Identification and characterization of outcome measures reported in animal models of epilepsy. Protocol for a systematic review of the literature.

Report of the TASK2 group of the AES/ILAE Translational Task Force

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Abstract

Current anti-seizure therapy is ineffective in approximately one-third of people with epilepsy and is often associated with substantial side effects. In addition, most current therapeutic paradigms offer treatment, but not cure, and no therapies are able to modify the underlying disease, that is can prevent or halt the process of epileptogenesis or alleviate the cognitive and psychiatric comorbidities. Preclinical research in the field of epilepsy has been extensive, but unfortunately, not all the animal models being used have been validated for their predictive value. The overall goal of TASK 2 of the AES/ILAE Translational Task Force is to organize and coordinate systematic reviews on selected topics regarding animal research in epilepsy. Here we describe our strategy. In the first part of the paper we provide an overview of the usefulness of systematic reviews and meta-analysis for preclinical research and explain the essentials for their conduct. Then, we describe in detail the protocol for a first systematic review, which will focus on the identification and characterization of outcome measures reported in animal models of epilepsy. The specific goals of this study are to define systematically the phenotypic characteristics of the most commonly used animal models, and to effectively compare these with the manifestations of human epilepsy. This will provide epilepsy researchers with detailed information on the strengths and weaknesses of epilepsy models, facilitating their refinement and the future research. Ultimately, this could lead to a refined use of relevant models for understanding the mechanism(s) of the epilepsies and developing novel therapies.

Key Words

Systematic reviews, Meta-analysis, Animal models

Key Point Box

- Systematic reviews provide a scientific approach to the collection, grading and interpretation of large volumes of data.
- A goal of the AES/ILAE Translational Task Force is to organize and coordinate systematic reviews regarding animal research in epilepsy.
- The first systematic review will focus on the characterization of outcome measures reported in animal models of epilepsy.
- This article describes in detail the protocol for this systematic review.

Introduction: systematic reviews and meta-analyses

Historically, the medium of scientific communication limited the access of researchers to data. Now the opposite holds: the internet and electronic citation repositories allow access to huge amounts of data that in most circumstances cannot be assimilated by a single individual. As each scientist has to be extremely selective in their reading, there is a high risk of acquisition bias. Moreover, the statistical power of single studies in basic and preclinical research is very often insufficient to draw solid conclusions, which may therefore be either falsely positive or negative. False negatives mean potentially valuable lines of research are shut down prematurely while false positives inflate our appreciation of the effect size.[1; 2] Widespread publication bias can also lead to a false impression of the potential value of an area of research.[3] This is especially so because positive results are more likely to be published in high impact journals and high impact journals are, by definition, more likely to be cited.

These problems have been addressed in clinical research by means of systematic reviews of the literature and meta-analysis of the data (**Figure 1**). Systematic reviews provide a scientific approach to the collection, grading and interpretation of large volumes of data. Detailing the search strategy used to explore the literature and defining inclusion and exclusion criteria allows readers to judge for themselves whether the writers have taken a rigorous approach to finding relevant data, providing a critical element of science: a defined methodology which allows others to confirm and extend the results. Meta-analysis allows aggregating and re-analyzing the data from systematic reviews. This can provide greater statistical power, leading to the discovery of effects that were not evident within single data sets, and can lessen the risk of chance associations (false positives).

In basic and preclinical research, the breadth of available data and heterogeneity of study design necessitate a different approach to the systematic review and meta-analysis process employed in clinical science. Assessments of risk of bias and estimation of the effect size for a single intervention can still be made, but the heterogeneity of design also facilitates study of the methodologies employed to model disease and of the underlying biological variables that influence outcome.[4-10]

Conduct of systematic reviews and meta-analyses on animal research

Protocol. The prospective registration of protocols is recognized as an important part of the conduct of systematic reviews in the clinical sciences. Establishing a protocol for the research we intend to conduct serves a number of important functions that reduce waste and minimizes the risk of biases. If protocols are registered in an open database, potential authors can determine if another group has already initiated a systematic review on the same topic and decide whether to proceed with their review. Prospective registration also minimizes the potential for publication bias by maintaining a permanent record of initiated reviews, regardless of publication status. Finally, by describing *a priori* the analyses to be performed, a protocol protects against "HARKing" (Hypothesizing After Results are Known). In this regard, PROSPERO (http://www.crd.york.ac.uk/PROSPERO/) is an international prospective registry for systematic review protocols in human health. Expanding PROSPERO's scope to include systematic reviews of preclinical studies will provide authors with a central source for registering and searching for protocols. In addition to such registration it is appropriate for protocols to be published in peer-reviewed journals to provide more details on the background/rationale and methods.

Search strategy. A comprehensive search strategy is essential to identify all studies relevant to a particular topic. The first steps in designing a comprehensive search strategy are: (i) translating the review questions into clear and simple ones, (ii) defining search components and (iii) building comprehensive search strategies which identify intersections of search components. SYRCLE (https://www.radboudumc.nl/Research/Organisationofresearch/Departments/cdl/SYRCLE/Pages/default.aspx) has developed several tools to specifically facilitate the search process for animal-based studies.[11-13]

One of the greatest challenges in designing a comprehensive search is to find the right balance between sensitivity and specificity. Sensitivity is the proportion of relevant articles identified by a search strategy as a percentage of all relevant articles on a given topic. It is a measure of the ability of a search strategy to identify all relevant articles. Specificity is the proportion of relevant articles identified by a search strategy as a percentage of all articles (relevant and irrelevant) identified by that search. In other words, it is a measure of the ability of a search strategy to exclude irrelevant articles. Searches with a high sensitivity often result in relatively low specificity and vice versa. To increase

sensitivity of the search, the Cochrane handbook for systematic reviews of interventions [14] suggests using multiple databases in a search. However, while clinical researchers have begun to grapple with the issues of coverage of different databases,[15-18] very little is known about the effect of search strategy, and database selection in particular, on the validity of systematic reviews in the context of animal studies.[19]

Approaches to statistical analysis. Different approaches can be used to pool data from individual studies and provide summary estimates of effect. While the fundamental principles are the same as for meta-analyses of human clinical trial data, some important differences have to be considered in animal research. Clinical research reviews are usually based on a small number of studies involving large cohorts of patients, investigating a fairly homogenous treatment effect. In contrast, animal research reviews often involve a large number of studies, each with a small number of cases and, in general, with substantial heterogeneity in the circumstances of testing (species, dose, timing of treatment, outcome assessed). While the purpose of clinical meta-analyses is usually to produce a better estimate of the treatment effect, such an estimate (e.g., improvement of drug outcome in a particular disease) has generally little meaning in animal studies. Rather, what is important are the associations between different aspects of experimental design and the observed effects, which might define the limits to efficacy; circumstances in which efficacy is not observed; the prevalence and impact of publication bias; and the impact of reporting of other risks of bias. Such an approach helps to define the reaction norms of the biological response.

Effect size may be represented as (i) a mean difference (i.e., all outcomes use the same scale); (ii) a standardized mean difference (i.e., the effect is scaled according to the observed variance); (iii) a normalized mean difference (i.e., the effect is scaled as a proportionate improvement in outcome). Because animal studies rarely use the same scale and because the observed variance is an imprecise measure of the population variance, a normalized mean difference (NMD) approach is often used. When this is not possible, analyses based on standardized mean difference (SMD) can be used, but have lower statistical power because of the measurement error in the estimation of effect sizes.

Sources of heterogeneity between different studies may be assessed through partitioning of the observed heterogeneity (i.e., the weighted sum of the squared deviations from the fixed effects estimate) into that occurring within groups and that occurring between groups. Alternatively, meta-regression seeks to build a univariate or multivariate regression equation, which minimizes the weighted squared deviations from the model. While both of these approaches have strengths, modeling

suggests that partitioning of heterogeneity is much less conservative, in statistical terms, than meta-regression (http://www.dcn.ed.ac.uk/camarades/files/CAMARADES%20Monograph%201.pdf).

In this project we will use the partitioning of heterogeneity to summarize SMD effect sizes and metaregression when NMD effect sizes are used.

Publication standards and meta-bias. As with any type of research, systematic reviews and meta-analyses are susceptible to bias. It was found in a systematic review of systematic reviews of preclinical studies that 30% specified a testable hypothesis, 27% performed a literature search without language restrictions, 17% assessed for the presence of publication bias, 50% assessed study validity, and 2% investigated sources of heterogeneity.[20] It is only through clear reporting of what was done that it is possible to assess the risk of bias. The potential users of the systematic reviews need to be able to assess whether the methodologies are sound and interpretations valid.

Protocol for a systematic review on animal research in epilepsy

There are many different topics of interest in pre-clinical epilepsy research, which are worthy subjects for systematic reviews and meta-analysis. As a start, our working group agreed that it would be relevant to identify and characterize the outcome measures that are the most frequently used to evaluate outcome in animal models of epilepsy. These are amongst the most important "tools of our trade" and it is critical to how they behave in different models of our disease and to understand which are fit for the task. This broad analysis will provide the foundations for more sharply focused reviews. Once we know which of our assessment tools are effective we can more effectively probe the relevance of our broad range of epilepsy and seizure models and the relative merits of individual corrective drugs.

Below, we provide a detailed protocol of this initial work, that has already been registered in the CAMARADES website (http://www.dcn.ed.ac.uk/camarades/files/epilepsy%20models%20protocol%20final.pdf).

Background

Epilepsy is one of the most common neurological diseases. It affects an estimated 1% of the population, i.e. over 50 million individuals worldwide. Approximately one in 25 people will develop epilepsy at some point in their lifetime and it is estimated that 2.4 million people are newly diagnosed with epilepsy each year (http://www.who.int/mediacentre/factsheets/fs999/en/).[21] In addition, neurological and psychiatric comorbidities heavily contribute to the disability of this population of patients. Since the introduction of the bromides as an anti-seizure drug almost two centuries ago, many effective therapies have been tested and introduced in clinical practice. These treatments have been referred to as ‘‘antiepileptic drugs’’ (AEDs), and about 20 of them have been developed during the last 30 years. However, all AEDs are symptomatic agents that, at best, control the most obvious manifestation of the disease, i.e. seizures. For this reason, the term “anti-seizure drugs” (ASDs) is now preferred.[22] However, seizures are still not adequately controlled in a third of the cases, ASDs often have side effects and no disease-modifying therapies (which remove the susceptibility to seizures) are yet available. Moreover, there are no therapies that specifically address the comorbidities of epilepsy. Therefore, an urgent demand exists to address these unmet clinical needs.[23; 24]

Preclinical research in epilepsy has strongly facilitated the discovery of ASDs with different mechanisms of action. Traditionally, these new therapies have been identified based on effects in preventing chemically (pentylenetetrazol, PTZ) or electrically (maximal electroshock, MES) induced acute seizures in otherwise normal animals or, more recently, in slowing kindling progression or increasing seizure threshold in fully kindled animals. These tests have been performed by individual research teams, by companies, or by the Epilepsy Therapy Screening Program (previously named the Anticonvulsant Screening Program, ASP), an initiative of the National Institute for Neurological Diseases and Stroke (NINDS). Some of the new ASDs introduced to the clinic as a result of this approach proved more tolerable, but the percentage of patients with drug-resistant epilepsy has not changed, nor have these new drugs proved effective in preventing epilepsy development in at-risk individuals or in treating co-morbidities.[25] Probably because testing for tolerability and safety has been done in acute (and not chronic) epilepsy models, current screening methods have often failed to elucidate which drugs will produce significant adverse effects. Additional models (such as 6 Hz and *status epilepticus* models) have been proposed as additions to the screening, but these have not been yet fully validated for their predictiveness of therapeutic effects in human disease.[24] In addition, the

similarities of their characteristics with the human symptoms (i.e., their face validity) has been questioned.[26; 27]

In summary, despite its important achievements, epilepsy therapy development still needs to address major existing unmet clinical gaps. To meet these demands, redesign of current translational approaches is needed. The aims of this systematic review are to define the phenotypic features of some commonly used models, in order to effectively compare these to the aspects of the human clinical condition they are intended to model. This will provide epilepsy researchers with detailed information of the most commonly used models for future research and also give ideas on how the existing models could be improved. Ultimately, this could lead to a refined use of relevant models for understanding the mechanism(s) of the epilepsies and identifying novel therapies. To achieve these goals, (1) we created a list of animal models of induced epilepsy (**Table 1**) based on a report of the United Kingdom's National Centre for Replacement, Refinement and Reduction of Animals in Research (NC3Rs) epilepsy working group[28] and on a preliminary screening of the literature run by the authors; the list will be further refined following a more detailed screening to identify how many articles are published per model and select a more limited group of representative models to examine in detail; (2) we identified relevant outcome measures that would allow ascertainment of the impact of therapeutic interventions (**Table 2**).

Objectives of the Systematic Review and Meta-Analysis

As described above, animal studies have been instrumental in the identification of new effective ASDs. The species that proved most useful in this respect were small rodents (mice and rats), but much valuable data has also been obtained in other species ranging from fish (zebrafish) to primates. In this study, we will collect and examine results obtained in mammalian species. Non-mammalian species will be excluded only because the phenotypical presentation of the disease in these species is far less complex than in humans, preventing an in-depth comparison.

Acquired epilepsies in humans are often caused by an initial epileptogenic insult (head trauma, episode of status epilepticus, stroke, brain infection, hyperthermia) that, after a latency period, may lead to the occurrence of spontaneous seizures and the diagnosis of epilepsy. All these epileptogenic insults can be reproduced in animals and, as in humans, may lead to spontaneous seizures after a latency period. Therefore, in this study we will consider all interventions intended by the authors to evoke acquired epilepsy. Interventions intended to induce a single seizure only will not be included.

Since this is not a therapeutic intervention, we will use here the term "epileptogenic insult". Data from animals not receiving the epileptogenic insult (sham-animals or animals receiving vehicle rather than epileptogenic drug or insult) will serve as the control population. We will categorize all reported outcomes as electrophysiological (EEG), behavioral, histological or imaging. Detailed analysis will focus on specific outcome measures selected on the basis of a survey among preclinical and clinical epilepsy experts (**Table 2**). The definitions and classifications of seizures are currently being developed by the TASK 1 of the AES/ILAE Translational Task Force, and are reported in detail in other articles of this supplement.

Specifically, our research questions are: (1) What outcome measures are commonly reported in animal experiments modeling epilepsy? (2) To what extent do the changes associated with model induction reflect human epilepsy (face validity)? (3) What is the statistical performance (power to detect a given effect size) of different outcome measures, and does this differ between models?
The protocol below describes the methodology we plan to use to pursue an answer to these questions.

Methods

Search strategy

Databases to search. Based on previous experience, we chose to search Pubmed and Embase databases.

Electronic search strategies. We will run the following search: [animal study string [12; 13] AND [all chronic models (named one by one)] AND [epilepsy]. The animal study strings are reported in the **Appendix**.

Study selection procedures

Screening phases. Pubmed and Embase search results will be downloaded to EndNote, and full text of articles retrieved when available using the automated EndNote feature (not available articles will be obtained via interlibrary loan or direct contact with authors). Unique results will be exported from Endnote as an XML file and uploaded into the SyRF application (http://app.syrf.org.uk/). Screening for inclusion/exclusion of titles and abstracts will be performed against the criteria below. Publications passing stage 1 will proceed to stage 2 according to the criteria below. The SyRF application allows that each screener is offered each record only once; and records are offered for screening until 2 reviewers agree on disposal (inclusion or exclusion).

Study selection criteria

Inclusion and exclusion criteria. Inclusion: controlled studies comparing outcomes between a group of animals in which the epileptogenic insult has been induced and a group of animals not receiving that epileptogenic insult. Exclusion: acute seizure models; studies on transgenic and knockout mice; drug efficacy studies where outcomes are compared only in cohorts which have received an intervention intended to model epilepsy (no non-epileptic control); publications that do not contain primary data (i.e. review articles).

Type of intervention. Any epileptogenic insult intended to induce a model of epilepsy (i.e. chronically reduced seizure threshold or induce spontaneous recurrent seizures or both).

Language restrictions. Only papers in English.

Publication date restrictions. None.

Exclusion criteria per selection phase. Stage 1. This will be a screening phase based only on title and abstract. Inclusion criteria: i) papers written in English; ii) concerning the induced epilepsy models listed in **Table 1**; iii) using mammalian species. Exclusion criteria: i) paper not written in English; ii) not concerning the induced epilepsy models listed in Table 2; iii) using non-mammalian species. Stage 2. This will be the extraction phase, in which information will be extracted from the full text. The outcome measure and the mode of information extraction are listed in **Table 2** and below ("Study characteristics to be extracted"). The reason for an extensive list of outcomes in Table 2 is proposed in an attempt to reduce a priori preconceptions of what outcomes are important, and we will employ statistical methods to account for multiplicity of comparisons.

Study characteristics to be extracted

All study characteristics will be extracted by two independent reviewers with discrepancies resolved by a third reviewer.

Study ID. Unique Study identifiers will be generated automatically for each article and will be linked to the basic information of the publication extracted from the online searching engines.

Study design characteristics (e.g. experimental groups, number of animals). Number of animals per experiment and per experimental cohort.

Animal model characteristics. For each experiment, and for each experimental cohort:

1. species, strain and where available breeder;
2. gender;
3. age (or weight as a surrogate if age is not given);
4. housing (singly- vs. group-housed).

Intervention characteristics. For each experiment, and for each experimental cohort:

1. method of induction of epilepsy;
2. timing, and, when appropriate, number and intensity of induction events (i.e. drug dose, electrical stimulation parameters, amount of infective agent, etc.).

Outcome measures. For each experiment:

1. category of outcome measure (see **Table 2**);
2. timing(s) of outcome assessment.

For each experimental cohort:

1. median or mean outcome at each time point;
2. variance or IQR of mean or median outcome.

Assessment risk of bias (internal validity) or study quality

Number of reviewers assessing the risk of bias/study quality in each study and resolution of discrepancies. Two reviewers will be assigned to assess the risk of bias and study quality. Discrepancies will be resolved by a third screener.

Criteria to assess the internal validity of included studies and/or other study quality measures. The study quality checklist described by CAMARADES[29] study will be adapted as follows:

- publication in peer-reviewed journal;
- random allocation to group;
- blinded conduct of the experiment;
- blinded assessment of outcome;
- sample size calculation;
- reporting of animals excluded from analysis and reasons for exclusion (e.g. health status, general conditions or other parameters);
- reported health status & general condition;
- monitoring duration longer than 1 week (for adult animal models);
- continuous (vs. discontinuous) monitoring (for adult animal models);
- video-EEG monitoring;

- information about the course of spontaneous recurrent seizures (i.e. progression / regression / remission).

Collection of outcome data

Type of data to be extracted for each outcome measure. See **Table 2**.

Methods for data extraction/retrieval. Multi-modal:

1. from text;
2. from graphs using a digital ruler software.

Data analysis and synthesis

How will data be combined/compared. Meta-analysis.

How it will be decided whether a meta-analysis will be performed. Meta-analysis will be performed where more than 10 experimental comparisons are available.

Effect measure. For dichotomous outcomes we will present odds ratios. For continuous outcomes we will use standardized mean difference.

Statistical model of analysis. Random effects meta-analysis.

Statistical methods to assess heterogeneity. I^2 and Q.

Which study characteristics will be examined as potential source of heterogeneity (subgroup analysis). Species; age at intervention; gender; housing (single vs. group; enrichment); time to outcome assessment; randomization, blinding.

Sensitivity analyses. Meta-regression.

Other meta-analysis details. To adjust for multiple testing of study design (n=4) and risk of bias (n=5) items we will use a Bonferroni-Holm correction.

Method to assess for risk of publication bias. Risk of publication bias analyses will be assessed using funnel plot assessment and Egger's regression. Trim and fill analysis will be used to assess the potential impact of publication bias.

Concluding remarks

As stated, we expect that the protocol outlined in this article will lead to a series of systematic reviews and meta-analyses on preclinical epilepsy research. Once we have completed the analysis on the general features of the models and of their similarities to the human epilepsy condition, the following obvious step will be to analyze the effects of FDA- and EMA-approved drugs using the same approach.

This study is expected to provide concrete evidence to inform attempts to improve the currently available models of chronic epilepsy and the conduct of preclinical epilepsy research. In addition, we believe that this will help providing a more realistic and translationally useable view of the data generated by preclinical epilepsy research, identifying areas that need further exploration and providing more solid bases for the initiation and design of clinical studies.

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expressed by the authors, however, do not necessarily represent the policy or position of the ILAE or AES.

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We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Tables

Table 1. Models of induced epilepsy

Electrical	Chemical	Physical
Electrically-induced status epilepticus (amygdala stimulation, perforant path stimulation)	Pilocarpine Lithium-pilocarpine	Traumatic brain injury (fluid percussion, controlled cortical impact) Penetrating brain injury
Kindling (corneal, hippocampal, amygdaloidal, PTZ)	Kainic acid (intraamygdala, intrahippocampal, intracerebroventricular, intraperitoneal, subcutaneous)	Hypoxia Hypoxic-ischemic encephalopathy Hyperthermia
	Tetanus toxin	Osmotic blood-brain barrier disruption
	Bicuculline intrahippocampal	Stroke
	Penicillin cortical	Albumin
	Ferric salts (intraamygdala or intraneocortex)	Prenatal teratogen, maternal stress and teratogen model of autism and epilepsy
		Neurocysticercosis
		Viral encephalitis (Theiler's murine encephalomyelitis virus model)
	Cobalt cortical	Multiple hit model of infantile spasms
		Tetrodotoxin model of spasm
		Undercut

Table 2. Outcome measures

Outcome	Scale	Measure
EEG		
Percent of tested animals with seizures	Ordinal	Quantitative measure: %
Frequency of spontaneous seizures	Ordinal	Quantitative measure: seizures/day
Duration of spontaneous seizures Cumulative duration of seizure per EEG session (time spent seizing)	Ordinal	Quantitative measure: sec
Frequency of interictal spikes	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
High frequency oscillations	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
Seizure threshold	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
Seizure spread	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
Behavior - seizures		
Percent of tested animals with seizures	Ordinal	Quantitative measure: %
Semiology of seizures (different types)	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
Frequency of convulsive seizures	Ordinal	Quantitative measure: seizures/day
Duration of convulsive seizures	Ordinal	Quantitative measure: sec
Severity of convulsive seizures	Nominal	Quantitative measure: Racine scale (specify the Racine scale variant employed in the study)
Post-ictal behavior (e.g. postictal depression or alterations in behavior following seizures)	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
Seizure threshold	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
Behavior - co-morbidities		
Anxiety- and depression-associated behavior		
Open field	Ordinal	Quantitative measure: entries in the central quadrants (number/min).
Elevated plus maze	Ordinal	Quantitative measure: entries in the open arms (number/min).
T maze	Ordinal	Quantitative measure: number of correct choices (% correct).
Forced swimming	Ordinal	Quantitative measure: immobility (% total time of observation).

Light/dark (black/white) box	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
Glucose preference	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
Weight change	Ordinal	Quantitative measure: weight gain per week.
Cognitive impairment		
Novel object recognition	Ordinal	Quantitative measure: % time exploring novel object.
Morris water maze	Ordinal	Quantitative measure: % time in target quadrant in the probe trial.
Barnes Maze	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
Nesting behavior	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
Autism		
Ultrasonic vocalization (USV)	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
Social exploration/interaction	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
Repetitive behavior	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
Sleep impairment		
Sleep EEG	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
Histological		
Cell death		
Hippocampal volume	Ordinal	Quantitative measure: % control volume.
Cell counting	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
Neuronal loss	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
Fluoro-Jade	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
TUNEL	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
Neurogenesis		
BrDU, Ki67, etc	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
Gliosis		
GFAP	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
Microgliosis		

Iba1, Cd11b	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
Neuroinflammation		
Cytokines, cyclooxygenase-2, …	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
Alterations in organization and morphology		
Granule cell dispersion	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
Sprouting of the mossy fibers (Timm, ZnT3, dynorphin)	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
Alterations in dendritic arborization	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
Blood-brain barrier integrity		
Albumin, IgG, …	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
Imaging		
MRI	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)
PET	Dichotomous	Qualitative measure: yes/no (just note if evaluated in the paper)

List of abbreviations

AED, antiepileptic drugs

ASD, anti-seizure drugs

MES, maximal electroshock

NMD, normalized mean difference

PTZ, pentylenetetrazol

SMD, standardized mean difference

USV, ultrasonic vocalization

Figure legends

Figure 1. The process of systematic reviews and meta-analyses. White circles represent individual papers containing different sets of data (circles, squares, hexagons, stars). Data that are relevant for the study (represented as blue squares) are extracted and combined in a meta-analysis.

Appendix

Animal study search string for Pubmed [12]

(“animal experimentation”[MeSH Terms] OR “models, animal”[MeSH Terms] OR “invertebrates”[MeSH Terms] OR “Animals”[Mesh:noexp] OR “animal population groups”[MeSH Terms] OR “chordata”[MeSH Terms:noexp] OR “chordata, nonvertebrate”[MeSH Terms] OR “vertebrates”[MeSH Terms:noexp] OR “amphibians”[MeSH Terms] OR “birds”[MeSH Terms] OR “fishes”[MeSH Terms] OR “reptiles”[MeSH Terms] OR “mammals”[MeSH Terms:noexp] OR “primates”[MeSH Terms:noexp] OR “artiodactyla”[MeSH Terms] OR “carnivora”[MeSH Terms] OR “cetacea”[MeSH Terms] OR “chiroptera”[MeSH Terms] OR “elephants”[MeSH Terms] OR “hyraxes”[MeSH Terms] OR “insectivora”[MeSH Terms] OR “lagomorpha”[MeSH Terms] OR “marsupialia”[MeSH Terms] OR “monotremata”[MeSH Terms] OR “perissodactyla”[MeSH Terms] OR “rodentia”[MeSH Terms] OR “scandentia”[MeSH Terms] OR “sirenia”[MeSH Terms] OR “xenarthra”[MeSH Terms] OR “haplorhini”[MeSH Terms:noexp] OR “strepsirhini”[MeSH Terms] OR “platyrrhini”[MeSH Terms] OR “tarsii”[MeSH Terms] OR “catarrhini”[MeSH Terms:noexp] OR “cercopithecidae”[MeSH Terms] OR “hylobatidae”[MeSH Terms] OR “hominidae”[MeSH Terms:noexp] OR “gorilla gorilla”[MeSH Terms] OR “pan paniscus”[MeSH Terms] OR “pan troglodytes”[MeSH Terms] OR “pongo pygmaeus”[MeSH Terms]) OR ((animals[tiab] OR animal[tiab] OR mice[Tiab] OR mus[Tiab] OR mouse[Tiab] OR murine[Tiab] OR woodmouse[tiab] OR rats[Tiab] OR rat[Tiab] OR murinae[Tiab] OR muridae[Tiab] OR cottonrat[tiab] OR cottonrats[tiab] OR hamster[tiab] OR hamsters[tiab] OR cricetinae[tiab] OR rodentia[Tiab] OR rodent[Tiab] OR rodents[Tiab] OR pigs[Tiab] OR pig[Tiab] OR swine[tiab] OR swines[tiab] OR piglets[tiab] OR piglet[tiab] OR boar[tiab] OR boars[tiab] OR “sus scrofa”[tiab] OR ferrets[tiab] OR ferret[tiab] OR polecat[tiab] OR polecats[tiab] OR “mustela putorius”[tiab] OR “guinea pigs”[Tiab] OR “guinea pig”[Tiab] OR cavia[Tiab] OR callithrix[Tiab] OR marmoset[Tiab] OR marmosets[Tiab] OR cebuella[Tiab] OR hapale[Tiab] OR octodon[Tiab] OR chinchilla[Tiab] OR chinchillas[Tiab] OR gerbillinae[Tiab] OR gerbil[Tiab] OR gerbils[Tiab] OR jird[Tiab] OR jirds[Tiab] OR merione[Tiab] OR meriones[Tiab] OR rabbits[Tiab] OR rabbit[Tiab] OR hares[Tiab] OR hare[Tiab] OR diptera[Tiab] OR flies[Tiab] OR fly[Tiab] OR dipteral[Tiab] OR drosophila[Tiab] OR drosophilidae[Tiab] OR cats[Tiab] OR cat[Tiab] OR carus[Tiab] OR felis[Tiab] OR nematoda[Tiab] OR nematode[Tiab] OR nematodes[Tiab] OR sipunculida[Tiab] OR dogs[Tiab] OR dog[Tiab] OR canine[Tiab] OR canines[Tiab] OR canis[Tiab] OR sheep[Tiab] OR sheeps[Tiab] OR mouflon[Tiab] OR mouflons[Tiab] OR ovis[Tiab] OR goats[Tiab] OR goat[Tiab] OR capra[Tiab] OR capras[Tiab] OR rupicapra[Tiab] OR rupicapras[Tiab] OR chamois[Tiab] OR haplorhini[Tiab] OR monkey[Tiab] OR monkeys[Tiab] OR anthropoidea[Tiab] OR anthropoids[Tiab] OR saguinus[Tiab] OR tamarin[Tiab] OR tamarins[Tiab] OR leontopithecus[Tiab] OR hominidae[Tiab] OR ape[Tiab] OR apes[Tiab] OR “pan paniscus”[Tiab] OR bonobo[Tiab] OR bonobos[Tiab] OR “pan troglodytes”[Tiab] OR gibbon[Tiab] OR gibbons[Tiab] OR siamang[Tiab] OR siamangs[Tiab] OR nomascus[Tiab] OR symphalangus[Tiab] OR chimpanzee[Tiab] OR chimpanzees[Tiab] OR prosimian[Tiab] OR prosimians[Tiab] OR “bush baby”[Tiab] OR bush babies[Tiab] OR galagos[Tiab] OR galago[Tiab] OR pongidae[Tiab] OR gorilla[Tiab] OR gorillas[Tiab] OR “pongo pygmaeus”[Tiab] OR orangutan[Tiab] OR orangutans[Tiab] OR lemur[Tiab] OR lemurs[Tiab] OR lemuridae[Tiab] OR horse[Tiab] OR horses[Tiab] OR equus[Tiab] OR cow[Tiab] OR calf[Tiab] OR bull[Tiab] OR chicken[Tiab] OR chickens[Tiab] OR gallus[Tiab] OR quail[Tiab] OR bird[Tiab] OR birds[Tiab] OR quails[Tiab] OR poultry[Tiab] OR poultries[Tiab] OR fowl[Tiab] OR fowls[Tiab] OR reptile[Tiab] OR reptilia[Tiab]

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Animal study search string for Embase [13]

exp animal experiment/ or exp animal model/ or exp experimental animal/ or exp transgenic animal/ or exp male animal/ or exp female animal/ or exp juvenile animal/ OR animal/ OR chordata/ OR vertebrate/ OR tetrapod/ OR exp fish/ OR amniote/ OR exp amphibia/ OR mammal/ OR exp reptile/ OR exp sauropsid/ OR therian/OR exp monotremate/ OR placental mammals/ OR exp marsupial/ OR Euarchontoglires/ OR exp Afrotheria/ OR exp Boreoeutheria/ OR exp Laurasiatheria/ OR exp Xenarthra/ OR primate/ OR exp Dermoptera/ OR exp Glires/ OR exp Scandentia/ OR Haplorhini/ OR exp prosimian/ OR simian/ OR exp tarsiiform/ OR Catarrhini/ OR exp Platyrrhini/ OR ape/ OR exp Cercopithecidae/ OR hominid/ OR exp hylobatidae/ OR exp chimpanzee/ OR exp gorilla/ OR exp orang utan/ OR (animal OR animals OR pisces OR fish OR fishes OR catfish OR catfishes OR sheatfish OR silurus OR arius OR heteropneustes OR clarias OR gariepinus OR fathead minnow OR

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