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Issues Related to Development of Anti-Epileptogenic Therapies

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We confirm that we have read the Journal's position on issues relating to ethical publication. This review is consistent with these guidelines.

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Summary

Several preclinical proof-of-concept studies have provided evidence for positive treatment effects on epileptogenesis. However, none of these hypothetical treatments has advanced to clinic. The experience in other fields of neurology such as stroke, Alzheimer's disease, or amyotrophic lateral sclerosis has indicated several problems in the design of pre-clinical studies which likely contribute to failures in translating the positive preclinical data to clinic. The Working Group on "Issues related to development of anti-epileptogenic therapies" of the International League Against Epilepsy and the American Society for Epilepsy has considered the possible problems that arise when moving from proof-of-concept antiepileptogenesis (AEG) studies to preclinical AEG trials, and eventually to clinical AEG trials. This article summarizes the discussions and provides recommendations on how to design a preclinical AEG monotherapy trial in adult animals. We specifically address study design, animal and model selection, number of studies needed, issues related to administration of the treatment, outcome measures, statistics, and reporting. In addition, we give recommendations for future actions to advance the pre-clinical AEG testing.

Keywords

disease modification; epilepsy; epileptogenesis; pre-clinical; protocol; therapy

Introduction

Several fields in neurology have recently made efforts for standardization of preclinical study designs in order to improve the replication of favorable results from preclinical treatment trials in the clinic. Examples include spinal cord injury (Steward et al., 2012), stroke (Fisher et al., 2009), amyotrophic lateral sclerosis (Ludolph et al., 2010), and Alzheimer's disease (http://www.alzdiscovery.org/wp-ontent/uploads/2011/01/alzheimersbestpracticesguidelines.pdf). ARRIVE (Animal research: Reporting *In Vivo* Experiments) guidelines were published by the National Center for the Replacement, Refinement and Reduction of Animals in Research (Kilkenny et al., 2010; comments by Muhlhausler et al., 2013). Moreover, the National Institute for Neurological Diseases and Stroke (NINDS) of the US National Institutes of Health (NIH) has urged investigators to improve the quality of pre-clinical and clinical research through rigorous study design and transparent reporting (Landis et al., 2012). These activities have been kindled by many previous failures in translation. For example, in stroke over 100 monotherapy or combination therapy trials with diverse investigational compounds have been done, many of which have shown beneficial effects in pre-clinical trials but none of them have advanced to clinical use (O'Collins et al., 2012). These unexpected failures have raised the question, why has the translation failed. The explanations range from lack of blinding and randomization to poor control of experimental conditions (*e.g.*, animal body temperature) to species differences to pharmacokinetic/pharmacodynamic (PK/PD) properties to differences between the experimental and clinical trial designs (Philip et al., 2009). One concerning observation in the field of spinal cord injury field has been the low reproducibility of data between different laboratories (Steward et al., 2012).

Several proof-of-concept experimental studies have shown positive treatment effects on epileptogenesis (Pitkänen, 2010; Löscher and Brandt, 2010; Pitkänen and Lukasiuk, 2011). So far, none of those treatments has been advanced to clinical investigations in humans. Therefore, the *ILAE/AES Working Group on "Issues related to the development of antiepileptogenic therapies"* examined the information available from other neurological fields, with the goal of anticipating the challenges that the field will face when trying to translate the data from preclinical antiepileptogenesis (AEG) trials to clinical trials in humans.

This article summarizes the work of the ILAE/AES Working Group, and its purpose is to give recommendations for designing a preclinical AEG monotherapy trial. During the course of the work, it became apparent that (a) issues related to conducting an AEG trial in immature animals and (b) implications of human AEG trial designs on preclinical studies, needed special attention. Therefore, two subgroups were established to specifically address these issues, and their work is also summarized here. We did not address PK/PD issues, assessment of adverse events, toxicology, assessment of comorbidities, methodology for video-EEG monitoring, statistics needed at different steps in preparation of the powered trial and data analysis, specifics related to non-pharmacological therapies, and regulatory issues as they are presented in accompanying articles (Brooks-Kayal et al., 2013; Galanopoulou et al., 2013).

Definitions

Definitions and terminologies used in this article (presented below) were adopted from Pitkänen (2010) and Galanopoulou et al. (2012). *Epileptogenesis* refers to the development and extension of tissue capable of generating spontaneous seizures, resulting in (a) development of an epileptic condition and/or (b) progression after the condition is established. Disease-modification has two components: AEG and co-morbidity modification. *AEG* treatment can be given prior to or after epilepsy onset. When an AEG treatment is given *prior* to epilepsy onset, it prevents or delays the development of epilepsy. If seizures occur, they may be fewer in frequency, shorter, or of milder severity. When such a treatment is given *after* the diagnosis of epilepsy, it can alleviate seizure severity, prevent or reduce the progression of epilepsy, or change the seizures from drug-resistant to drug-sensitive. Cure is achieved when there is a complete and permanent reversal of epilepsy, such that no seizures occur after treatment withdrawal. *Comorbidity-modifying* treatment alleviates or reverses the symptomatic development or progression of epilepsy-related co-morbidities such as anxiety, depression, somato-motor impairment, or cognitive decline. Both AEG and co-morbidity modifying treatments can also alleviate or reverse the associated pathology. Development of comorbidity-modifying therapy is considered in an accompanying article (Brooks-Kayal et al., 2013).

Entry criteria from a proof-of-concept study to a preclinical trial

The different phases of the development of AEG treatments are summarized in Fig. 1. Prior to undertaking a proof-of-concept AEG study, a thorough understanding of the nature of epilepsy in the target model is required. For example, the variability in latency to epilepsy onset (*i.e.*, delay from the insult to the appearance of the first unprovoked seizure) and proportion of animals that develop epilepsy should be well-characterized for the model in the hands of the investigator. This will be important in calculating sample size, ensuring that the study is not underpowered, and determining the duration of observation required.

Before initiating the statistically powered, detailed preclinical AEG trial (see below), there should be evidence from a proof-of-concept study that the compound crosses the blood-brain-barrier (BBB), is tolerated in rodents (testing in one gender is enough), and that treatment with the compound modifies the epilepsy phenotype. If the target is known, there should be evidence of target engagement in the brain.

Design of an anti-epileptogenic preclinical trial

Table 1 summarizes the major points related to the design of a preclinical AEG monotherapy trial.

Study design

The recommendation is that the preclinical AEG study should be blinded, in regard to treatment, animal groups, and data analysis to minimize bias. An AEG study should be placebo-controlled. An active AEG treatment as a comparator should be used when it is available. A single-center study was considered sufficient. As compared to a multicenter trial, a single-center study would be less expensive, as there would be no need to standardize methodologies between different study sites. It was recognized that conducting a powered preclinical AEG trial in one center requires a large monitoring unit and its availability for testing of treatments.

As the AEG treatment can be started at different time points during epileptogenesis, including the time after epilepsy onset, the studies should be designed accordingly (Fig. 2).

Selection of study subjects

The rat was considered to be the primary species to be used in preclinical AEG trials, as rats possess several advantages over mice. For example, the larger size of the head and brain is advantageous for insertion of EEG electrodes and MRI analysis, respectively, and repeated blood sampling is possible. It was considered of particular importance that the genetic background of study subjects is specified in the methods of all reports of study results. Efficacy in more than one species or genetic background was considered as an added value. Even though the recommendations presented here refer to testing of compounds in adult rodents, it was considered very important to address the age-specificities in AEG trial study designs (see “Future recommendations”). Finally, demonstration of AEG efficacy in one gender is considered adequate before advancing to a clinical study. However, if the resources are available, AEG testing should be conducted in both males and females.

It was noted that in some cases there could be specific advantages related to the use of genetically modified rats/mice in preclinical AEG testing.

Model selection and data replication

Model selection should be appropriate for the epilepsy syndrome targeted by the AEG treatment. It is considered sufficient to use only a single model per study. If the initial study is successful, a second study should be performed in the same model in a different independent lab to confirm the results, and designed based on the data from the first study. It does not need to be a full replication of the first study but it should (a) verify the efficacy and (b) optimize dosing. Also, if the target is known, it should ensure the target relevance and engagement during the treatment period. Before advancing to clinic, testing in a second and different model (*i.e.*, another model of the same epilepsy syndrome), preferably in a separate species, is needed

Timing of drug administration, dosing, and duration of treatment

An AEG study should demonstrate that it achieved active drug concentrations during the treatment. This can be done by demonstrating that target expression was operant during treatment, and the target was affected by the treatment. Duration of target relevance should also guide the assessment of the therapeutic window for the initiation of the treatment, and for how long the treatment should be continued (Fig. 2). It was noted that documentation of drug exposure and target engagement is necessary to exclude the possibility of a false-negative outcome.

To confirm an AEG effect, the wash-out-time should be long enough to demonstrate that (1) the drug is no longer present in the (brain) target organ and (2) the known transient treatment-related PD effects have washed-out at the time of assessment of primary AEG

outcomes. Under certain circumstance as in the genetic epilepsies, continuous treatment may be needed to prevent the development of epileptogenic pathology. In these cases, the wash-out requirement does not apply.

In the initial testing, a single dose is considered adequate. In the replication study, more than one dose should be used to establish a dose-response relationship. There should be no difference in the formulation and administration route between the placebo (vehicle) and the treatment. It was noted that vehicles with possible disease-modifying effects such as ethanol should be avoided.

Comparison of different routes of administration or assessment of long-term safety or toxicology are not needed as part of the initial pre-clinical AEG trial, as it is anticipated that they will be performed at later stages of drug development. It should be investigated whether the active concentration of the drug has antiseizure effect to avoid confusion in data interpretation between the antiepileptic (AED) and AEG effects.

Outcome measures

The assessment of epilepsy outcome should be based on video-EEG monitoring. The two primary outcome measures are: (1) reduction in seizure frequency (reported as seizures/recording period) and (2) percentage of seizure-free animals per recording period. In addition to reporting the mean seizure frequency in different treatment groups, one could also report, for example, the percentage of animals with over 50% reduction in seizure frequency. To obtain reliable data, monitoring should be performed at the stable phase of the natural history of epilepsy in a given model. As secondary outcome measures, one can analyze the effect of AEG treatment on (1) seizure duration and (2) seizure type. In some cases, an effect on only a single seizure type (*e.g.*, generalized convulsive seizures) can be clinically relevant.

The difficulty in using spontaneous seizures as an endpoint was acknowledged. One concern is: how long should the monitoring be continued? One suggestion was that monitoring should last at least three times the average inter-seizure interval in a given model. Some members expressed concern that this was too short of a monitoring period. It is also possible that the effect is seen in a subpopulation (endophenotype) of a study cohort, for example, in animals with a specific level of severity of brain damage or a specific pattern of epilepsy phenotype. Demonstration of an effect on only a subpopulation of the data set would, however, require a larger study population. Moreover, in many models [e.g., cerebral stroke or traumatic brain injury (TBI)], the seizure frequency is low, requiring much longer monitoring periods. In any case, the duration of seizure monitoring will necessarily be limited in duration, and thus it will always be difficult to exclude the subsequent occurrence of epilepsy. Therefore, novel methods are needed to diagnose epileptogenesis in a definitive manner with a limited duration of monitoring.

The use of several other possible outcome measures as endpoints in AEG trials was discussed. Latency to the 1st seizure was not considered as a primary outcome because it is very labor intensive to establish, requiring long-lasting 24/7 video-EEG monitoring with cortical/subcortical electrodes, and there can be large inter-animal variability and significant species (genotype) differences. Latency to the peak seizure frequency was not considered to be a strong endpoint as it is also highly variable between the animals, is age-dependent, requires extensive long-term video-EEG monitoring, and seizure clustering can compromise the analysis. Assessment of seizure susceptibility or seizure threshold could be useful in proof-of-concept studies, but it is not clear how this measure translates to actual epilepsy. The idea of using drug-refractoriness was considered a clinically relevant endpoint, but unpractical in preclinical trials. Using disease progression as an outcome is complicated

because of large inter-animal variability, but it was considered as one alternative measure at later stages of therapy development. Structural endpoints were considered as possible endpoints at later stages of drug-development. Analysis would be costly and would require assessment at both the beginning and end of the study as well as the comparison of responders and non-responders. Co-morbidities should be profiled, but not used as a primary outcome in an AEG preclinical trial. Seizure-related death was not considered a realistic endpoint because of its low incidence, although reporting such events would be informative

Data analysis

The rationale and justification for statistical analysis of data should be defined before initiation of the study. Determination of animal numbers should be based on power analysis. Inclusion and exclusion criteria should be predefined and described in the study report. If the study has to be conducted with multiple animal cohorts (*e.g.*, the limitations of the video-EEG facility does not allow monitoring of all animals at the same time), analysis of multiple cohorts should be predefined. One should quantify the accuracy of the endpoints to avoid false-positive and false-negative findings. One should provide information on external validity (i.e., control data similar to other studies) and experimental variability (similar to other studies). It is advisable to include a statistician in the research team early in the planning phase.

Reporting

Study design, exclusions, missing data, and flow of the animals throughout the study should be described in detail in the experimental design and methods. It is important to report both positive and negative outcomes, for example, to avoid unnecessary replication of unsuccessful studies. One should discuss the study limitations such as the possibility of false- positive or false-negative findings, and should consider species differences when attempting to translate from rats to humans. The investigator should also attempt to define which study population could most likely benefit from the treatment. Possible interactions with other treatments the patient may be receiving should be addressed. Finally, one should report any conflicts-of-interest the investigators may have.

Issues related to conducting preclinical AEG studies in immature animals

In studies aimed at finding an AEG treatment for pediatric epilepsy syndromes, it is important that the data are not derived from AEG trials performed in adult animal models. The studies should be done in models of pediatric epilepsy syndromes, such as models of infantile spasms, Dravet's syndrome, or tuberous sclerosis (Auvin et al., 2012; Galanopoulou, 2013). Table 2 summarizes factors that require special consideration when planning a preclinical AEG trial in models of pediatric epilepsy syndromes. These features necessitate separate preclinical studies in immature animals to examine drug metabolism and pharmacokinetics. Particular attention is needed to confirm that the target is expressed at a given developmental age. Studies should take into account the natural history of age-specific syndromes. For example, spontaneous remission needs to be considered, which would also affect the selection of the therapeutic window for treatment. In species selection, rats were preferred over mice, although in genetic epilepsy syndromes mice will become the species of choice. Use of other species such as dogs, cats, pigs, and marmosets is presumably going to be unrealistic because of costs. When conducting an AEG trial in immature animals, one should pay particular attention that (1) there are both AEG and vehicle treated pups within the same litter, and (2) pups from several litters from different dams are included in each study cohort. We recognize that with current techniques, continuous video-EEG recording is not feasible in young rodents and study designs need to address that only several hours of EEG may be available daily. It was emphasized that outcome measures need to be tailored for each syndrome.

In conclusion, to advance preclinical AEG development, one needs to develop hardware and infrastructure for video-EEG monitoring in immature animals. In addition, there remain many pediatric syndromes that lack adequate animal models. Under what circumstances can a preclinical AEG clinical trial be performed for a specific syndrome, for which there is no animal model? The overall conclusion was that there is a need for a separate Working Group that would prepare more detailed recommendations related to issues surrounding preclinical AEG trials in immature animals.

Assessing antiepileptogenic activity in humans: the quest for predictive preclinical trial design

Assessing the AEG activity of agents in humans is fraught with many problems, and there are lessons to be learned from previous clinical trials with AEDs (Schmidt, 2012). The reasons why placebo-controlled, double-blind, randomized trials of established anti-seizure agents have had minimal success are manifold (see Sloviter 2011, Mani et al, 2012). One option to minimize the risk of failure in future AEG trials in humans may lie in improving the predictive ability of preclinical trials.

Preclinical AEG trial designs would vary depending on whether the experimental compound has AEG as well as anti-seizure activity. An appropriate control group for AEG drugs with anti-seizure effects would be a standard anti-convulsant known to be effective in a given epilepsy model. Examples would be to compare the experimental AEG with levetiracetam in a model of human focal epilepsy or with ethosuximide in a model of human absence epilepsy. Following washout of both drugs, animals would be followed until the onset of spontaneous seizures.

Monitoring seizure frequency or remission over extended time-periods in humans is currently done by counting seizure frequency based on seizure calendars which has limited reliability (Fisher et al., 2012). Most preclinical studies would include assessing seizure frequency or remission after washout as their primary outcome parameter. Therefore, trials in experimental animals will thus need to include intensive video-EEG monitoring, which may impractical and costly for routine screening and even for extended follow-up.

Proof-of-concept studies may be used to identify the optimal treatment window for the compound as well as to explore surrogate endpoints (EEG features, MRI changes) that can be validated in future preclinical trials. Identifying biomarkers of compound efficacy in experimental animals that can be assessed in humans will be important in early clinical development of the AEG treatment (*e.g.*, phase IIa). The lack of reliable and practical surrogate endpoints of efficacy in humans may prevent even the most promising drugs from entering clinical development. This is particularly relevant in conditions with a long latent period such as post-traumatic epilepsy.

For treatments which demonstrate a positive effect on latency to epilepsy onset in humans, which could be measured, for example, as a time period to the n^{th} seizure, a long-term follow up to assess AEG activity will be needed. The clinical trial design has to include a randomized treatment phase versus a control, either placebo or preferably a standard AED, to assess anti-seizure effects, if any. In addition, it is very important to study AEG effects after drug washout (Schmidt, 2012).

The epilepsy research community needs to establish criteria for what we perceive as a clinically useful AEG effect. Does lowering seizure frequency or remission after washout for a follow-up of 3 months present an added benefit for an agent over current treatment? Or do we require demonstrating a longer duration of effect? In measuring efficacy of anti-seizure agents, a 50% reduction of seizure frequency or remission are considered to be

clinically valid estimates of added benefit over controls. Does this also apply for AEG effects? Other potentially important endpoints for an AEG therapy include the proportion of animals remaining seizure-free at the end of a suitable observation period.

There is emerging evidence that the natural history of epilepsies in humans is more complex than previously thought. Although two-thirds of human patients follow stable patterns, that is, become seizure-free early and remain seizure-free (49%), or have refractory epilepsy all their life (19%), one in every three patients has an unstable course of epilepsy. Patients with an unstable course will either enter remission only after many years of having seizures (19%) or will relapse and develop uncontrolled seizures (14%) despite continued treatment (Schmidt and Sillanpää, 2012). Preliminary data suggest that patterns of uncontrolled epilepsy may not be amenable to treatment with current anti-seizure drugs (Geerts et al., 2012), and thus, may present a new therapeutic area for disease-modifying agents. The recognition of patterns of human epilepsy presents still another clinical challenge for preclinical trial design as it is not known whether patterns of epilepsy also exist in experimental animals.

In summary, preclinical trial design may become as difficult and time-consuming as trials in human epilepsy unless we come up with suitable biomarkers for easy and inexpensive assessment of seizures in animals. This applies particularly to testing AEG activity which requires prolonged follow-up after termination of the treatment. Furthermore defining what constitutes an added benefit over current treatment needs further consideration.

Bottlenecks in conducting AEG trials

One of the greatest methodological issues slowing down the progress of AEG development is the performance and analysis of long-term video-EEG monitoring, a “gold-standard” method to reveal the critical outcome measures in AEG studies. In particular, there is a great need for hardware to be used in monitoring of immature animals. The number of monitoring units per center is often too low, allowing only a few animals to be monitored at the same time. Also, there is need for a higher throughput in EEG analysis software to speed up the assessment of outcomes after data collection. In the AEG trials it is of particular importance to minimize the false negative and false positive detection of seizures in EEG analysis.

The other obstacles include methods for administration of treatments for a longer period of time, as rapid drug elimination in rodents often makes it difficult to maintain active drug levels (Ali et al., 2012). Several methods, including implantation of minipumps or drug administration via the drinking water or food have been described, but these methods are not without problems (Löscher, 2007; Ali et al., 2012). Also, lack of biomarkers to predict therapy outcome in experimental models (and humans) was considered as a major limiting factor for current AEG studies. It was considered of utmost importance to generate funding instruments to gain support for solving these problems.

Future recommendations

The *ILAE/AES Working Group on “Issues related to the development of antiepileptogenic therapies”* considered it important that the future development of AEG treatments for pediatric epilepsies does not rely on AEG development in adults. Therefore, the recommendation is to setup a special working group to address preclinical AEG trials in immature animals. Second, considering the cost and the need for special skills and standardized methodologies, it might be time to initiate a discussion about whether AEG testing should be centralized or whether it should continue to be done in multiple different academic and industry laboratories.

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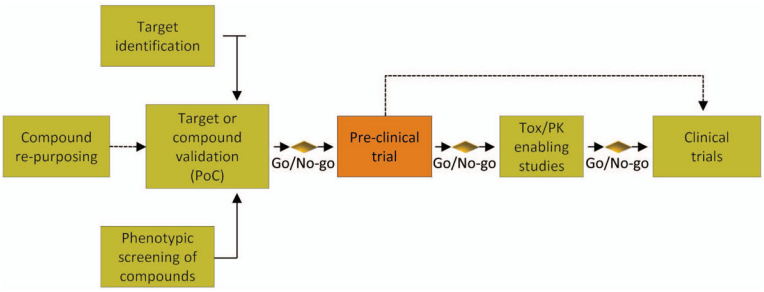

Figure 1.
Different phases of pre-clinical drug discovery. “Compound repurposing” refers to studying small molecules approved to treat other diseases or conditions whether they would be safe and effective antiepileptogenic drugs. Abbreviations: PK, pharmacokinetic studies; PoC, proof-of-concept study; Tox, toxicological studies.

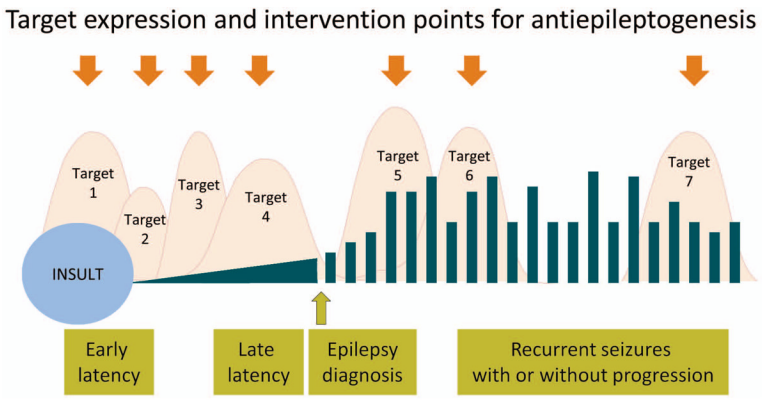

Figure 2.

Schematic graph demonstrating the epileptogenic process and points for antiepileptogenic interventions. Different targets can be expressed, depending on the stage of the epileptogenic process. Target expression can vary in magnitude and duration, and there can be temporal overlap in their expression. Note that as epileptogenesis continues even after epilepsy diagnosis, antiepileptogenic treatments can be started even after the appearance of spontaneous seizures.

Table 1

Key features of a preclinical antiepileptogenesis (AEG) monotherapy trial in adult rodents.

Study-design

- single center
- blinded
- placebo-controlled
- statistically powered

Animals

- adult rats (either gender)

Experimental model

- selection of a model appropriate for the syndrome targeted by the tested treatment

Number of studies

- 1st study: pre-clinical study to show efficacy
- 2nd study: a replication study in a different laboratory guided by data from the first study in a given model
- 3rd study: testing in another model of the same syndrome before advancing to clinic

Timing, dosing and duration of treatment (if target is known)

- based on target relevance
- evidence for exposure and engagement of the target by the treatment should be provided

Outcome measures

- primary outcome measures
  - seizure frequency
  - percentage of animals seizure free during the period of seizure monitoring
- secondary outcome measures
  - seizure duration
  - seizure type

Statistics

- predefined
- inclusion of statistician into the team from the beginning

Reporting

- both positive and negative outcome

Table 2

Specific requirements for pre-clinical antiepileptogenesis (AEG) trial in immature animals.

Factors affecting target expression

- Different ratio between neurons and astrocytes
- Developmental differences in the expression and function of proteins, enzymes, receptors, ion channels, transporters
- Developmental differences in rates of neurogenesis and apoptosis, as well as in the effects of brain injury on these events
- Different resistance to changes in cell environment that may accompany seizures (e.g., hypoxia/anoxia)
- Shift from predominantly anaerobic to aerobic metabolism with progressive maturation of the enzymes of energy metabolism, the active synthesis of amino acid neurotransmitters and the compartmentalization of glutamate metabolism implying active exchanges of glutamate/glutamine/GABA between astrocytes and neurons
- Age-related differences in the effects of seizures, precipitating events, and their treatments on the processes implicated in epileptogenesis

Factors affecting drug dosing and duration of treatment

- Different permeability of the BBB and hence access to the brain
- Different distribution within the brain (ratio lipids/water lower)
- Different pharmacokinetics related to the immaturity of the hepatic enzymes of drug metabolism

Factors affecting selection and interpretation of outcome measures

- EEG recordings may not be possible with current technology in newborn rodents (especially mice) due to size and fragility of skull.
- Prolonged and continuous classical video-EEG recordings on the same animal are not possible in developing animals due to growing size of skull/brain and the need for the pups to be cared by the dam. Alternative solutions may affect the design of study, power analysis, and interpretation of results.
- Developmental differences in the connectivity and function of cortical and subcortical networks involved in seizure control and their interactions with other systems (e.g., endocrine and immune systems)
- Age-specific expression of early life seizures or developmental differences in the evolution of the phenotype of early life epileptic syndromes may need to be accounted for in the design and interpretation of AEG studies in immature animals.