl. J. Med. 365, 919–926.
- Landis, S.C., Amara, S.G., Asadullah, K., Austin, C.P., Blumenstein, R., Bradley, E.W., Crystal, R.G., Darnell, R.B., Ferrante, R.J., Fillit, H., Finkelstein, R., Fisher, M., Gendelman, H.E., Golub, R.M., Goudreau, J.L., Gross, R.A., Gubitz, A.K., Hesterlee, S.E., Howells, D.W., Huguenard, J., Kelner, K., Koroshetz, W., Krainc, D., Lazic, S.E., Levine, M.S., MacLeod, M.R., McCall, J.M., Iii, R.T.M., Narasimhan, K., Noble, L.J., Perrin, S., Porter, J.D., Steward, O., Unger, E., Utz, U., Silberberg, S.D., 2012. A call for transparent reporting to optimize the predictive value of preclinical research. Nature 490, 187–191. https://doi.org/10.1038/nature11556
- Larhammar, D., Wraith, A., Berglund, M.M., Holmberg, S.K.S., Lundell, I., 2001. Origins of the many NPY-family receptors in mammals. Peptides 22, 295–307. https://doi.org/10.1016/S0196-9781(01)00331-X
- Lavenex, P., Amaral, D.G., 2000. Hippocampal-neocortical interaction: A hierarchy of associativity. Hippocampus 10, 420–430.

- Ledri, M., Sorensen, A.T., Madsen, M.G., Christiansen, S.H., Ledri, L.N., Cifra, A., Bengzon, J., Lindberg, E., Pinborg, L.H., Jespersen, B., Gotzsche, C.R., Woldbye, D.P.D., Andersson, M., Kokaia, M., 2015. Differential Effect of Neuropeptides on Excitatory Synaptic Transmission in Human Epileptic Hippocampus. J. Neurosci. 35, 9622–9631. https://doi.org/10.1523/JNEUROSCI.3973-14.2015
- Leite, J.P., Babb, T.L., Pretorius, J.K., Kuhlman, P.A., Yeoman, K.M., Mathern, G.W., 1996. Neuron loss, mossy fiber sprouting, and interictal spikes after intrahippocampal kainate in developing rats. Epilepsy Res. 26, 219–231. https://doi.org/10.1016/S0920-1211(96)00055-1
- Lemmon, V.P., Ferguson, A.R., Popovich, P.G., Xu, X.M., Snow, D.M., Igarashi, M., Beattie, C.E., Bixby, J.L., 2014. Minimum information about a spinal cord injury experiment: A proposed reporting standard for spinal cord injury experiments. J. Neurotrauma 31, 1354–1361. https://doi.org/10.1089/neu.2014.3400
- Lévesque, M., Avoli, M., 2013. The kainic acid model of temporal lobe epilepsy. Neurosci. Biobehav. Rev. 37, 2887–2899. https://doi.org/10.1016/j.neubiorev.2013.10.011
- Li, S., Xu, B., Martin, D., Racine, R.J., Fahnestock, M., 2002. Glial cell line-derived neurotrophic factor modulates kindling and activation-induced sprouting in hippocampus of adult rats. Exp. Neurol. 178, 49–58. https://doi.org/10.1006/exnr.2002.8036
- Lin, L.F., Doherty, D.H., Lile, J.D., Bektesh, S., Collins, F., 1993. GDNF: a glial cell linederived neurotrophic factor for midbrain dopaminergic neurons. Science (80-.). 260, 1130 LP – 1132.
- Linard, B., Ferrandon, A., Koning, E., Nehlig, A., Raffo, E., 2010. Ketogenic diet exhibits neuroprotective effects in hippocampus but fails to prevent epileptogenesis in the lithium-pilocarpine model of mesial temporal lobe epilepsy in adult rats. Epilepsia 51, 1829–1836. https://doi.org/10.1111/j.1528-1167.2010.02667.x
- Lindvall, O., Wahlberg, L.U., 2008. Encapsulated cell biodelivery of GDNF: A novel clinical strategy for neuroprotection and neuroregeneration in Parkinson's disease? Exp. Neurol. 209, 82–88. https://doi.org/10.1016/j.expneurol.2007.08.019
- Löscher, W., 2017. Animal Models of Seizures and Epilepsy: Past, Present, and Future Role for the Discovery of Antiseizure Drugs. Neurochem. Res. 42, 1873–1888. https://doi.org/10.1007/s11064-017-2222-z
- Löscher, W., 2016. Fit for purpose application of currently existing animal models in the discovery of novel epilepsy therapies. Epilepsy Res. 126, 157–184. https://doi.org/10.1016/j.eplepsyres.2016.05.016
- Löscher, W., 2002. Current status and future directions in the pharmacotherapy of epilepsy. Trends Pharmacol. Sci. 23, 113–118. https://doi.org/10.1016/S0165-6147(00)01974-X

- Löscher, W., Gernert, M., Heinemann, U., 2008. Cell and gene therapies in epilepsypromising avenues or blind alleys? Trends Neurosci. 31, 62–73. https://doi.org/10.1016/j.tins.2007.11.012
- Löscher, W., Köhling, R., 2010. Functional, metabolic, and synaptic changes after seizures as potential targets for antiepileptic therapy. Epilepsy Behav. 19, 105–113. https://doi.org/10.1016/j.yebeh.2010.06.035
- Löscher, W., Schmidt, D., 2011. Modern antiepileptic drug development has failed to deliver: Ways out of the current dilemma. Epilepsia 52, 657–678. https://doi.org/10.1111/j.1528-1167.2011.03024.x
- Lothman, E.W., Collins, R.C., Ferrendelli, J.A., 1981. Kainic acid-induced limbic seizures: Electrophysiologic studies. Neurology 31, 806.
- Magiorkinis, E., Diamantis, A., Sidiropoulou, K., Panteliadis, C., 2014. Highights in the History of Epilepsy: The Last 200 Years. Epilepsy Res. Treat. 2014, 1–13. https://doi.org/10.1155/2014/582039
- Magiorkinis, E., Sidiropoulou, K., Diamantis, A., 2010. Hallmarks in the history of epilepsy: Epilepsy in antiquity. Epilepsy Behav. 17, 103–108. https://doi.org/10.1016/j.yebeh.2009.10.023
- Marescaux, C., Vergnes, M., Depaulis, A., 1992. Genetic absence epilepsy in rats from Strasbourg — A review BT - Generalized Non-Convulsive Epilepsy: Focus on GABA-B Receptors, in: Marescaux, C., Vergnes, M., Bernasconi, R. (Eds.), . Springer Vienna, Vienna, pp. 37–69.
- Margerison, J.H., Corsellis, J.A.N., 1966. Epilepsy and the temporal lobes: A clinical, electroencephalographic and neuropathological study of the brain in epilepsy, with particular reference to the temporal lobes. Brain 89, 499–530. https://doi.org/10.1093/brain/89.3.499
- Marks, C., Belluscio, L., Ibáñez, C.F., 2012. Critical Role of GFRα1 in the Development and Function of the Main Olfactory System. J. Neurosci. 32, 17306–17320. https://doi.org/10.1523/JNEUROSCI.1522-12.2012
- Marksteiner, J., Lassmann, H., Saria, A., Humpel, C., Meyer, D.K., Sperk, G., 1990a. Neuropeptide Levels after Pentylenetetrazol Kindling in the Rat. Eur. J. Neurosci. 2, 98–103. https://doi.org/10.1111/j.1460-9568.1990.tb00385.x
- Marksteiner, J., Prommegger, R., Sperk, G., 1990b. Effect of anticonvulsant treatment on kainic acid-induced increases in peptide levels. Eur. J. Pharmacol. 181, 241–246. https://doi.org/10.1016/0014-2999(90)90084-J
- Marksteiner, J., Sperk, G., Maas, D., 1989. Differential increases in brain levels of neuropeptide Y and vasoactive intestinal polypeptide after kainic acid-induced seizures in the rat. Naunyn-Schmiedeberg's Arch.Pharmacol. 339, 173–177.
- Martin, D., Miller, G., Rosendahl, M., Russell, D.A., 1995. Potent inhibitory effects of glial derived neurotrophic factor against kainic acid mediated seizures in the rat. Brain Res. 683, 172–178. https://doi.org/10.1016/0006-8993(95)00369-2
- McCown, T.J., 2016. Gene Therapy for Epilepsy Translational Neuroscience: Fundamental Approaches for Neurological Disorders, in: Tuszynski, M.H. (Ed.), . Springer US,

Boston, MA, pp. 155-165. https://doi.org/10.1007/978-1-4899-7654-3_9

- Michel, M.C., Beck-Sickinger, A., Cox, H., Doods, H.N., Herzog, H., Larhammar, D., Quirion, R., Schwartz, T., Westfall, T., 1998. XVI. International union of pharmacology recommendations for the nomenclature of neuropeptide Y, peptide YY, and pancreatic polypeptide receptors. Pharmacol. Rev. 50, 143–150.
- Mikuni, N., Babb, T.L., Chakravarty, D.N., Wylie Christi, 1999. Time course of transient expression of GDNF protein in rat granule cells of the bilateral dentate gyri after unilateral intrahippocampal kainic acid injection. Neurosci. Lett. 262, 215–218. https://doi.org/10.1016/S0304-3940(99)00074-9
- Mizuguchi, H., Xu, Z., Ishii-Watabe, A., Uchida, E., Hayakawa, T., 2000. IRES-Dependent Second Gene Expression Is Significantly Lower Than Cap-Dependent First Gene Expression in a Bicistronic Vector. Mol. Ther. 1, 376–382. https://doi.org/10.1006/mthe.2000.0050
- Moran, N.F., Poole, K., Bell, G., Solomon, J., Kendall, S., McCarthy, M., McCormick, D., Nashef, L., Sander, J., Shorvon, S.D., 2004. Epilepsy in the United Kingdom: Seizure frequency and severity, anti-epileptic drug utilization and impact on life in 1652 people with epilepsy. Seizure 13, 425–433. https://doi.org/10.1016/j.seizure.2003.10.002
- Nanobashvili, A., Airaksinen, M.S., Kokaia, M., Rossi, J., Asztély, F., Olofsdotter, K., Mohapel, P., Saarma, M., Lindvall, O., Kokaia, Z., 2000. Development and persistence of kindling epilepsy are impaired in mice lacking glial cell line-derived neurotrophic factor family receptor α2. Proc. Natl. Acad. Sci. U. S. A. 97, 12312–12317. https://doi.org/10.1073/pnas.97.22.12312
- Nanobashvili, A., Kokaia, Z., Lindvall, O., 2003. Generalization of rapidly recurring seizures is suppressed in mice lacking glial cell line-derived neurotrophic factor family receptor α2. Neuroscience 118, 845–852. https://doi.org/10.1016/S0306-4522(02)00998-3
- Nederpelt, I., Bunnik, J., IJzerman, A.P., Heitman, L.H., 2016. Kinetic Profile of Neuropeptide–Receptor Interactions. Trends Neurosci. 39, 830–839. https://doi.org/10.1016/j.tins.2016.09.008
- Noè, F., Pool, A.H., Nissinen, J., Gobbi, M., Bland, R., Rizzi, M., Balducci, C., Ferraguti, F., Sperk, G., During, M.J., Pitkänen, A., Vezzani, A., 2008. Neuropeptide Y gene therapy decreases chronic spontaneous seizures in a rat model of temporal lobe epilepsy. Brain 131, 1506–1515. https://doi.org/10.1093/brain/awn079
- Noe, F., Vaghi, V., Balducci, C., Fitzsimons, H., Bland, R., Zardoni, D., Sperk, G., Carli, M., During, M.J., Vezzani, A., 2010. Anticonvulsant effects and behavioural outcomes of rAAV serotype 1 vector-mediated neuropeptide y overexpression in rat hippocampus. Gene Ther. 17, 643–652. https://doi.org/10.1038/gt.2010.23

- Noe, F.M., Sorensen, A.T., Kokaia, M., Vezzani, A., 2012. Gene therapy of focal onset epilepsy using adeno-associated virus vector-mediated overexpression of neuropeptide Y. Jasper's Basic Mech. epilepsies 1–13.
- Nutt, J.G., Burchiel, K.J., Comella, C.L., Jankovic, J., Lang, A.E., Laws, E.R., Lozano, A.M., Penn, R.D., Simpson, R.K., Stacy, M., Wooten, G.F., 2003. Randomized, double-blind trial of glial cell line-derived neurotrophic factor (GDNF) in PD. Neurology 60, 69–73. https://doi.org/10.1212/WNL.60.1.69
- O'Brien, T.J., Ben-Menachem, E., Bertram, E.H., Collins, S.D., Kokaia, M., Lerche, H., Klitgaard, H., Staley, K.J., Vaudano, E., Walker, M.C., Simonato, M., 2013. Proposal for a "phase II" multicenter trial model for preclinical new antiepilepsy therapy development. Epilepsia 54, 70–74. https://doi.org/10.1111/epi.12300
- O'Dell, C.M., Das, A., Wallace, G., Ray, S.K., Banik, N.L., 2012. Understanding the basic mechanisms underlying seizures in mesial temporal lobe epilepsy and possible therapeutic targets: A review. J. Neurosci. Res. 90, 913–924. https://doi.org/10.1002/jnr.22829
- O'Hare, M.M.T., Tenmoku, S., Aakerlund, L., Hilsted, L., Johnsen, A., Schwartz, T.W., 1988. Neuropeptide Y in guinea pig, rabbit, rat and man. Identical amino acid sequence and oxidation of methionine-17. Regul. Pept. 20, 293–304. https://doi.org/10.1016/0167-0115(88)90064-X
- Olesen, M. V, Christiansen, S.H., Gøtzsche, C.R., Nikitidou, L., Kokaia, M., Woldbye, D.P.D., 2012. Neuropeptide Y Y1 receptor hippocampal overexpression via viral vectors is associated with modest anxiolytic-like and proconvulsant effects in mice. J. Neurosci. Res. 90, 498–507. https://doi.org/10.1002/jnr.22770
- Paolone, G., Falcicchia, C., Lovisari, F., Kokaia, M., Bell, W.J., Fradet, T., Barbieri, M., Wahlberg, L.U., Emerich, D.F., Simonato, M., 2019. Long-Term, Targeted Delivery of GDNF from Encapsulated Cells Is Neuroprotective and Reduces Seizures in the Pilocarpine Model of Epilepsy. J. Neurosci. 39, 2144–2156. https://doi.org/10.1523/JNEUROSCI.0435-18.2018
- Patrício, M.I., Barnard, A.R., Green, A.L., During, M.J., Sen, A., MacLaren, R.E., 2018. A clinical-grade gene therapy vector for pharmacoresistant epilepsy successfully overexpresses NPY in a human neuronal cell line. Seizure 55, 25–29. https://doi.org/10.1016/j.seizure.2017.12.005
- Patrylo, P.R., Van Den Pol, A.N., Spencer, D.D., Williamson, A., 1999. NPY inhibits glutamatergic excitation in the epileptic human dentate gyrus. J. Neurophysiol. 82, 478–483. https://doi.org/10.1152/jn.1999.82.1.478
- Paxinos, G., Watson, C., 2007. The Rat Brain in Stereotaxic Coordinates Sixth Edition. Elsevier Acad. Press 170, 547–612. https://doi.org/10.1016/0143-4179(83)90049-5
- Perucca, E., French, J., Bialer, M., 2007. Development of new antiepileptic drugs: challenges, incentives, and recent advances. Lancet Neurol. 6, 793–804. https://doi.org/10.1016/S1474-4422(07)70215-6
- Picot, M.C., Baldy-Moulinier, M., Daurès, J.P., Dujols, P., Crespel, A., 2008. The prevalence of epilepsy and pharmacoresistant epilepsy in adults: A population-based study in a Western European country. Epilepsia 49, 1230–1238. https://doi.org/10.1111/j.1528-

1167.2008.01579.x

- Pillay, S., Meyer, N.L., Puschnik, A.S., Davulcu, O., Diep, J., Ishikawa, Y., Jae, L.T., Wosen, J.E., Nagamine, C.M., Chapman, M.S., Carette, J.E., 2016. An essential receptor for adeno-associated virus infection. Nature 530, 108–112. https://doi.org/10.1038/nature16465
- Pitkänen, A., 2010. Therapeutic approaches to epileptogenesis Hope on the horizon. Epilepsia 51, 2–17. https://doi.org/10.1111/j.1528-1167.2010.02602.x
- Pitkänen, A., Buckmaster, P., Galanopoulou, A.S., Moshé, S.L., 2017. Models of seizures and epilepsy. Academic Press.
- Pitkänen, A., Engel, J., 2014. Past and Present Definitions of Epileptogenesis and Its Biomarkers. Neurotherapeutics 11, 231–241. https://doi.org/10.1007/s13311-014-0257-2
- Pitkänen, A., Sutula, T.P., 2002. Is epilepsy a progressive disorder? Prospects for new therapeutic approaches in temporal-lobe epilepsy. Lancet Neurol. 1, 173–181. https://doi.org/10.1016/S1474-4422(02)00073-X
- Pohlmann-Eden, B., Beghi, E., Camfield, C., Camfield, P., 2006. The first seizure and its management in adults and children. Br. Med. J. 332, 339–342. https://doi.org/10.1136/bmj.332.7537.339
- Powell, K.L., Fitzgerald, X., Shallue, C., Jovanovska, V., Klugmann, M., Von Jonquieres, G., O'Brien, T.J., Morris, M.J., 2018. Gene therapy mediated seizure suppression in Genetic Generalised Epilepsy: Neuropeptide Y overexpression in a rat model. Neurobiol. Dis. 113, 23–32. https://doi.org/10.1016/j.nbd.2018.01.016
- Qian, J., Colmers, W.F., Saggau, P., 1997. Inhibition of synaptic transmission by neuropeptide Y in rat hippocampal area CA1: modulation of presynaptic Ca2+ entry. J. Neurosci. 17, 8169–8177. https://doi.org/1997/10/23 00:01
- Racine, R.J., 1972. Modification of seizure activity by electrical stimulation: II. Motor seizure. Electroencephalogr. Clin. Neurophysiol. 32, 281–294. https://doi.org/10.1016/0013-4694(72)90177-0
- Raedt, R., Van Dycke, A., Van Melkebeke, D., De Smedt, T., Claeys, P., Wyckhuys, T., Vonck, K., Wadman, W., Boon, P., 2009. Seizures in the intrahippocampal kainic acid epilepsy model: Characterization using long-term video-EEG monitoring in the rat. Acta Neurol. Scand. 119, 293–303. https://doi.org/10.1111/j.1600-0404.2008.01108.x
- Raedt, R., Van Dycke, A., Vonck, K., Boon, P., 2007. Cell therapy in models for temporal lobe epilepsy. Seizure 16, 565–578. https://doi.org/10.1016/j.seizure.2007.05.003

- Rattka, M., Brandt, C., Löscher, W., 2013. The intrahippocampal kainate model of temporal lobe epilepsy revisited: Epileptogenesis, behavioral and cognitive alterations, pharmacological response, and hippoccampal damage in epileptic rats. Epilepsy Res. 103, 135–152. https://doi.org/10.1016/j.eplepsyres.2012.09.015
- Ravizza, T., Balosso, S., Vezzani, A., 2011. Inflammation and prevention of epileptogenesis. Neurosci. Lett. 497, 223–230. https://doi.org/10.1016/j.neulet.2011.02.040
- Redrobe, J.P., Dumont, Y., St-Pierre, J.A., Quirion, R., 1999. Multiple receptors for neuropeptide Y in the hippocampus: Putative roles in seizures and cognition. Brain Res. 848, 153–166. https://doi.org/10.1016/S0006-8993(99)02119-8
- Reeben, M., Laurikainen, A., Hiltunen, J.O., Castrén, E., Saarma, M., 1998. The messenger RNAs for both glial cell line-derived neurotrophic factor receptors, c-ret and GDNFRα, are induced in the rat brain in response to kainate-induced excitation. Neuroscience 83, 151–159. https://doi.org/10.1016/S0306-4522(97)00361-8
- Reichmann, F., Holzer, P., 2016. Neuropeptide Y: A stressful review. Neuropeptides 55, 99–109. https://doi.org/10.1016/j.npep.2015.09.008
- Reschke, C.R., Silva, L.F.A., Norwood, B.A., Senthilkumar, K., Morris, G., Sanz-Rodriguez, A., Conroy, R.M., Costard, L., Neubert, V., Bauer, S., Farrell, M.A., O'Brien, D.F., Delanty, N., Schorge, S., Pasterkamp, R.J., Rosenow, F., Henshall, D.C., 2017. Potent Anti-seizure Effects of Locked Nucleic Acid Antagomirs Targeting miR-134 in Multiple Mouse and Rat Models of Epilepsy. Mol. Ther. - Nucleic Acids 6, 45–56. https://doi.org/10.1016/j.omtn.2016.11.002
- Riban, V., Bouilleret, V., Pham-Lê, B.T., Fritschy, J.M., Marescaux, C., Depaulis, A., 2002. Evolution of hippocampal epileptic activity during the development of hippocampal sclerosis in a mouse model of temporal lobe epilepsy. Neuroscience 112, 101–111. https://doi.org/10.1016/S0306-4522(02)00064-7
- Riban, V., Fitzsimons, H.L., During, M.J., 2009. Gene therapy in epilepsy. Epilepsia 50, 24– 32. https://doi.org/10.1111/j.1528-1167.2008.01743.x
- Richichi, C., Lin, E.-J.D., Stefanin, D., Colella, D., Ravizza, T., Grignaschi, G., Veglianese, P., Sperk, G., During, M.J., Vezzani, A., 2004. Anticonvulsant and Antiepileptogenic Effects Mediated by Adeno-Associated Virus Vector Neuropeptide Y Expression in the Rat Hippocampus. J. Neurosci. 24, 3051 LP – 3059. https://doi.org/10.1523/JNEUROSCI.4056-03.2004
- Rocha, S.M., Cristovão, A.C., Campos, F.L., Fonseca, C.P., Baltazar, G., 2012. Astrocytederived GDNF is a potent inhibitor of microglial activation. Neurobiol. Dis. 47, 407– 415. https://doi.org/10.1016/j.nbd.2012.04.014
- Röder, C., Schwarzer, C., Vezzani, A., Gobbi, M., Mennini, T., Sperk, G., 1996. Autoradiographic analysis of neuropeptide Y receptor binding sites in the rat hippocampus after kainic acid-induced limbic seizures. Neuroscience 70, 47–55. https://doi.org/10.1016/0306-4522(95)00332-D

- Sajdyk, T.J., Shekhar, A., Gehlert, D.R., 2004. Interactions between NPY and CRF in the amygdala to regulate emotionality. Neuropeptides 38, 225–234. https://doi.org/10.1016/j.npep.2004.05.006
- Sato, M., 1982. Kindling: An Experimental Model of Epilepsy. Psychiatry Clin. Neurosci. 36, 440–441. https://doi.org/10.1111/j.1440-1819.1982.tb03123.x
- Schachter, S.C., 2007. Currently Available Antiepileptic Drugs. Neurotherapeutics 4, 4–11. https://doi.org/10.1016/j.nurt.2006.11.005
- Scheffer, I.E., Berkovic, S., Capovilla, G., Connolly, M.B., French, J., Guilhoto, L., Hirsch, E., Jain, S., Mathern, G.W., Moshé, S.L., Nordli, D.R., Perucca, E., Tomson, T., Wiebe, S., Zhang, Y.-H., Zuberi, S.M., 2017. ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. Epilepsia 58, 512–521. https://doi.org/doi:10.1111/epi.13709
- Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y.J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., Liceiri, K., Tomancak, P., A., C., 2012.
 Fiji: An open source platform for biological image analysis. Nat. Methods 9, 676–682. https://doi.org/10.1038/nmeth.2019.Fiji
- Schmidt-Kastner, R., Tomac, A., Hoffer, B., Bektesh, S., Rosenzweig, B., Olson, L., 1994. Glial cell-line derived neurotrophic factor (GDNF) mRNA upregulation in striatum and cortical areas after pilocarpine-induced status epilepticus in rats. Mol. Brain Res. 26, 325–330. https://doi.org/10.1016/0169-328X(94)90106-6
- Schwarzer, C., Kofler, N., Sperk, G., 1998. Up-regulation of neuropeptide Y-Y2receptors in an animal model of temporal lobe epilepsy. Mol. Pharmacol. 53, 6–13. https://doi.org/10.1124/mol.53.1.6
- Schwarzer, C., Sperk, G., Samanin, R., Rizzi, M., Gariboldi, M., Vezzani, A., 1996. Neuropeptides-immunoreactivity and their mRNA expression in kindling: functional implications for limbic epileptogenesis. Brain Res. Brain Res. Rev. 22, 27–50.
- Sharma, A.K., Jordan, W.H., Reams, R.Y., Greg Hall, D., Snyder, P.W., 2008. Temporal profile of clinical signs and histopathologic changes in an F-344 rat model of kainic acid-induced mesial temporal lobe epilepsy. Toxicol. Pathol. 36, 932–943. https://doi.org/10.1177/0192623308326093
- Sharma, A.K., Reams, R.Y., Jordan, W.H., Miller, M.A., Thacker, H.L., Snyder, P.W., 2007. Mesial temporal lobe epilepsy: Pathogenesis, induced rodent models and lesions. Toxicol. Pathol. 35, 984–999. https://doi.org/10.1080/01926230701748305
- Shuman, T., Aharoni, D., Cai, D.J., Lee, C.R., Chavlis, S., Page-Harley, L., Vetere, L.M., Feng, Y., Yang, C.Y., Mollinedo-Gajate, I., Chen, L., Pennington, Z.T., Taxidis, J., Flores, S.E., Cheng, K., Javaherian, M., Kaba, C.C., Rao, N., La-Vu, M., Pandi, I., Shtrahman, M., Bakhurin, K.I., Masmanidis, S.C., Khakh, B.S., Poirazi, P., Silva, A.J., Golshani, P., 2020. Breakdown of spatial coding and interneuron synchronization in epileptic mice. Nat. Neurosci. https://doi.org/10.1038/s41593-019-0559-0
- Sillanpää, M., Schmidt, D., 2008. Seizure clustering during drug treatment affects seizure outcome and mortality of childhood-onset epilepsy. Brain 131, 938–944. https://doi.org/10.1093/brain/awn037

- Silva, A.P., Xapelli, S., Grouzmann, E., Cavadas, C., 2005. The putative neuroprotective role of neuropeptide Y in the central nervous system. Curr. Drug Targets CNS Neurol. Disord. 4, 331–347. https://doi.org/10.2174/1568007054546153
- Sim, L.J., Selley, D.E., Childers, S.R., 1995. In vitro autoradiography of receptor-activated G proteins in rat brain by agonist-stimulated guanylyl 5'-[gamma-[35S]thio]-triphosphate binding. Proc. Natl. Acad. Sci. U. S. A. 92, 7242–6. https://doi.org/10.1073/pnas.92.16.7242
- Simonato, M., 2014. Gene therapy for epilepsy. Epilepsy Behav. 38, 125–130. https://doi.org/10.1016/j.yebeh.2013.09.013
- Simonato, M., Bennett, J., Boulis, N.M., Castro, M.G., Fink, D.J., Goins, W.F., Gray, S.J., Lowenstein, P.R., Vandenberghe, L.H., Wilson, T.J., Wolfe, J.H., Glorioso, J.C., 2013. Progress in gene therapy for neurological disorders. Nat. Rev. Neurol. 9, 277–291. https://doi.org/10.1038/nrneurol.2013.56
- Simonato, M., Brooks-Kayal, A.R., Engel, J., Galanopoulou, A.S., Jensen, F.E., Moshé, S.L., O'Brien, T.J., Pitkanen, A., Wilcox, K.S., French, J.A., 2014. The challenge and promise of anti-epileptic therapy development in animal models. Lancet Neurol. 13, 949–960. https://doi.org/10.1016/S1474-4422(14)70076-6
- Simonato, M., Löscher, W., Cole, A.J., Dudek, F.E., Engel, J., Kaminski, R.M., Loeb, J.A., Scharfman, H., Staley, K.J., Velíšek, L., Klitgaard, H., 2012. Finding a better drug for epilepsy: Preclinical screening strategies and experimental trial design. Epilepsia 53, 1860–1867. https://doi.org/10.1111/j.1528-1167.2012.03541.x
- Sloviter, R.S., Zappone, C.A., Bumanglag, A. V., Norwood, B.A., Kudrimoti, H., 2007. On the relevance of prolonged convulsive status epilepticus in animals to the etiology and neurobiology of human temporal lobe epilepsy. Epilepsia 48, 6–10. https://doi.org/10.1111/j.1528-1167.2007.01335.x
- Smith, D.H., Hicks, R.R., Johnson, V.E., Bergstrom, D.A., Cummings, D.M., Noble, L.J., Hovda, D., Whalen, M., Ahlers, S.T., Laplaca, M., Tortella, F.C., Duhaime, A.C., Dixon, C.E., 2015. Pre-Clinical Traumatic Brain Injury Common Data Elements: Toward a Common Language Across Laboratories. J. Neurotrauma 32, 1725–1735. https://doi.org/10.1089/neu.2014.3861
- Snowball, A., Chabrol, E., Wykes, R.C., Shekh-Ahmad, T., Cornford, J.H., Lieb, A., Hughes, M.P., Massaro, G., Rahim, A.A., Hashemi, K.S., Kullmann, D.M., Walker, M.C., Schorge, S., 2019. Epilepsy gene therapy using an engineered potassium channel. J. Neurosci. https://doi.org/10.1523/JNEUROSCI.1143-18.2019
- Sobayo, T., Mogul, D.J., 2013. Rapid onset of a kainate-induced mirror focus in rat hippocampus is mediated by contralateral AMPA receptors. Epilepsy Res. 106, 35–46. https://doi.org/10.1016/j.eplepsyres.2013.03.010
- Sørensen, A.T., Kanter-Schlifke, I., Carli, M., Balducci, C., Noe, F., During, M.J., Vezzani, A., Kokaia, M., 2008a. NPY gene transfer in the hippocampus attenuates synaptic plasticity and learning. Hippocampus 18, 564–574. https://doi.org/10.1002/hipo.20415
- Sørensen, A.T., Kanter-Schlifke, I., Lin, E.J.D., During, M.J., Kokaia, M., 2008b. Activitydependent volume transmission by transgene NPY attenuates glutamate release and LTP

in the subiculum. Mol. Cell. Neurosci. 39, 229–237. https://doi.org/10.1016/j.mcn.2008.06.014

- Sørensen, A.T., Nikitidou, L., Ledri, M., Lin, E.-J.J.D., During, M.J., Kanter-Schlifke, I., Kokaia, M., 2009. Hippocampal NPY gene transfer attenuates seizures without affecting epilepsy-induced impairment of LTP. Exp. Neurol. 215, 328—333. https://doi.org/10.1016/j.expneurol.2008.10.015
- Soud, K., Jørgensen, S.H., Woldbye, D.P.D., Sørensen, A.T., 2018. The C-terminal flanking peptide of neuropeptide Y (NPY) is not essential for seizure-suppressant actions of prepro-NPY overexpression in male rats. J. Neurosci. Res. 362–372. https://doi.org/10.1002/jnr.24350
- Stanić, D., Mulder, J., Watanabe, M., Hökfelt, T., 2011. Characterization of NPY Y2 receptor protein expression in the mouse brain. II. Coexistence with NPY, the Y1 receptor, and other neurotransmitter-related molecules. J. Comp. Neurol. 519, 1219– 1257. https://doi.org/10.1002/cne.22608
- Steward, O., Popovich, P.G., Dietrich, W.D., Kleitman, N., 2012. Replication and reproducibility in spinal cord injury research. Exp. Neurol. 233, 597–605. https://doi.org/10.1016/j.expneurol.2011.06.017
- Stone, K., 2010. Comparative effectiveness research in neurology: healthcare reform will increase the focus on finding the most effective--and affordable--treatment. Ann. Neurol. 68, A10.
- Sun, J.Y., Anand-Jawa, V., Chatterjee, S., Wong, K.K., 2003. Immune responses to adenoassociated virus and its recombinant vectors. Gene Ther. 10, 964–976. https://doi.org/10.1038/sj.gt.3302039
- Tang, F., Hartz, A.M.S., Bauer, B., 2017. Drug-resistant epilepsy: Multiple hypotheses, few answers. Front. Neurol. 8, 1–19. https://doi.org/10.3389/fneur.2017.00301
- Tatemoto, K., Carlquist, M., Mutt, V., 1982. Neuropeptide Y—a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. Nature 296, 659–660.
- Thompson, H.J., Lifshitz, J., Marklund, N., Grady, M.S., Graham, D.I., Hovda, D.A., Mcintosh, T.K., 2005. Lateral fluid percussion brain injury: A 15-year review and evaluation. J. Neurotrauma 22, 42–75. https://doi.org/10.1089/neu.2005.22.42
- Thorsell, A., Mathé, A.A., 2017. Neuropeptide Y in alcohol addiction and affective disorders. Front. Endocrinol. (Lausanne). 8. https://doi.org/10.3389/fendo.2017.00178
- van den Pol, A.N., 2012. Neuropeptide Transmission in Brain Circuits. Neuron 76, 98–115. https://doi.org/10.1016/j.neuron.2012.09.014
- Vezzani, A., Michalkiewicz, M., Michalkiewicz, T., Moneta, D., Ravizza, T., Richichi, C., Aliprandi, M., Mulé, F., Pirona, L., Gobbi, M., Schwarzer, C., Sperk, G., 2002. Seizure susceptibility and epileptogenesis are decreased in transgenic rats overexpressing neuropeptide Y. Neuroscience 110, 237–243. https://doi.org/10.1016/S0306-4522(01)00581-4
- Vezzani, A., Sperk, G., 2004. Overexpression of NPY and Y2 receptors in epileptic brain tissue: An endogenous neuroprotective mechanism in temporal lobe epilepsy? Neuropeptides 38, 245–252. https://doi.org/10.1016/j.npep.2004.05.004

- Vezzani, A., Sperk, G., Colmers, W.F., 1999. Neuropeptide Y : emerging evidence for a functional role in seizure modulation. Trends Neurosci. 22, 25–30. https://doi.org/S0166-2236(98)01284-3 [pii]
- Vincent, P., Mulle, C., 2009. Kainate receptors in epilepsy and excitotoxicity. Neuroscience 158, 309–323. https://doi.org/10.1016/j.neuroscience.2008.02.066
- Walker MC, White HS, S.J., 2002. Disease modification in partial epilepsy. Brain 125, 1937–1950.
- Weinberg, M.S., Samulski, R.J., McCown, T.J., 2013. Adeno-associated virus (AAV) gene therapy for neurological disease. Neuropharmacology 69, 82–88. https://doi.org/10.1016/j.neuropharm.2012.03.004
- West, S., Nolan, S.J., Cotton, J., Gandhi, S., Weston, J., Sudan, A., Ramirez, R., Newton, R., 2015. Surgery for epilepsy. Cochrane Database Syst. Rev.
- Whitlock, J.R., Heynen, A.J., Shuler, M.G., Bear, M.F., 2006. Learning induces long-term potentiation in the hippocampus. Science (80-.). 313, 1093–1097. https://doi.org/10.1126/science.1128134
- WHO, 2006. Neurological disorders: public health challenges. Geneva: World Health Organization.
- Wickham, J., Ledri, M., Bengzon, J., Jespersen, B., Pinborg, L.H., Englund, E., Woldbye, D.P.D., Andersson, M., Kokaia, M., 2019. Inhibition of epileptiform activity by neuropeptide Y in brain tissue from drug-resistant temporal lobe epilepsy patients. Sci. Rep. 9, 1–11. https://doi.org/10.1038/s41598-019-56062-1
- Wieser, H.-G., ILAE Commission on Neurosurgery of Epilepsy, 2004. ILAE Commission Report. Mesial temporal lobe epilepsy with hippocampal sclerosis, in: Epilepsia. p. 695—714. https://doi.org/10.1111/j.0013-9580.2004.09004.x
- Wilcox, K.S., West, P.J., Metcalf, C.S., 2019. The Current Approach of the Epilepsy Therapy Screening Program Contract Site for Identifying Improved Therapies for the Treatment of Pharmacoresistant Seizures in Epilepsy. Neuropharmacology 107811. https://doi.org/https://doi.org/10.1016/j.neuropharm.2019.107811
- Wilkinson, M.D., Dumontier, M., Aalbersberg, Ij.J., Appleton, G., Axton, M., Baak, A.,
 Blomberg, N., Boiten, J.W., da Silva Santos, L.B., Bourne, P.E., Bouwman, J., Brookes,
 A.J., Clark, T., Crosas, M., Dillo, I., Dumon, O., Edmunds, S., Evelo, C.T., Finkers,
 R., Gonzalez-Beltran, A., Gray, A.J.G., Groth, P., Goble, C., Grethe, J.S., Heringa, J., t
 Hoen, P.A.C., Hooft, R., Kuhn, T., Kok, R., Kok, J., Lusher, S.J., Martone, M.E.,
 Mons, A., Packer, A.L., Persson, B., Rocca-Serra, P., Roos, M., van Schaik, R., Sansone,
 S.A., Schultes, E., Sengstag, T., Slater, T., Strawn, G., Swertz, M.A., Thompson, M.,
 Van Der Lei, J., Van Mulligen, E., Velterop, J., Waagmeester, A., Wittenburg, P.,
 Wolstencroft, K., Zhao, J., Mons, B., 2016. Comment: The FAIR Guiding Principles
 for scientific data management and stewardship. Sci. Data 3, 1–9.
 https://doi.org/10.1038/sdata.2016.18
- Woldbye, D.P.D., Ängehagen, M., Gøtzsche, C.R., Elbrønd-Bek, H., Sørensen, A.T., Christiansen, S.H., Olesen, M. V., Nikitidou, L., Hansen, T.V.O., Kanter-Schlifke, I., Kokaia, M., 2010. Adeno-associated viral vector-induced overexpression of neuropeptide y Y2 receptors in the hippocampus suppresses seizures. Brain 133, 2778–

2788. https://doi.org/10.1093/brain/awq219

- Woldbye, D.P.D., Larsen, P.J., Mikkelsen, J.D., Klemp, K., Madsen, T.M., Bolwig, T.G., 1997. Powerful inhibition of kainic acid seizures by neuropeptide Y via Y5- like receptors. Nat. Med. 3, 761–764. https://doi.org/10.1038/nm0797-761
- Yoo, Y.M., Lee, C.J., Lee, U., Kim, Y.J., 2006. Neuroprotection of adenoviral-vectormediated GDNF expression against kainic-acid-induced excitotoxicity in the rat hippocampus. Exp. Neurol. 200, 407–417. https://doi.org/10.1016/j.expneurol.2006.02.132
- Zachrisson, O., Mathé, A.A., Stenfors, C., Lindefors, N., 1995. Limbic effects of repeated electroconvulsive stimulation on neuropeptide Y and somatostatin mRNA expression in the rat brain. Mol. Brain Res. 31, 71–85. https://doi.org/10.1016/0169-328X(95)00033-O
- Zincarelli, C., Soltys, S., Rengo, G., Rabinowitz, J.E., 2008. Analysis of AAV serotypes 1-9 mediated gene expression and tropism in mice after systemic injection. Mol. Ther. 16, 1073–1080. https://doi.org/10.1038/mt.2008.76

Advancing gene therapy for epilepsy



Esbjörn Melin graduated from Lunds Tekniska Högskola in 2013 with a master of science in Engineering Nanoscience. He started his doctoral studies at Lund University in 2014, in the Experimental Epilepsy Group, under the supervision of Professor Mérab Kokaia. During his PhD he has investigated the seizure suppressing effects of gene therapy strategies against temporal lobe epilepsy in pre-clinical studies.



FACULTY OF MEDICINE

Epilepsy Centre Department of clinical sciences, Lund

Lund University, Faculty of Medicine Doctoral Dissertation Series 2020:24 ISBN 978-91-7619-884-1 ISSN 1652-8220

