Epiglia

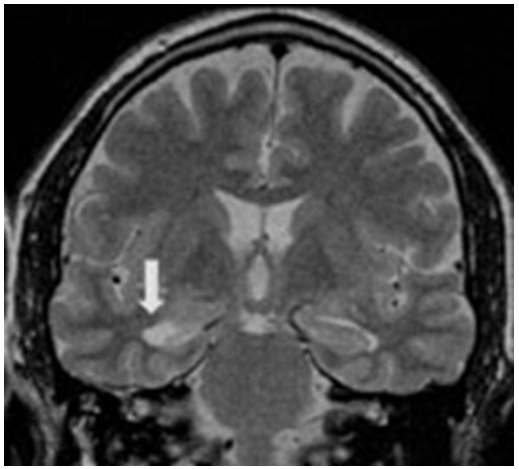
Genetic targets of epileptogenesis and pharmaco-resistance in brain glial cells;
a translational research project on the genetic and molecular pathways of Temporal Lobe Epilepsy and Febrile Seizures

Epiglia – 4 collaborating centers

- Dep of Neurology, Oslo Univ Hospital – Rikshospitalet, Oslo, Norway
  (Erik Taubøll, Kjell Heuser, Erlend Nagelhus)
- Institute of cellular Neurosciences, University of Bonn, Germany
  (Group leader: prof Christian Steinhäuser)
- Kuopio Epilepsy Center, Univ of Eastern Finland, Finland
  (Group leader: prof Reetta Kälviäinen)
- University Medical Center, Utrecht
  (Group leader: Prof. Pierre de Graan)

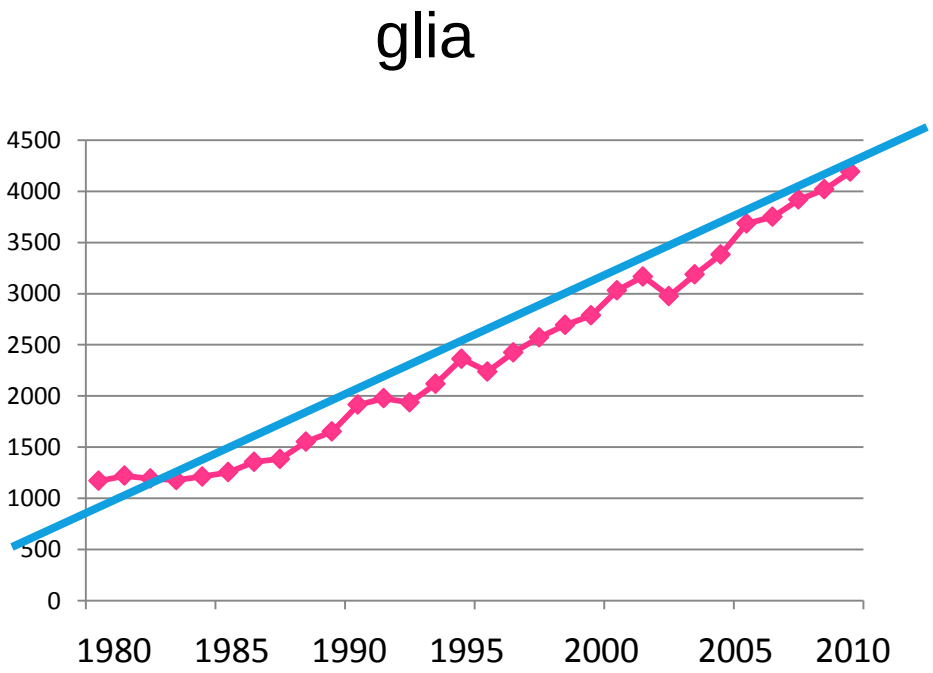
Clinical background

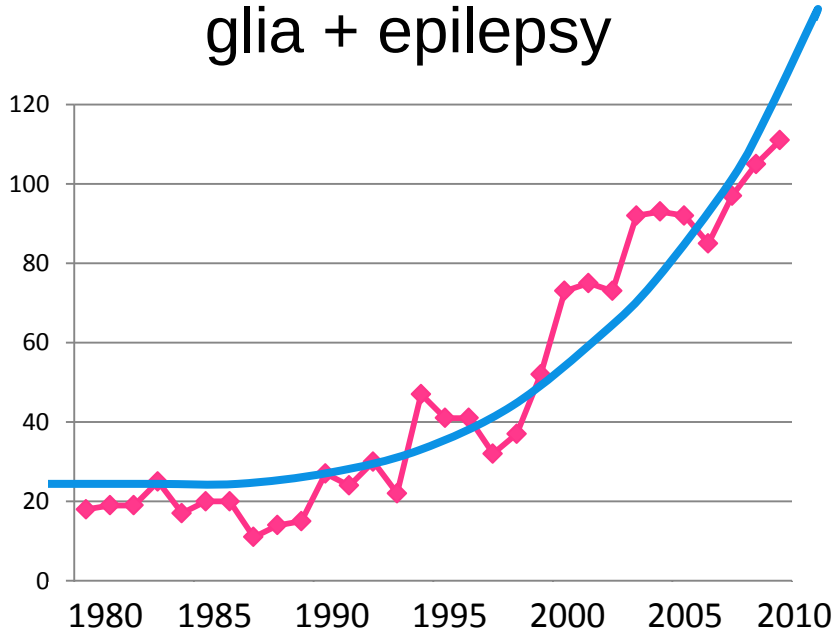
- TLE represents a major health problem!
- Accounts for 1/3 of all epilepsy patients
- Chronic condition
- Several subgroups of TLE with MTLE-HS as the most severe
- Often pharmacoresistant
- **Need for new treatment strategies!**



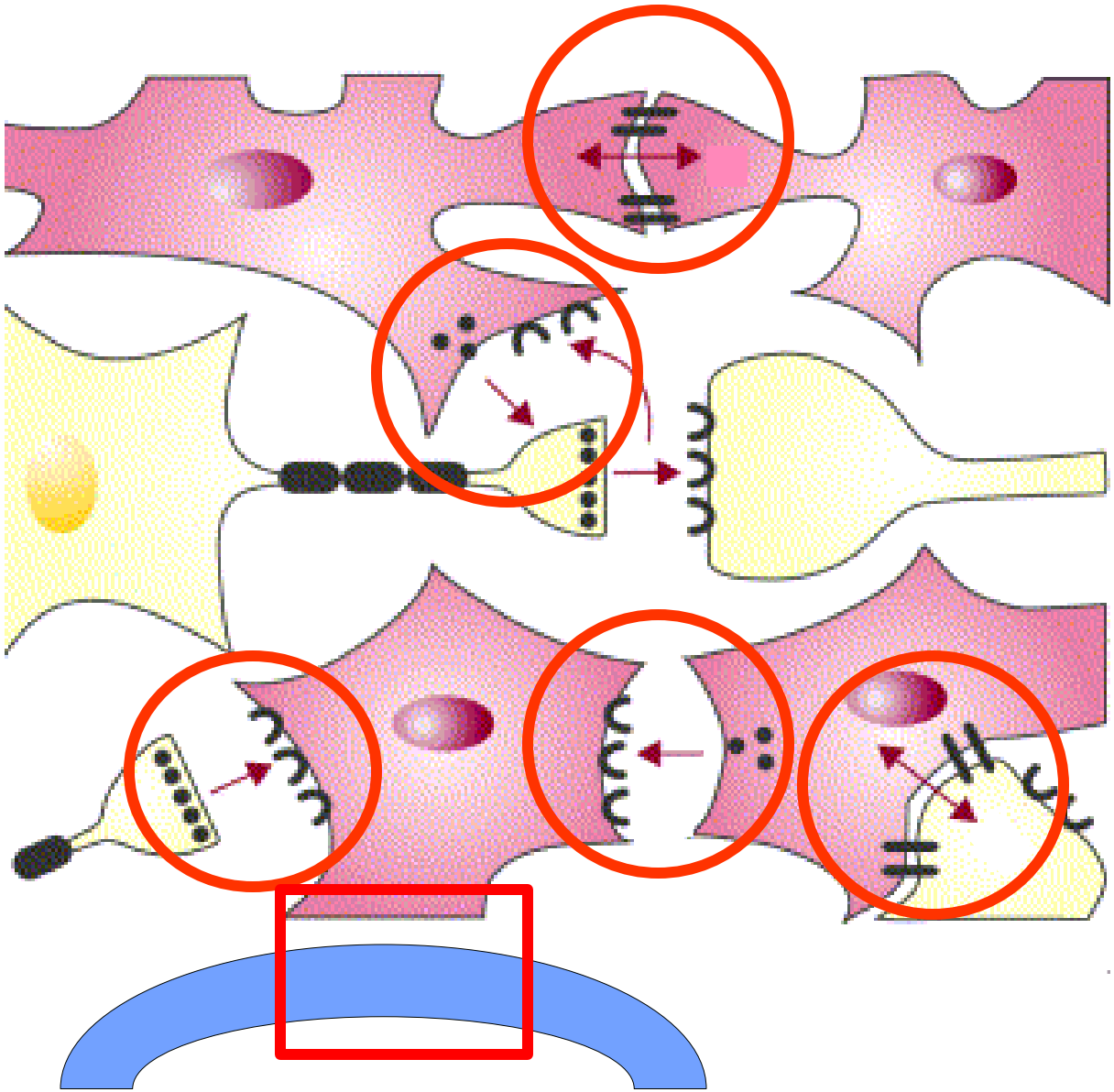
Increasing interest in glia and epilepsy

Medline publications





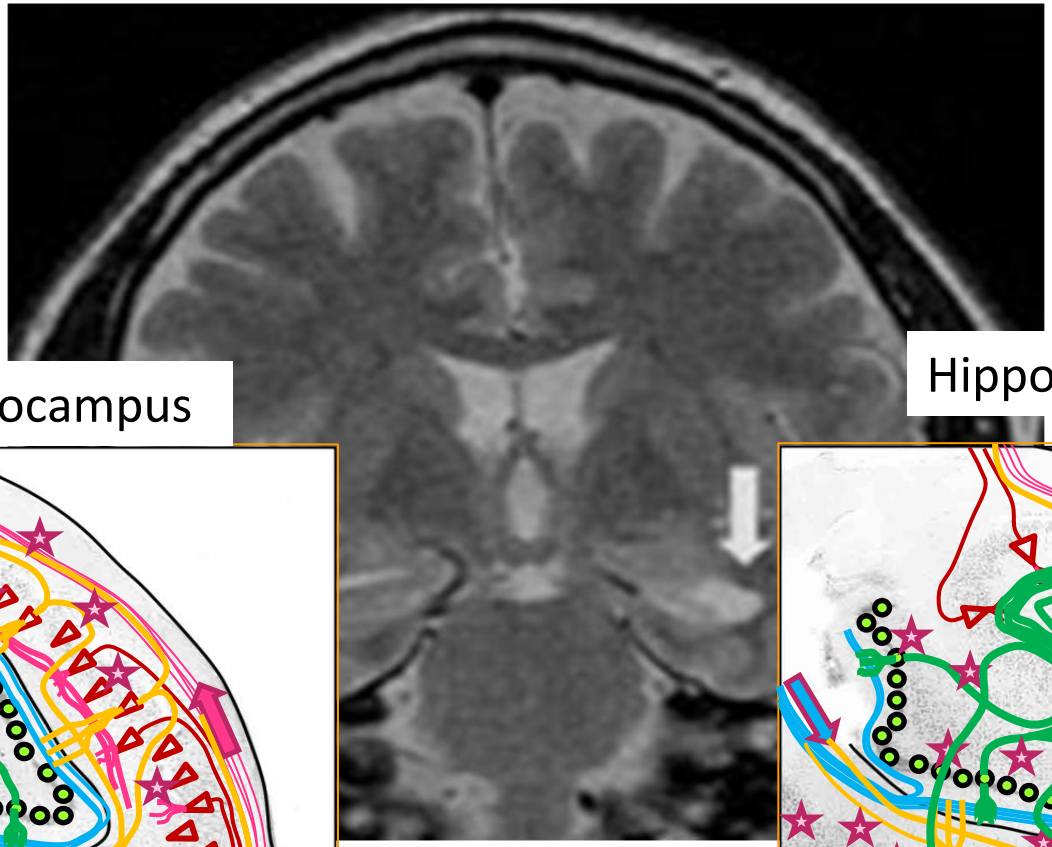
Neuron-glia crosstalk



- Modification of synaptic transmission
- Electrotonic coupling through gap junctions
- Presynaptic neurons-postsynaptic glial cells
- Glia-glia transmission
- Gap-junction coupling – neuron-glia(?)
- Buffering of ions and water, glia-blood vessels

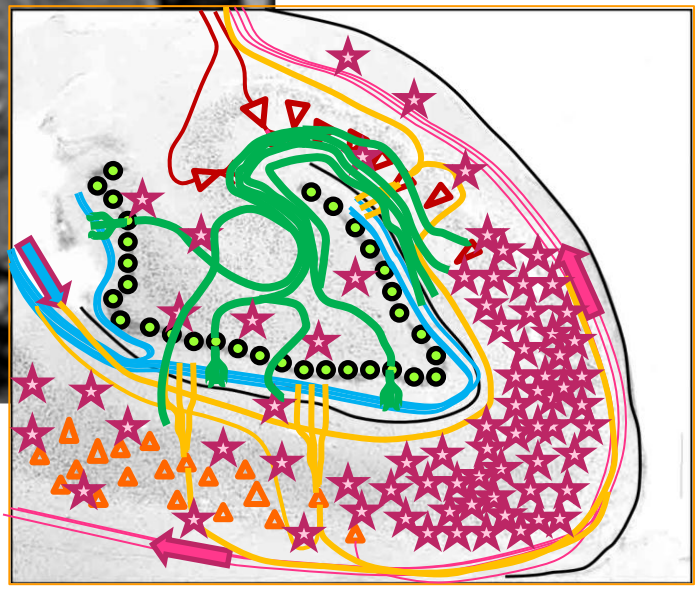
Bezzi and Volterra, 2001

Hippocampal sclerosis
– highly epileptogenic glial tissue scar



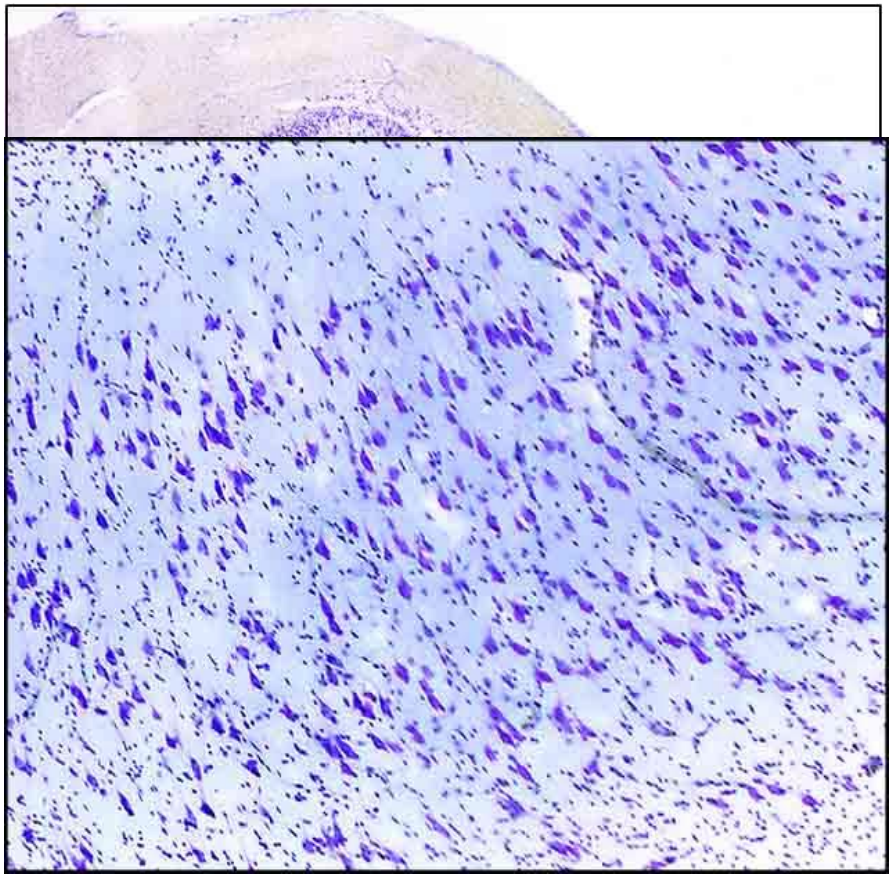
Normal hippocampus

Hippocampal sclerosis

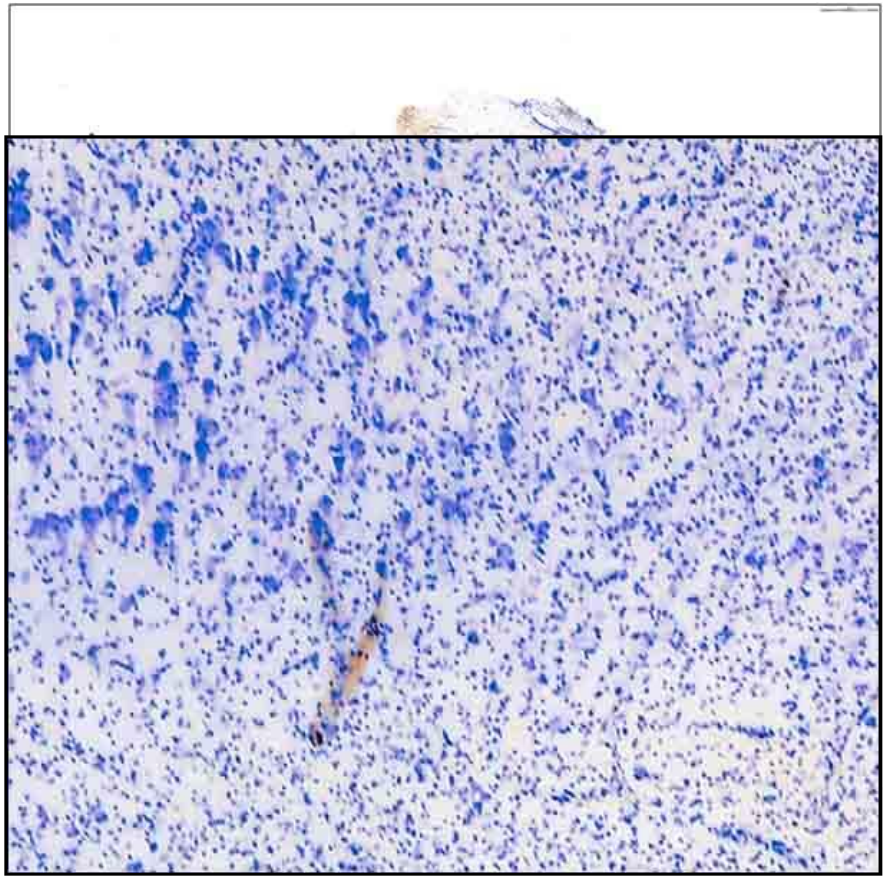


Glial changes in hippocampal sclerosis

NORMAL



MTS



Epiglia – hypothesis

Our hypothesis based on:

1. The important role for glia/astrocytes in regulation of brain excitability

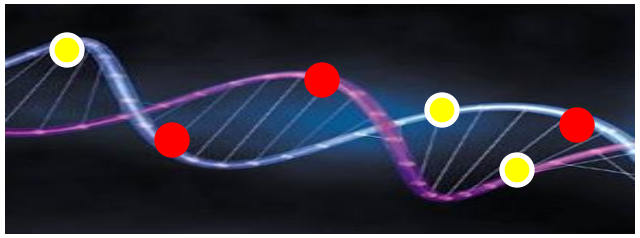
2. The important role of genetics in different TLE subgroups and in FS

Main hypothesis:

Astrocytes play an important role in epileptogenesis and in generation, spread and maintenance of seizures in TLE and its different subgroups

Epiglia – main areas of research

- Genetic association studies based on human data and material from all centers, focusing on targets in brain glia (e.g. AQP4, Kir4.1, glutamine synthetase, connexin 30/43)
- MTLE-HS and FS mouse models
- Functional studies on human epileptic tissue



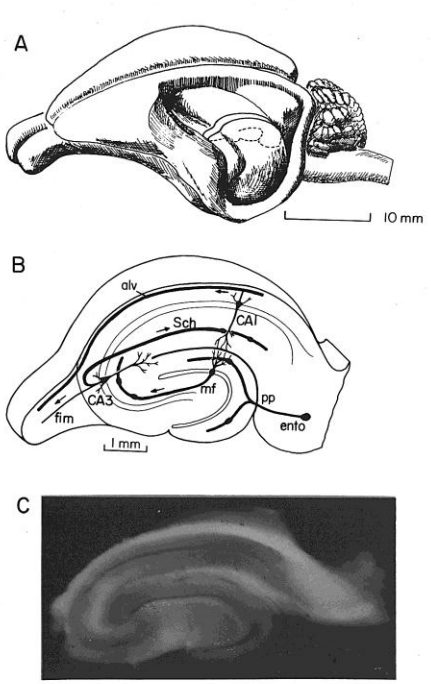
IP-1, Oslo

The group consist of:

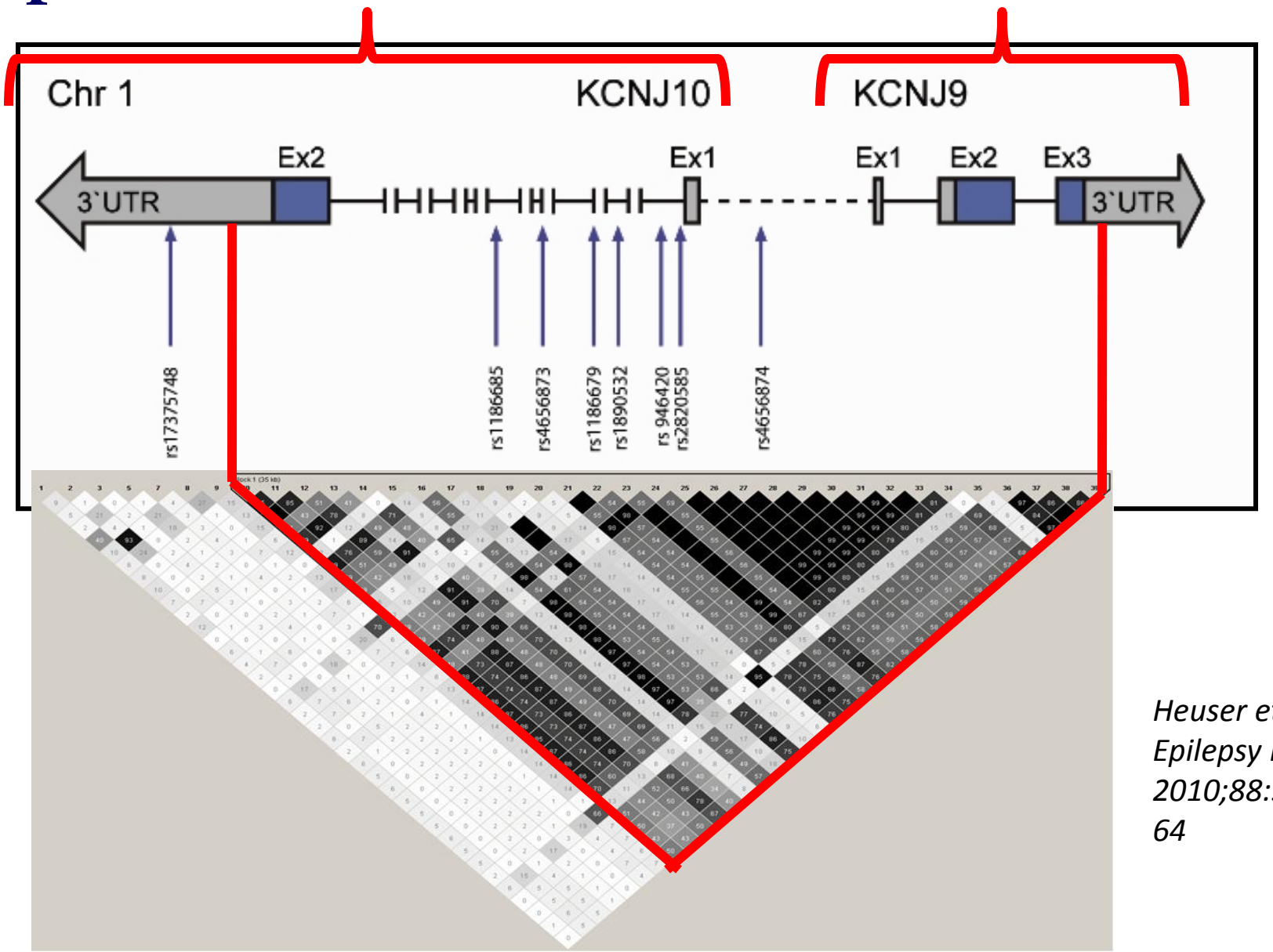
- Epilepsy Research Group, Dep of Neurology, Oslo University Hospital – Rikshospitalet
- Centre for Molecular Medicine Norway and Nordic EMBL Partnership, Univ of Oslo
- Institute of Neurophysiology, Univ of Oslo
- Dep of Neurosurgery, Oslo University Hospital – Rikshospitalet

Methods available

- Mouse models of MTLE-HS. Will set up and use the intracortical kainic acid model developed by the Bonn group
- Slice preparations, neurophysiology
- Multiphoton laser scanning microscopy
- Surgical tissue from patients with MTLE-HS



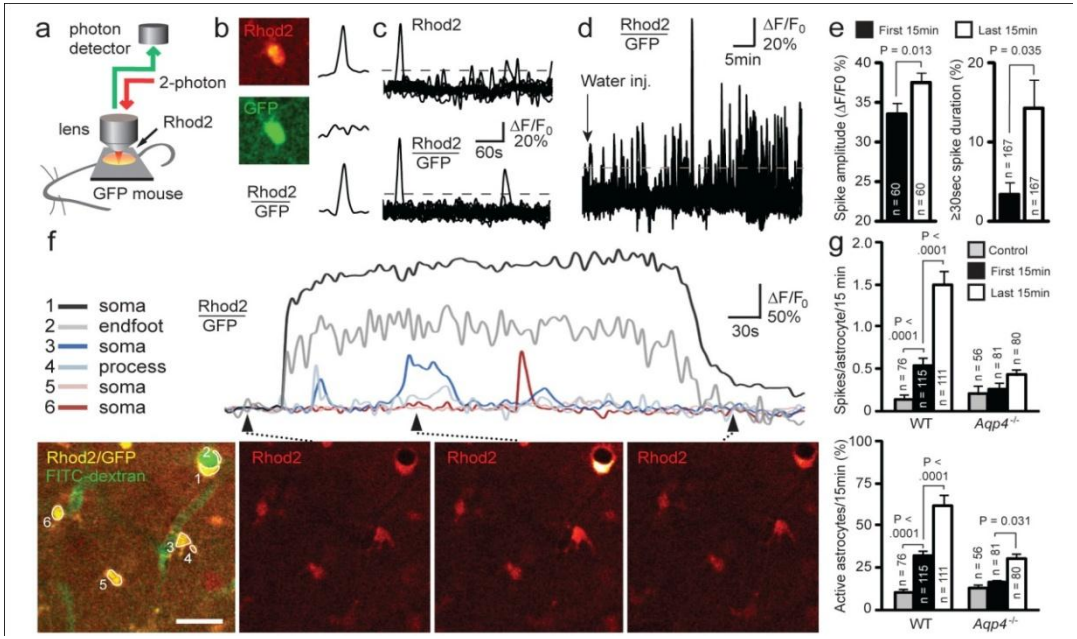
Kir4.1 channel polymorphisms in TLE patients with childhood febrile seizures



Heuser et al. Epilepsy Res 2010;88:55-64

AQP4 enhances Ca^{2+} signaling in astrocytes in experimental brain edema

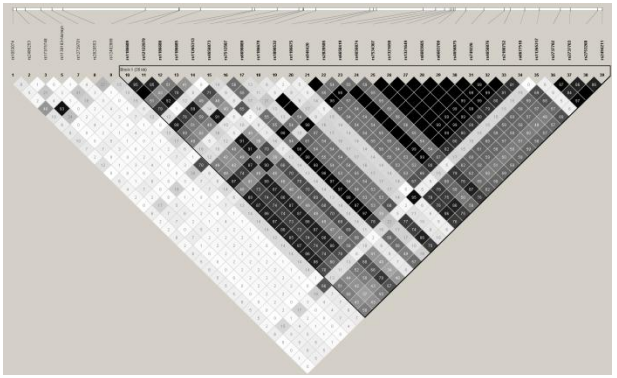
(Thrane, ... & Nagelhus, Proc. Natl. Acad. Sci. U S A. 2011 Jan 11;108(2):846-51



Q: Will increased AQP4 expression in MTLE-HS contribute to Ca hyperactivity?

Work packages

- Study factors that promote epileptogenesis in a mouse model of MTLE-HS (i.c. kainic injection). Can these factors be modulated by novel AEDs or antiinflammatory drugs? Wild type and AQP4 (or other) KO mice will be used.
- Study Ca activity in astrocytes in sclerotic versus non-sclerotic hippocampi in patients with TLE
- Collaborate on association studies on glial targets in well defined phenotypes



IP-2, Bonn
Group leader prof: Christian Steinhäuser

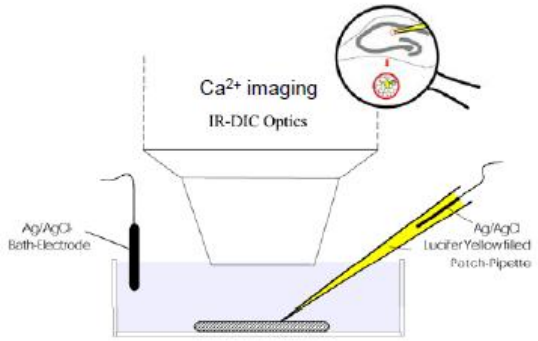
Deciphering the impact of astroglial Kir4.1 dysfunction in MTLE

Institute of Cellular Neurosciences

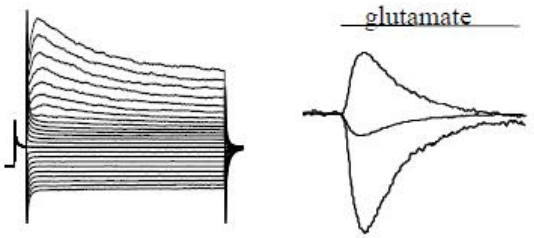
universitätbonn

Methods

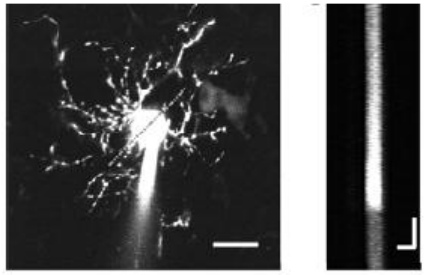
Patch-clamp in acute brain slices, isolated cells

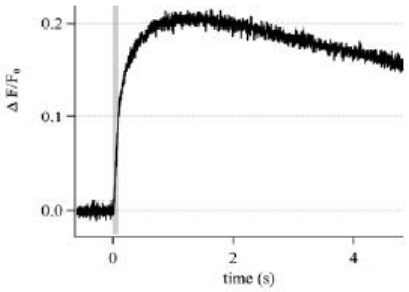


Elektrophysiology

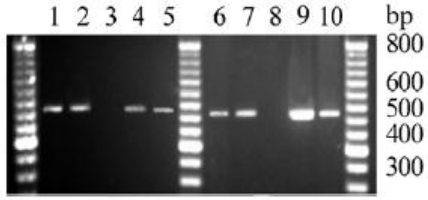


2P LSM



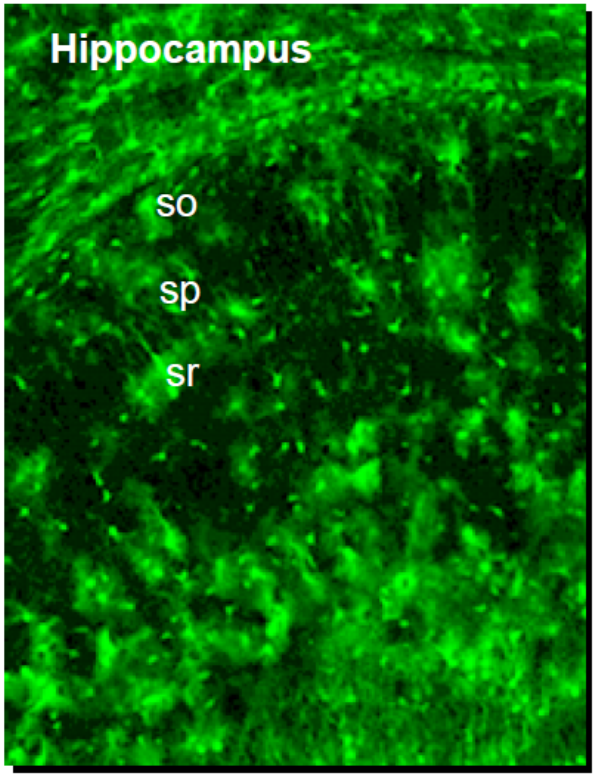


Single-cell sqRT-PCR



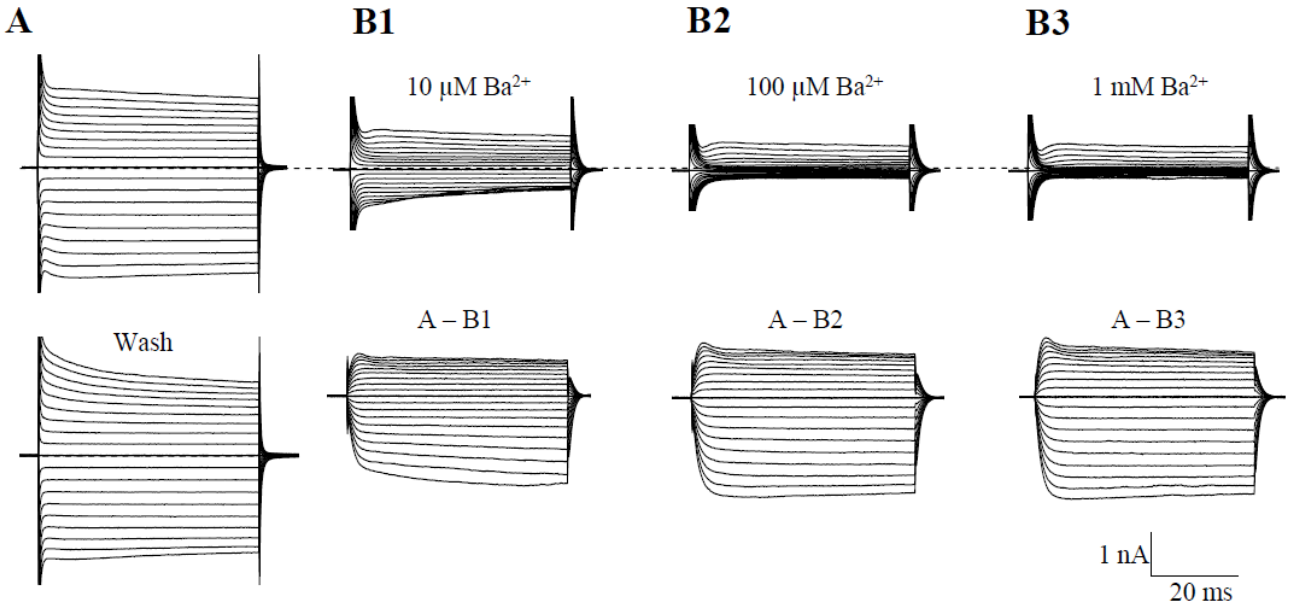
Conditional mutagenesis, transgenics (M. Theis)

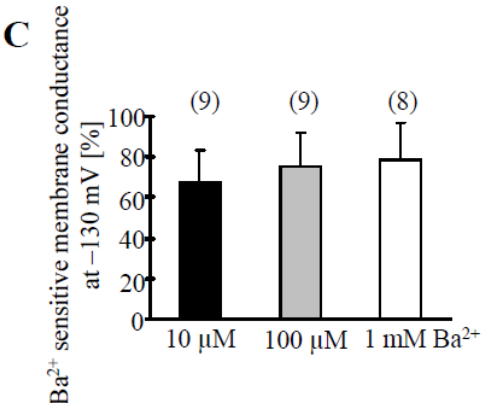
Transgenic mice, viral vectors

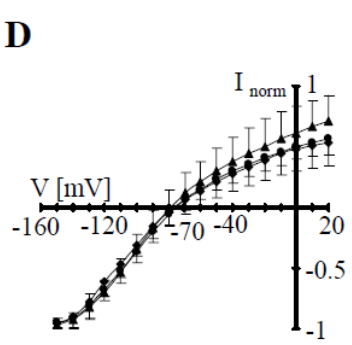


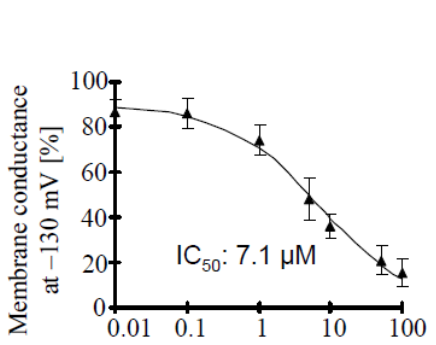
Type of transgene	Transgenic lines
Fluorescent indicator mice	NG2kiEYFP, PLP-EGFP; NG2DsRed, Thy-1-YFP, Thy1-Syt1/ECFP, hGFAP-EGFP, Cx43kiECFP; CX3CR1-EGFP, ALDH1L1-EGFP
knockout	DAOO loss of function; Pu1 KOs devoid of microglia; Kir4.1 KO; Cx30 KO; P2X7 KO; P2X4 KO, iNOS KO, CD11b KO, GNE KO; ASA KO; NAAGS-1 KO, NAAGS-2 KO, Cx47 KO; Ear2 KO, DBH KO; B7-H1 KO; PD-1 KO, TLR4 KO
Cell-type specific knockout	NG2-CreERT2, floxed Kir4.1 ; SRR KO (neuronal and glial); Astrocytic deletion of Asc-1; Cx43kiCreERT, Cx30-CreERT2, floxed Ezrin; GFAP-cre, floxed Cx43; Cx43kiCreERT, Cx30-CreERT2, floxed Cx30; floxed Cx30A88V, floxed Cx43G138R, Cx43K258Stop, floxed Cx43K378Stop; IDR related genes
Cell-type specific overexpression	glial DAOO overexpression, G72 BAC transgenics, glial serine racemase overexpression; APP, APP/PS1 transgenics, astrocytic DN-IkappaBalpha; Abeta-BRI mouse, IDE related genes
Cell-type specific transgene activation	NG2-CreERT2, floxed channelrhodopsin-2; NG2kiCreERT2, Rosy, Z/EG;
Tet System	NG2-tTA, tetO-Lck-GCaMP2; GFAP-tTA, tetO-DN-Ezrin and tetO-activated-Ezrin; GFAP-tTA, tetOCPEB3-EGFP, tetO-DN-CPEB, tetO-Cx43, tetO-GS, tetO-GLT-1, tetO-AQ4, tetO-Dys
Lentiviral transgenics	Overexpression of miRNAs and miRNA inhibitors in astrocytes

K^+ channel analysis in freshly isolated astrocytes



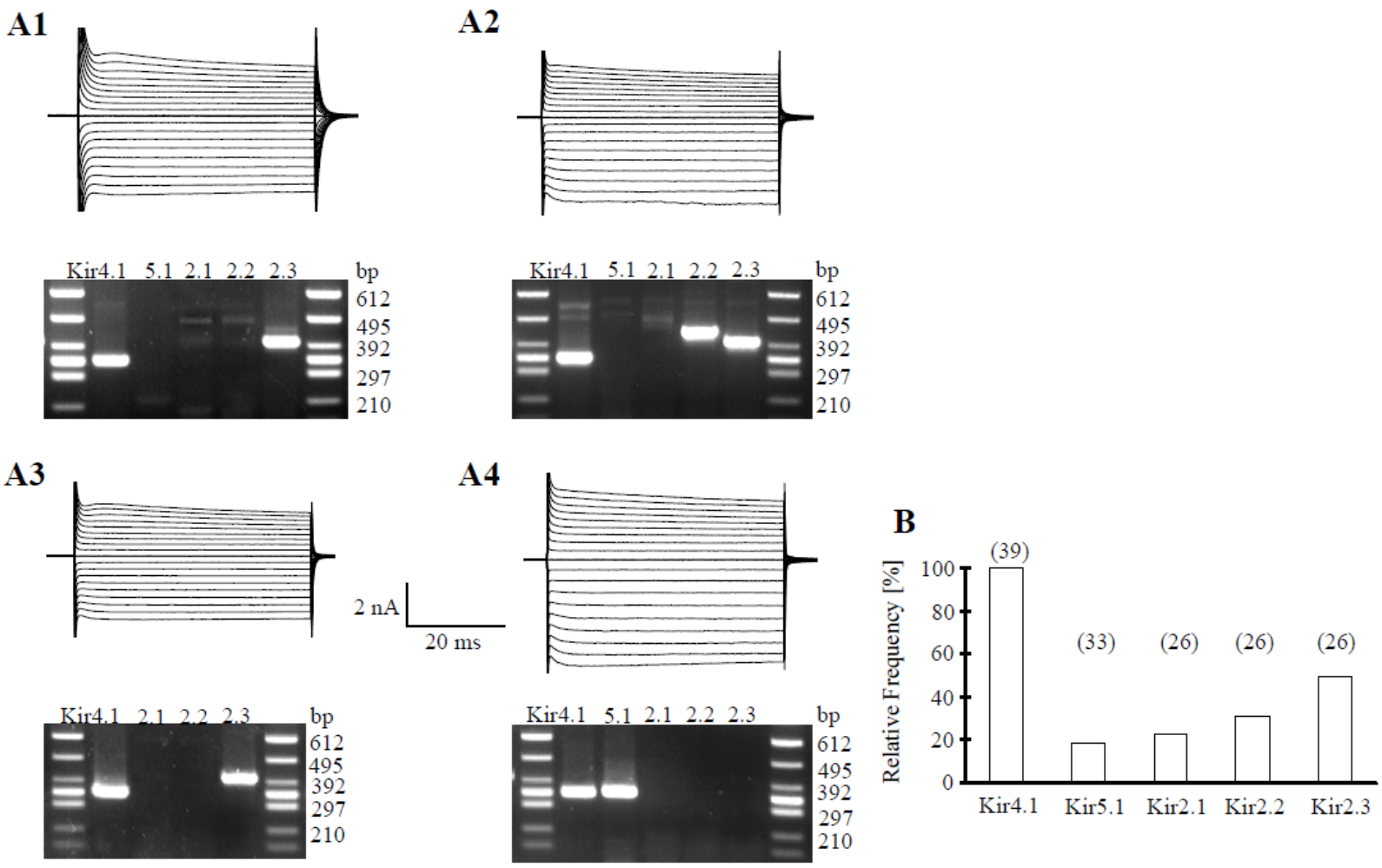






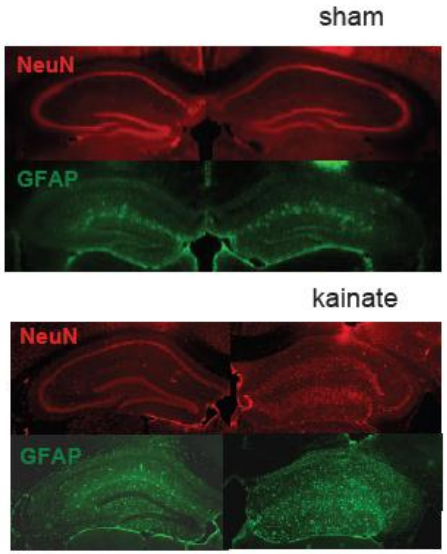
Seifert et al., J Neurosci, 2009

Astrocytes express Kir4.1



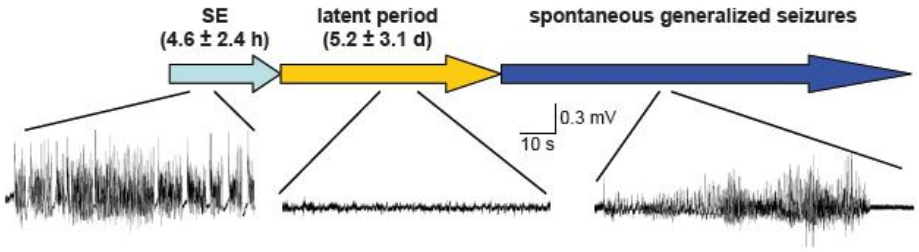
Unilateral intra*cortical* kainate injection: an animal model of TLE

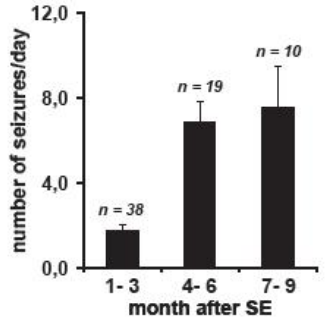
Established with the help of A. Becker, Bonn



Seizure frequency as a function of time after SE

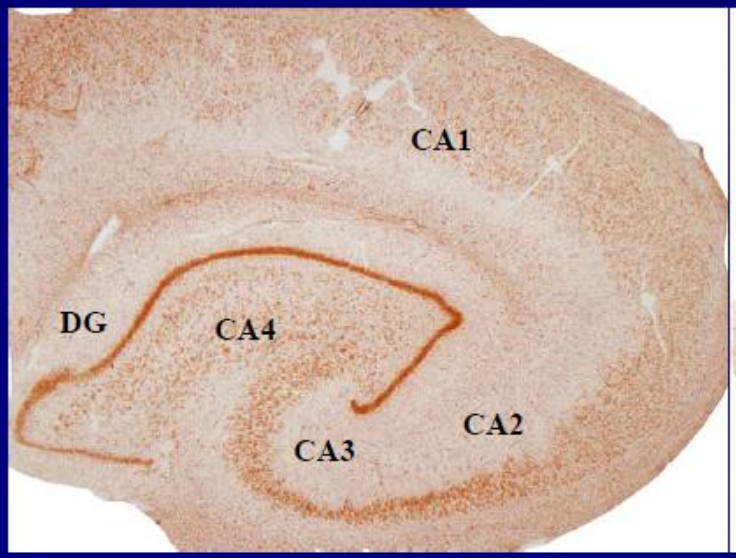
Time course of seizure activity after kainate treatment



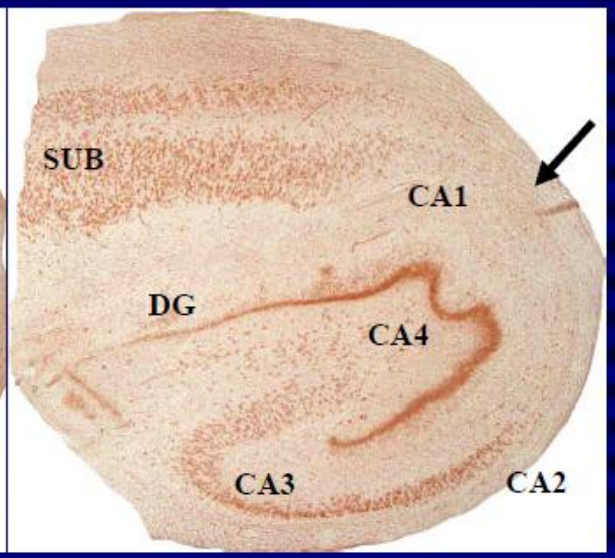


Neuropathology of hippocampal sclerosis

Control/Lesion-ass. Epilepsy



Sclerosis



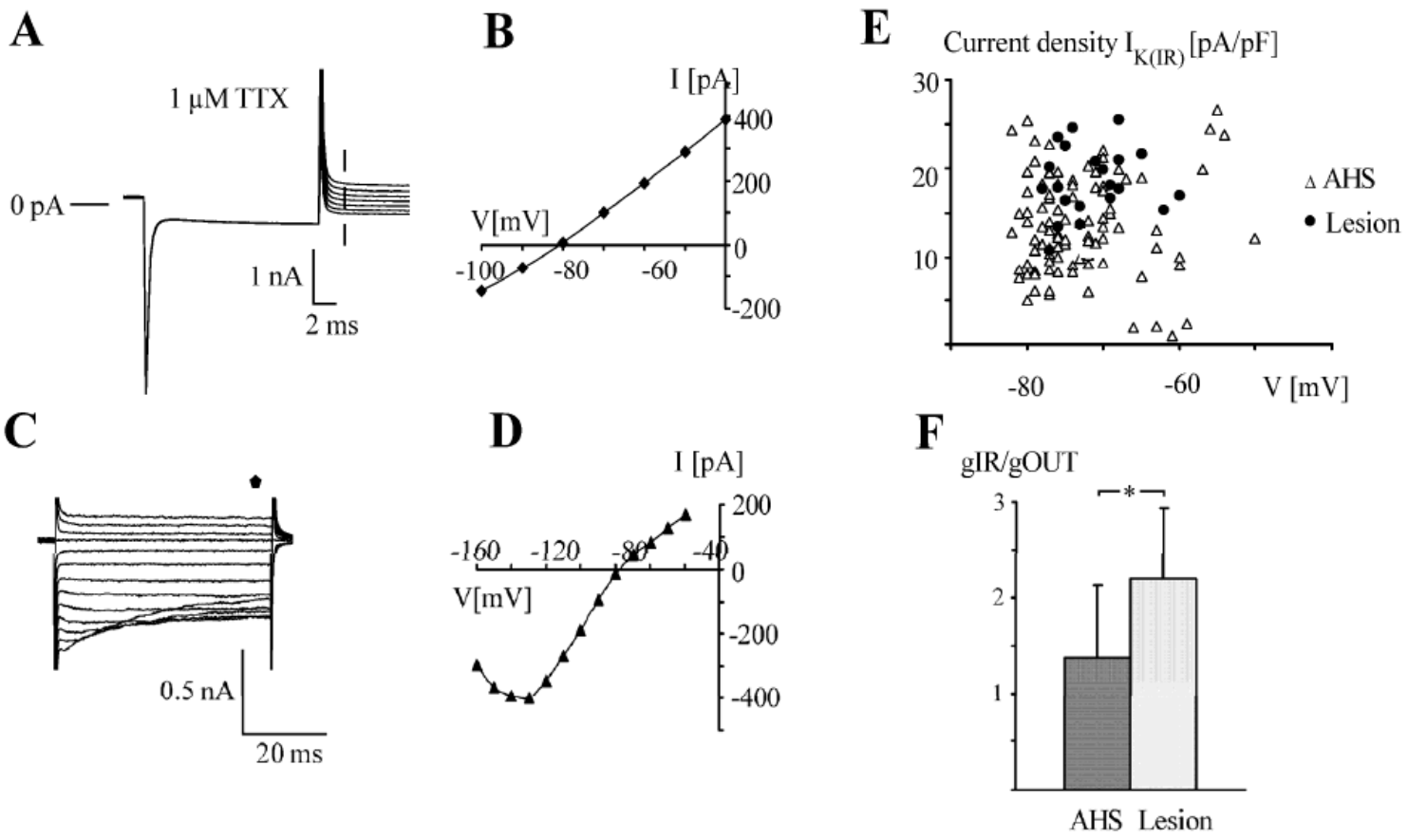
(Blümcke et al., 99)

Questions:

- Considering lesion-tissue as a kind of 'control': Do astrocytes in sclerosis display modified properties that might be of relevance to epileptogenesis?

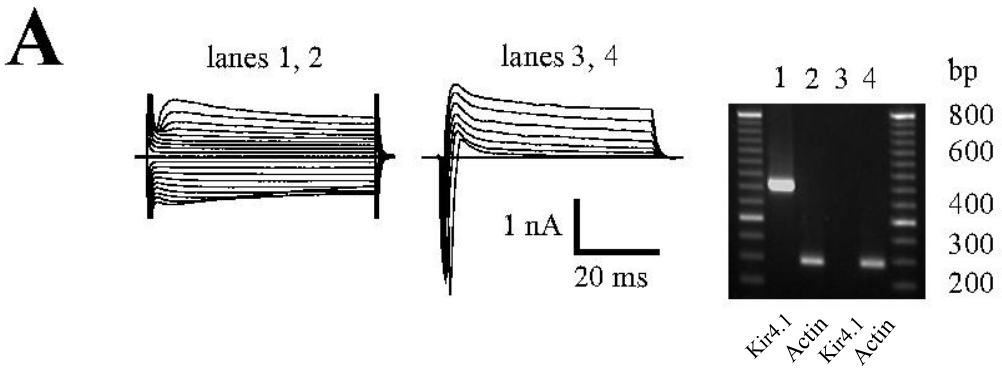
- **Compare Kir4.1 expression in human MTLE-HS w/o antecedent febrile seizures**

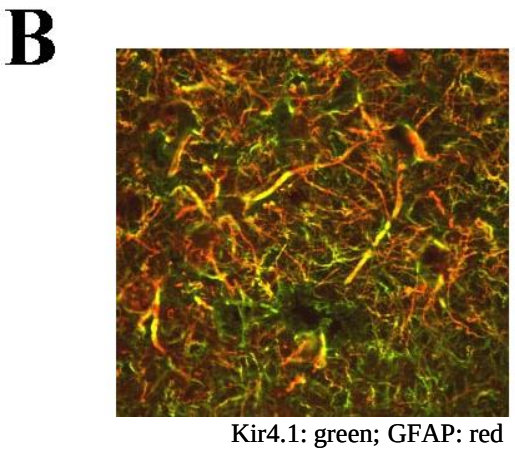
Reduced glial Kir currents in HS



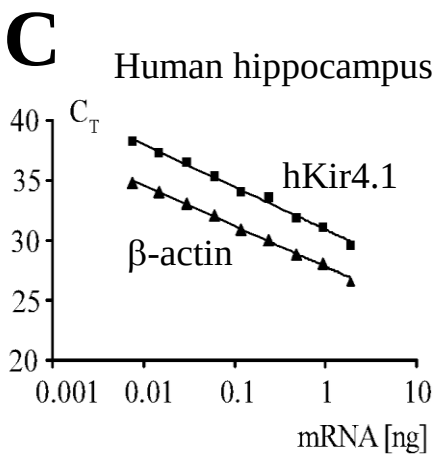
Hinterkeuser et al., 2000

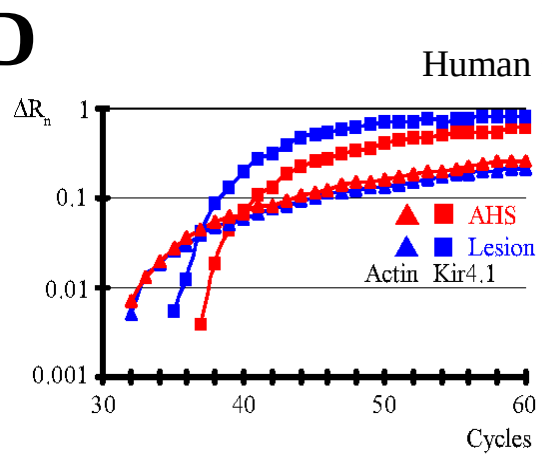
Kir4.1 is downregulated in sclerotic human hippocampus

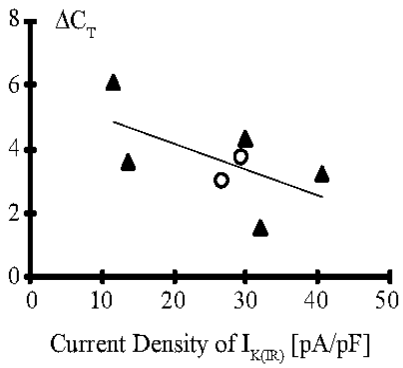




Kir4.1: green; GFAP: red







Work plan EuroEpinomics

WP1: Assess time course of altered Kir4.1 expression after SE in the KA mouse model of MTLE-HS

- quantify Kir4.1 current densities and mRNA levels with post-recording single cell sqRT-PCR in the latent period, 3 months and 9 months post SE
- Western blot analyses at the 3 time points
- rescue with 17ß-oestradiol?

WP2: Determine impact of astroglial Kir4.1 dysfunction on seizure generation, mice with inducible deletion of Kir4.1 in astrocytes

- compare K^+ buffering in Cx43kiCreERT/+; Kir4.1fl/fl mice (SC stimulation, astrocyte recording w/o 0.1 mM Ba^{2+})
- EEG recording / videomonitoring of Cx43kiCreERT/+; Kir4.1fl/fl mice
- apply KA model Cx43kiCreERT/+; Kir4.1fl/fl mice (score of SE, duration of latent phase, frequency of spont. seizures in chronic phase, morpohological changes, coupling)

WP3: Comparison of Kir4.1 expression in human MTLE-HS with and without antecedent FS

- compare current densities in glial cells from TLE-FS and non TLE-FS patients; re-evaluate earlier data
- comparison of Western blots, sqRT-PCR

Institute of Cellular Neurosciences

IP-3, Kuopio
Group leader: prof Reetta Kälviäinen

Main topic:
Risk factors for temporal lobe epilepsy;

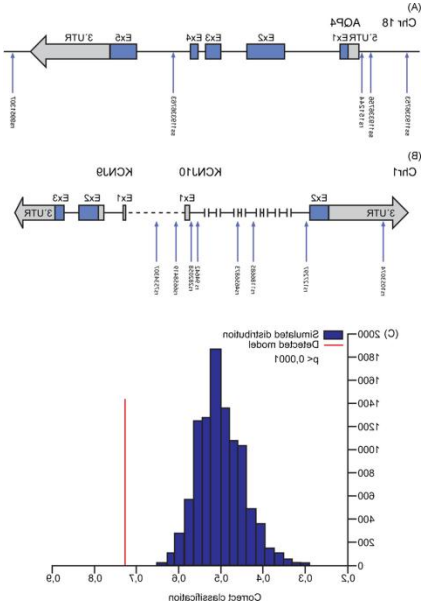
Gene association study of different temporal lobe epilepsy subtypes, especially those related with antecedent febrile seizures.

Why association studies in TLE?

- TLE denotes a location, not an entity
- TLE consists of several subgroups
- Different subgroups may have different causes and pathogenesis
- Response to treatment differs
- Different subgroups may need different treatment

Relation to FS

- TLE-FS may constitue a unique entity distinct from TLE without FS
- TLE and FS, common genetic basis?



Association analysis in TLE patients with/without FS

From: Heuser et al. Epi Res, 2010

Material

Will use patient and control materials from all 4 sites.

Altogether:

- >1000 TLE patients + controls extensively phenotyped
- DNA/RNA from most patients
- Brain tissue, > 500 patients

Work plan, Kuopio

WP1. Integration of the phenotypic data from all four cohorts into a common database.

WP2. Extraction of DNA from blood samples

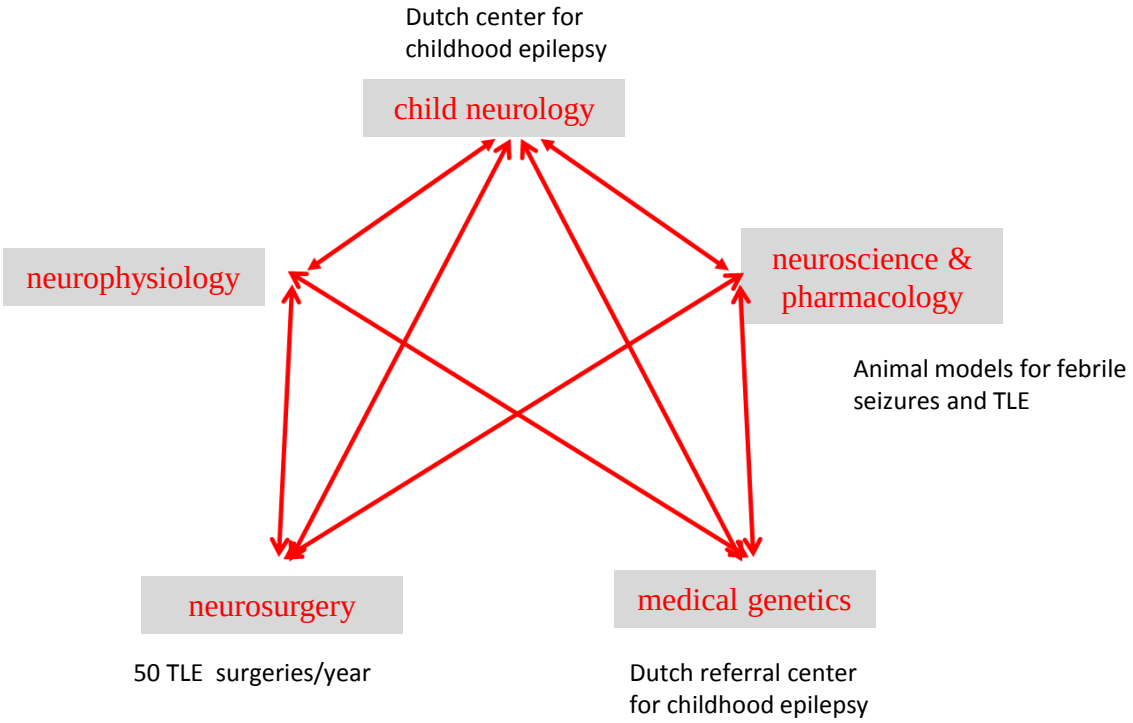
WP3. Association studies, focus on targets in glia as AQP4, Kir4.1, others according to further discussions

AP-1, Utrecht
Group leader: prof. Pierre de Graan

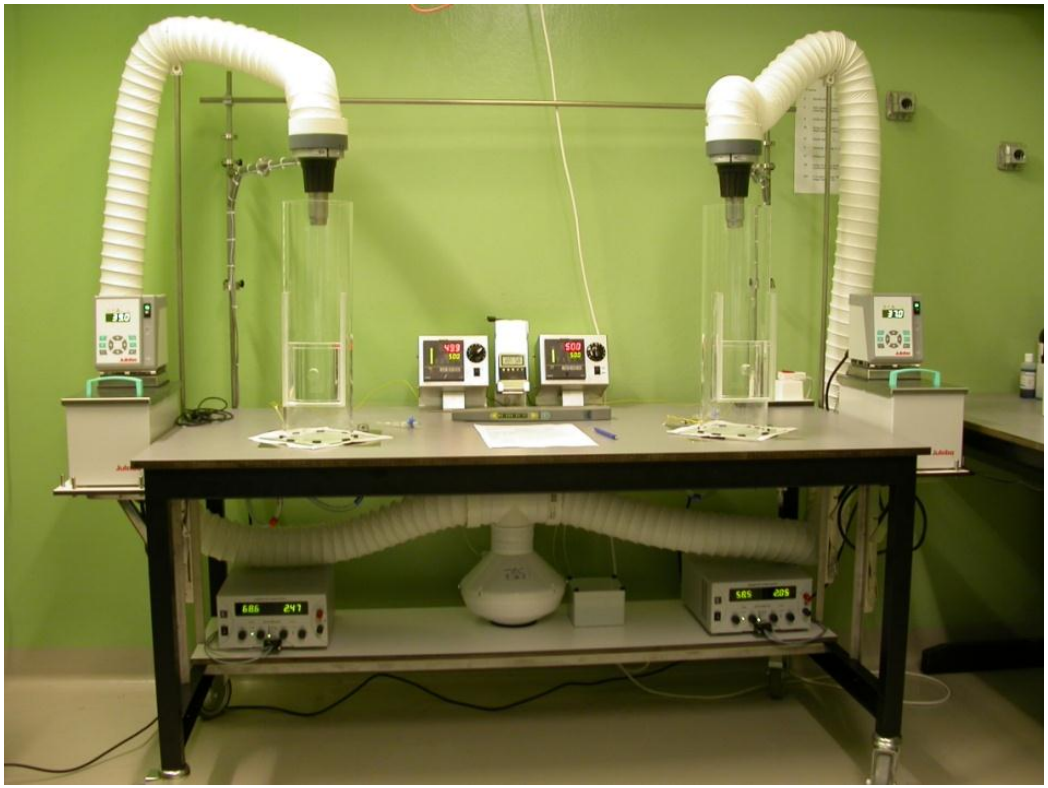
Febrile seizures and epileptogenesis in mice and men

University Medical Center Utrecht, Utrecht, Holland

Epilepsy UMCU



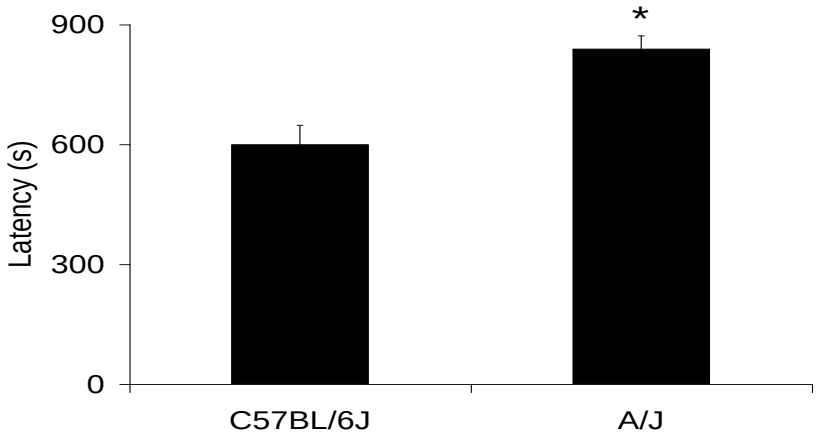
Mouse Febrile Seizure model



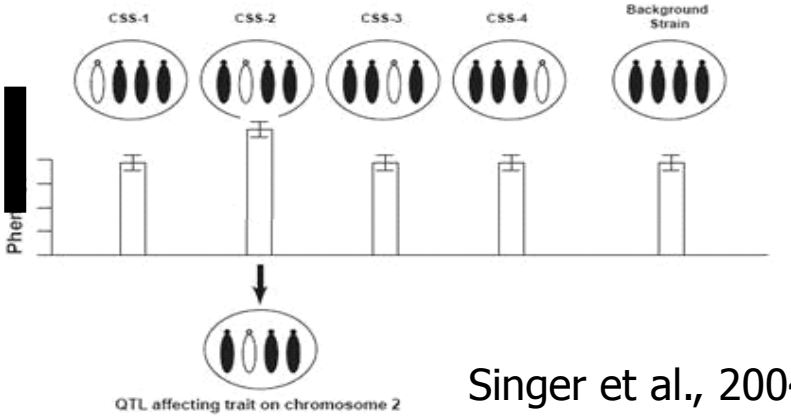
Mouse pups (p14) are exposed to a warm-air stream to induce experimental febrile seizures

Febrile seizure susceptibility is defined as the latency to tonic-clonic convulsions

Forward genetics: Chromosome substitution strains (CSS)



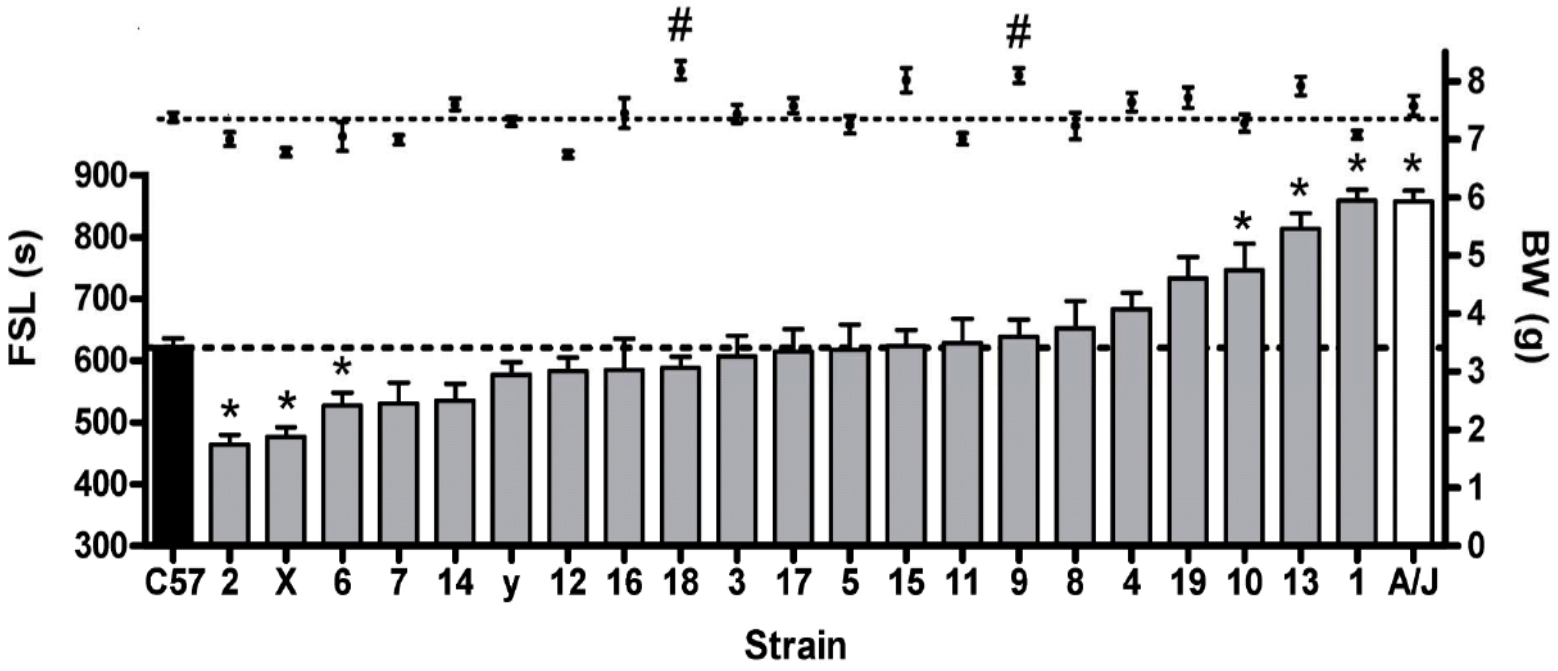
- Donor and host differ in FS susceptibility



Singer et al., 2004

- Substitution strains with donor phenotype carry QTL for FS

Febrile seizures susceptibility of mouse strains in the CSS panel



*significant difference compared to C57BL/6J p<0.003

Identification of 6 chromosomes carrying FS susceptibility genes.

Hessel et al., GBB (2009)

Workpackages - AP Utrecht

- Mapping febrile seizure QTLs on mouse chromosome 1 and 2
- Candidate gene selection (bioinformatics)
- Candidate gene identification
- Functional interference studies in mice
- Sequencing of candidate gene in human TLE patients with and without FS

Epiglia – overall collaboration

- Individual projects in each site focusing on glial mechanisms in epileptogenesis in TLE and FS
- Joint projects. 1) human association studies, 2) collaboration on mouse models on TLE and FS.

Main focus:

1. Bonn: Study the impact of Kir4.1 on epileptogenesis and seizure activity in a mouse model of TLE (i.c. kainic acid), and in human tissue
2. Utrecht: Focus on FS, and FS as a possible origin of TLE. Mice and humans
3. Kuopio: Focus on human genetic association studies
4. Oslo: Coordinative role. Will establish the i.c. kainic acid mouse model for studies on epileptogenesis, participate in association studies

Epiglia CRP meeting

Thank you!