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Revealing the mechanisms of epileptogenesis to design innovative treatments – what are the tools?

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Dublin, European Forum on Epilepsy Research
26.05.2013

Perspectives for novel treatments

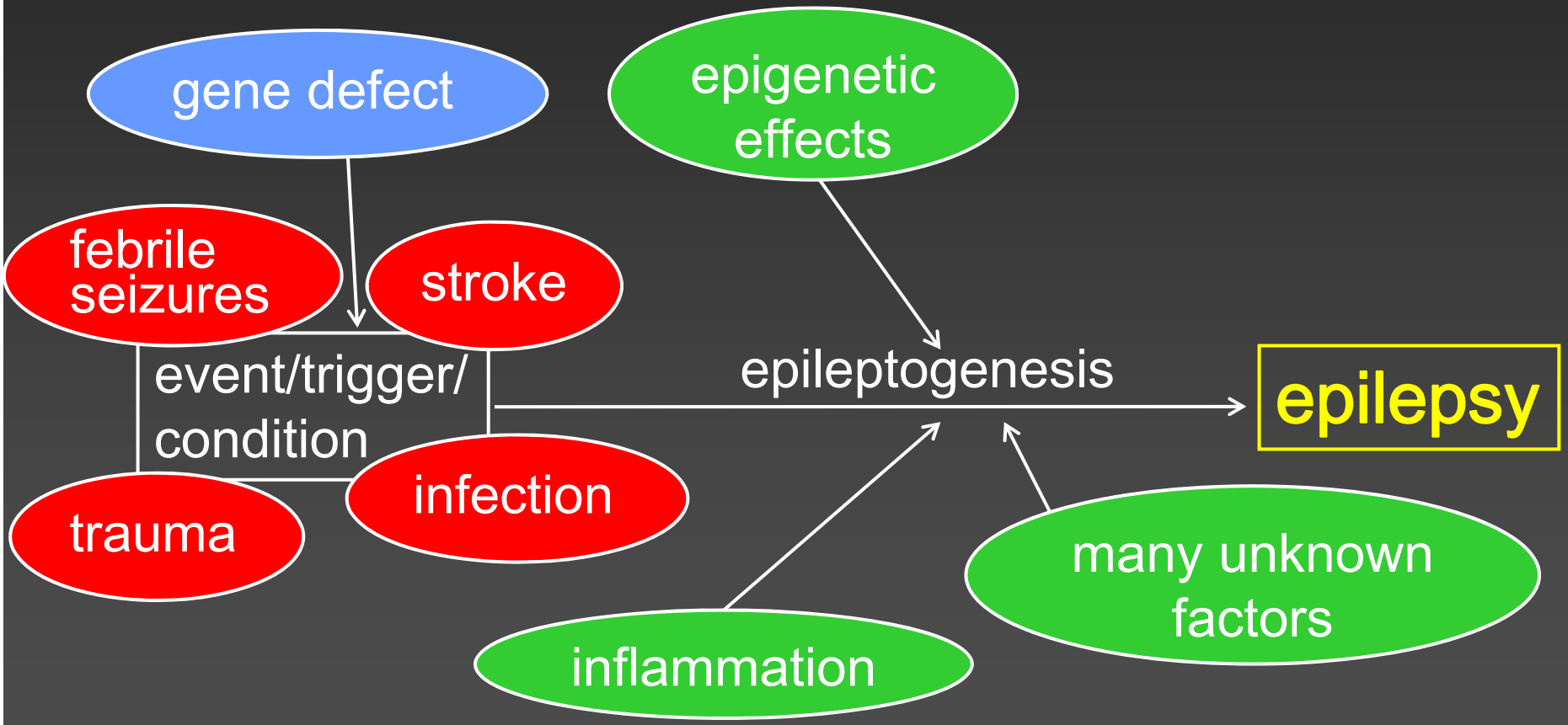
Two groups of epilepsy patients who are severely affected and deserve novel treatments urgently:

- patients with pharmacoresistent focal epilepsies (prototype with adequate animal models: temporal lobe epilepsy)
- patients with epileptic encephalopathies, severe epilepsies of childhood often with mental decline and other symptoms

Approaches to find novel therapies:

- understand epileptogenesis to design preventive/disease-modifying treatments
- design novel drug screening assays, two examples:
  - promoter screening of relevant genes
  - zebrafish models as screening tools
- use bioinformatics to identify novel targets

Epileptogenesis



Examples for antiepileptogenic / disase-modifying treatments

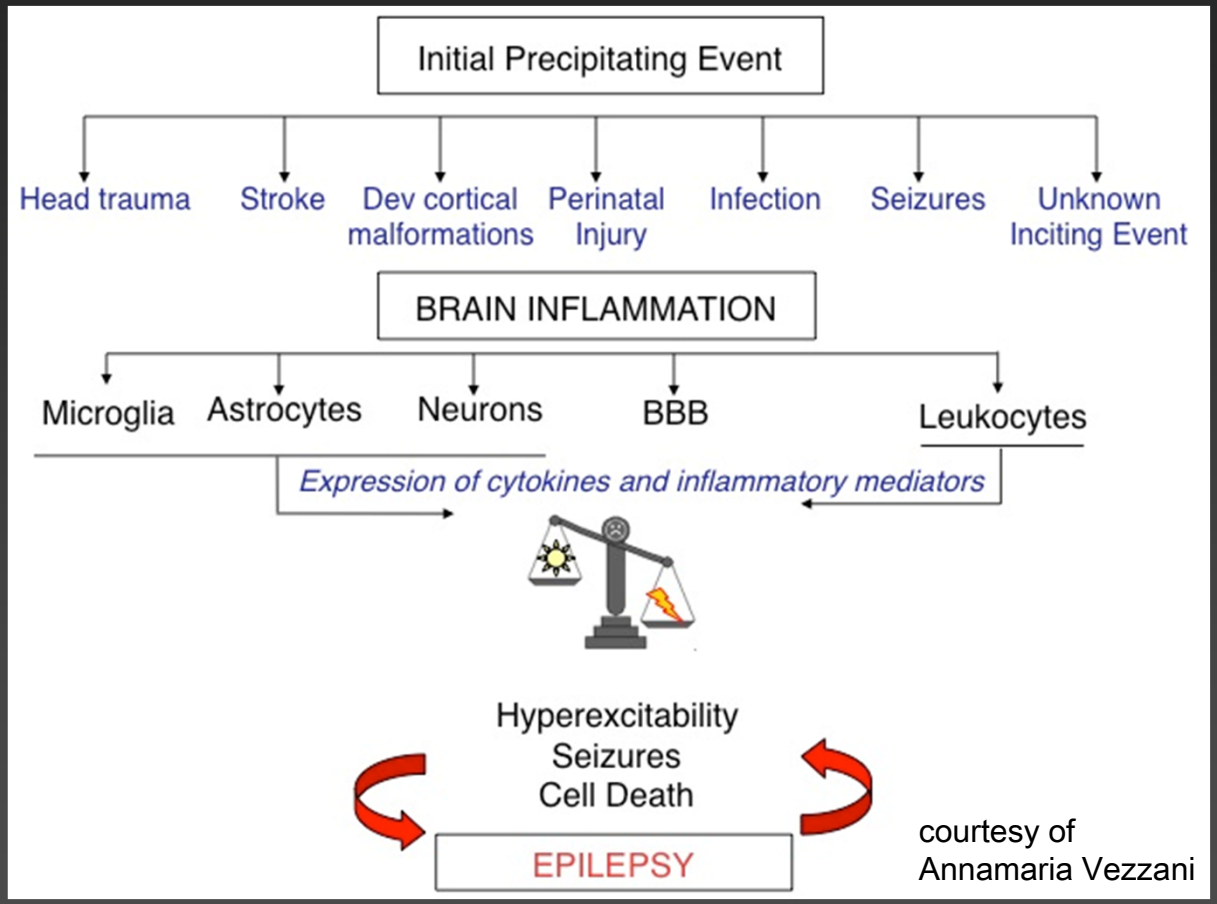
Temporal lobe (and other focal) epilepsies:

- in many animal models, knock-out or pharmacological manipulation of relevant targets is able to reverse epileptogenesis
- so far no clinical examples

Genetic epilepsies / epileptic encephalopathies:

- ketogenic diet can improve cognitive function and epilepsy in patients with glucose transporter type 1 defects
- stiripentol does not only treat seizures but seems to slow disease progression in Dravet syndrome

Inflammatory mechanisms in epileptogenesis



courtesy of Annamaria Vezzani

Molecules:

Toll-like receptor 4

High mobility group box 1

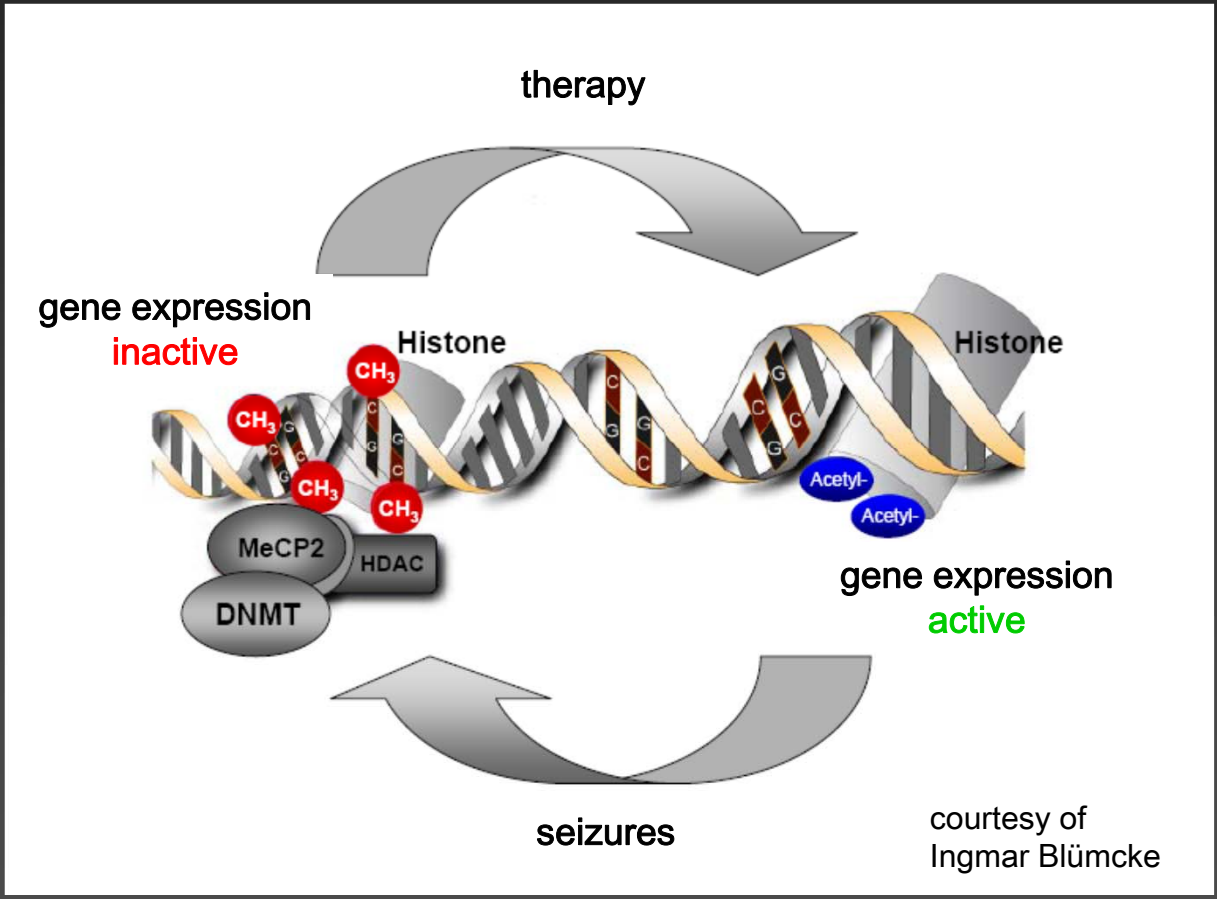
Interleukins

Maroso et al. Nat Med 2010;16:413-9.

Vezzani et al. Nat Rev Neurol 2011;7:31-40

Potential future antiepileptogenic therapy:
anti-inflammatory agents (existing and newly developed drugs)

Epigenetic mechanisms in epileptogenesis



potential targets:

reelin promoter

ion channel promoters

- $K_V4.2$
- HCN1
- $Ca_V3.2$

Kobow et al. J Neuropath Exp Neurol 2009:68:356-64.

Bernard et al. Science 2004;305:532-5.

Jung et al. J Neurosci 2007;27:13012-21.

Becker et al. J Neurosci 2008;28:13341-53.

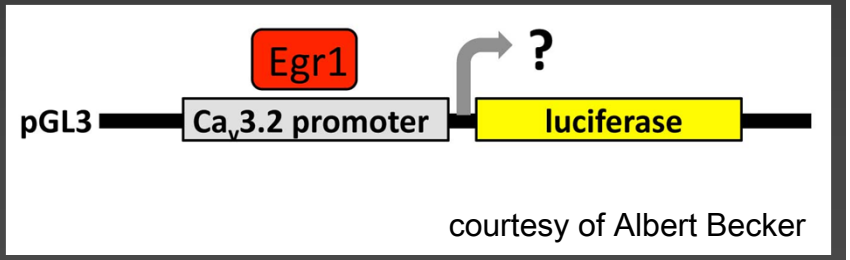
Potential future antiepileptogenic therapy:

HDAC inhibitors (valproate, newly developed drugs)

Manipulation of gene promoters

Influencing epileptogenesis in a model of temporal lobe epilepsy:

- $Ca_V3.2$ calcium channels are upregulated during epileptogenesis (presumably via upregulation of a transcription factor: Egr1)
- epileptogenesis is largely reduced in $Ca_V3.2$ knockout mice
- finding new therapeutic strategies:
  - establish promoter-reporter assays for high-throughput screening to find compounds suppressing $Ca_V3.2$ expression as antiepileptogenic therapy
  - viral transfer of Egr1 suppressors



courtesy of Albert Becker

Potential future antiepileptogenic therapies:

$Ca_V3.2$ promoter manipulations by small molecules or viral transfer

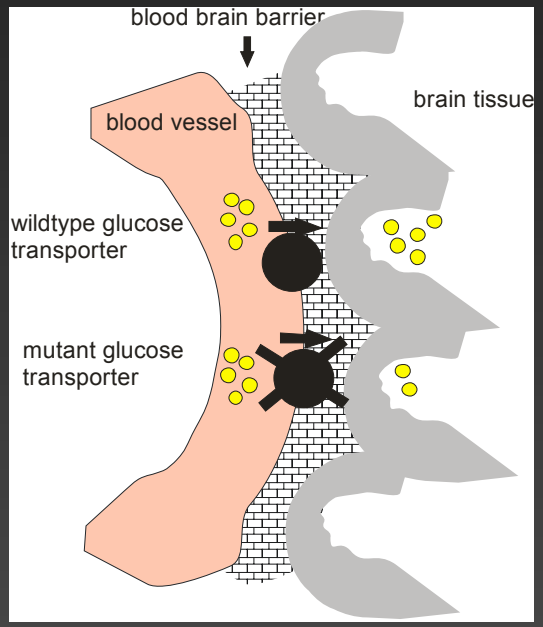
→ transferable to other promoters

Example of successful causative therapy in a genetic epilepsy and movement disorder: glucose transporter type 1 defects (GLUT1)

video child

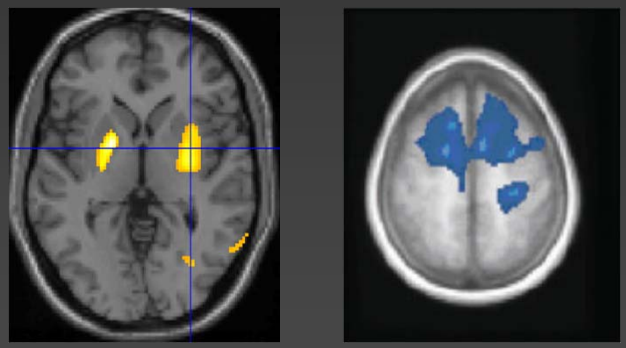
Weber et al., J Clin Invest 2008

Defective glucose transport across the blood-brain barrier – pathophysiology and therapy



Pathophysiology:

- energy deficit in the basal ganglia after physical exertion induces involuntary movements
- permanent frontal metabolic deficit induces seizures



Translation into an existing therapy

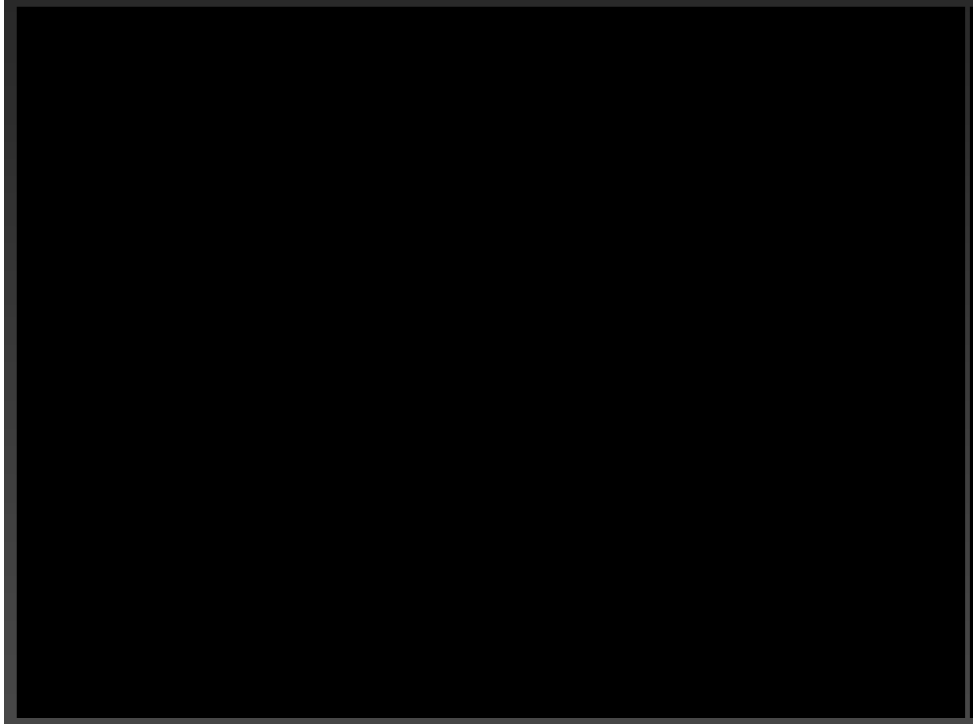
ketogenic diet: circumvent glucose as energy carrier
→ remission of seizures and episodic involuntary movements
→ dramatic improvement of cognitive function

Advantages of zebrafish as an *in vivo* drug discovery model

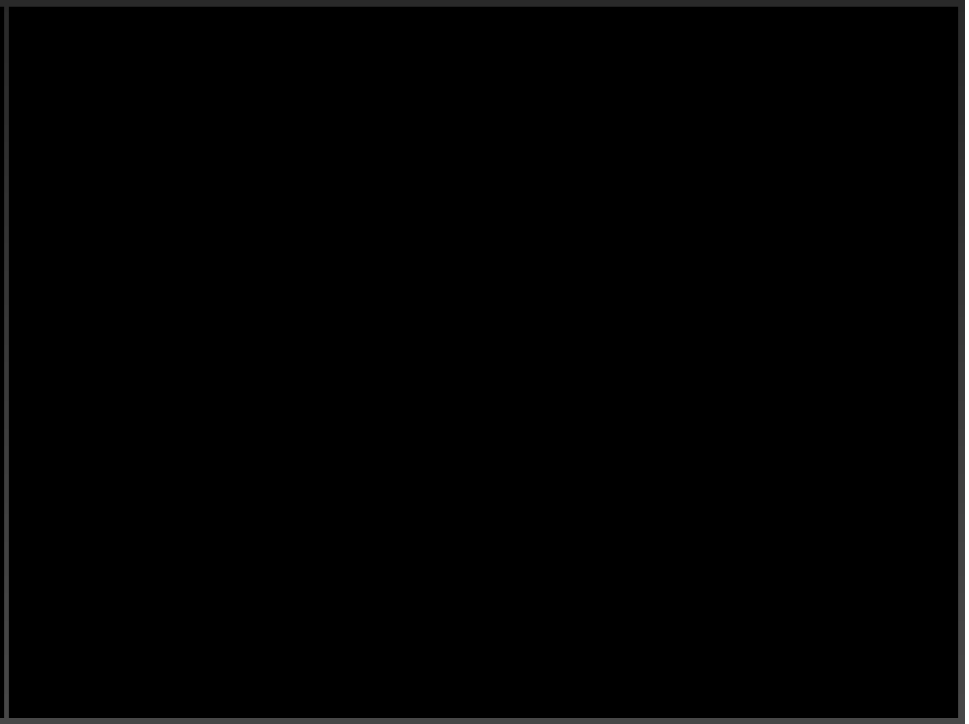
- Genetic, physiologic and pharmacologic homologies to humans
- High fecundity and small size
- Fast development ex utero
- Optical transparency
- Only µg amounts of compounds needed
- Compounds readily absorbed (skin, GI tract, gills)

courtesy of Alex Crawford and Camila Esguerra

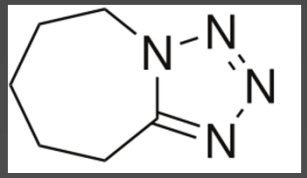
Zebrafish seizure assay



control

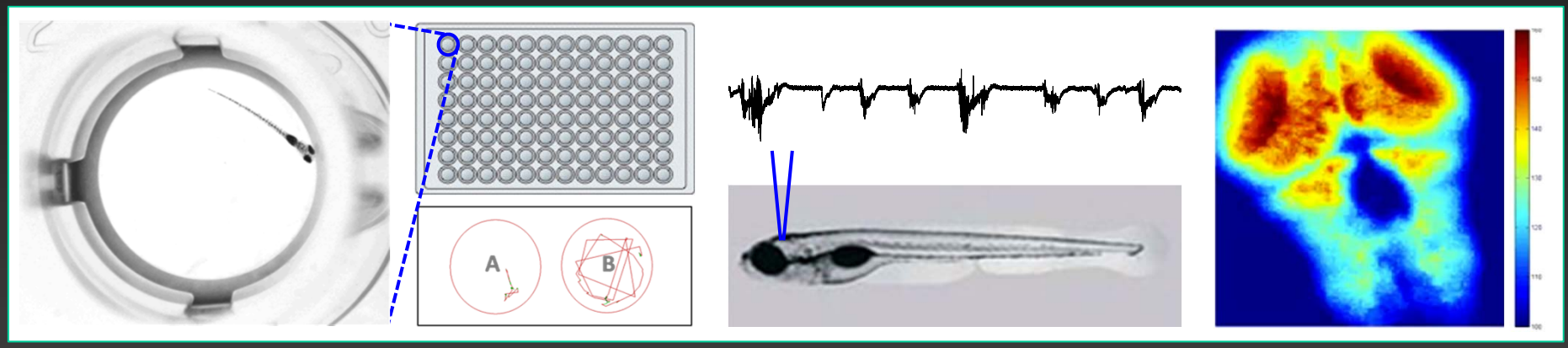


pentylenetetrazol-treated



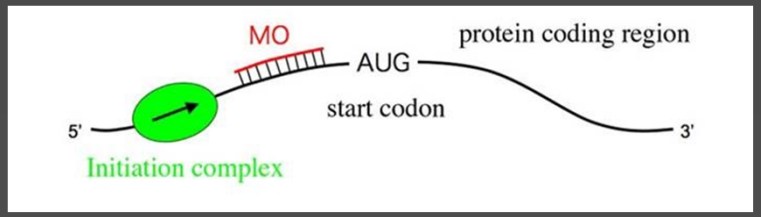
courtesy of Alex Crawford and Camila Esguerra

High-throughput, *in vivo* CNS assays in zebrafish



Behavioral screens Electrographic screens Whole-brain Ca^{2+} imaging

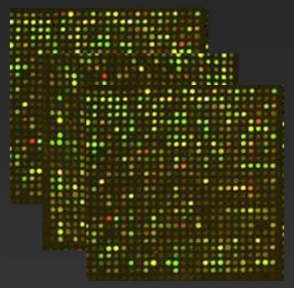
Rapid development of larvae allows not only to screen for anti-seizure but also for antiepileptogenic activity of small molecules:
- establish chemoconvulsant models with epileptogenic phase of few days
- establish genetic models with epileptogenic/(pre-)treatment phase



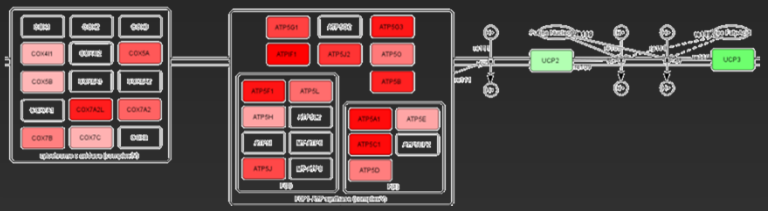
Rapid knockdown of genes using antisense morpholino oligomers (MO)

courtesy of Alex Crawford and Camila Esguerra

Use of bioinformatics to search for novel candidate genes / targets (example Parkinson's disease)

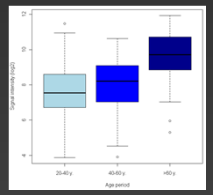


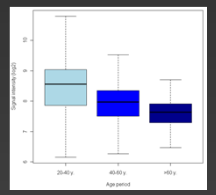
Meta-analysis of public transcriptomics data



Pathway visualization & enrichment

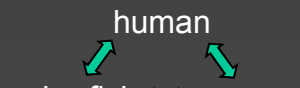
Integration with BrainAtlas data

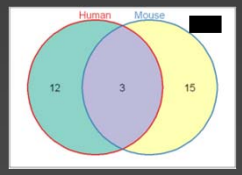




Identify joint gene deregulation in aging and PD

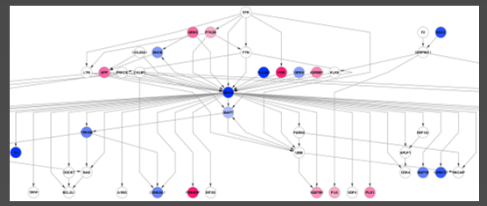
Phenolog candidates & GWAS SNPs

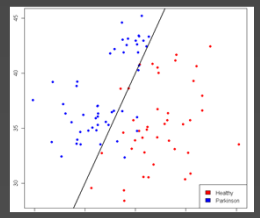




Combine evidence to prioritize candidate genes

Network & Machine learning analysis





Build and interpret combinatorial marker models

courtesy of Rudi Balling and Reinhard Schneider

Roadmap to find new therapeutic strategies:
identification - selection - validation of novel target candidates

understanding epilepsy

identification & selection of targets

development and application of target modulation strategies: viral transfer, knockdown, knockout, knockin

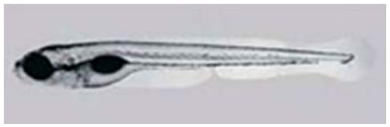
high-throughput

medium-/low-throughput

Specific changes in genetic and acquired epileptogenesis:
Genomics
Epigenomics
Metabolomics
Transcriptomics
Proteomics
Inflammation

Systems biology / bioinformatic modeling:
Cellular and molecular pathways,
junction points,
metabolic maps

zebrafish screening

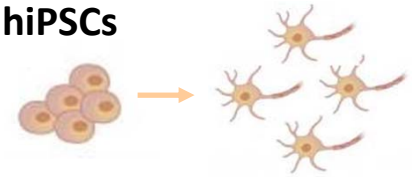


- chemoconvulsant
- genetic

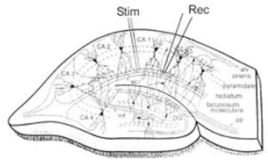
promoter screening

rodent models

hiPSCs



human tissue



Clinical trials

Conclusion

New tools in experimental research provide a fantastic chance to be translated into novel treatment options for people with epilepsy