

Summary

- Report of an expert working group convened to identify opportunities for refining rodent models of epilepsy and seizures.
- Recommendations to give researchers, veterinarians and animal care staff the tools to minimise pain, suffering, distress and lasting harm.
- Practical guidance on model choice, induction procedures, *in vivo* recordings, perioperative care, welfare assessment, humane endpoints, social housing, environmental enrichment, reporting and data sharing.
- Implementation of the recommendations should also improve the quality of animal studies in epilepsy research and maximise the use of animals.

Survey results

The survey enabled the Working Group to obtain an overview of the current areas of epilepsy research, the types of animal model used, their limitations and opportunities to refine adverse effects. Selected results are presented below.

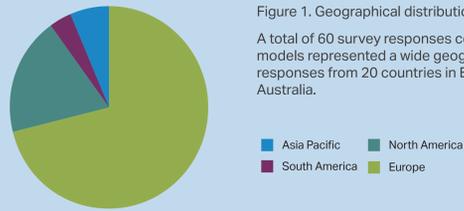


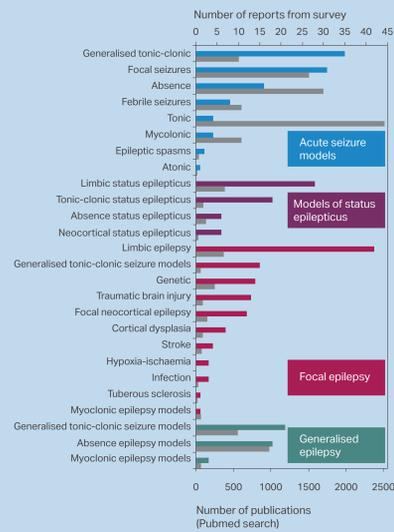
Figure 1. Geographical distribution of survey respondents

A total of 60 survey responses covering a broad range of animal models represented a wide geographical distribution with responses from 20 countries in Europe, North America, Asia and Australia.

Asia Pacific North America
South America Europe

Figure 2. Areas of epilepsy research represented in the survey.

Coloured bars are representative of the number of respondents in the survey conducting research in each area of epilepsy research. Grey bars are representative of the number of publications in the scientific literature based upon a PubMed search.



Data gaps and future research

The Working Group identified a number of research areas where increased knowledge and technological development would facilitate refinement and best practice in the use of animal models of epilepsy and seizures. Some of these areas are detailed below:

Lifetime experience of animal

- Developing improved approaches to understanding the experiences of animals used in the study of epilepsy; in particular, during and in between seizures and following a period of status epilepticus.

In vivo recordings

- Technological advancement of electrophysiological devices with increased biocompatibility and reliability to allow more data to be acquired per animal.
- Automated analysis tools to allow high throughput analysis of EEG data and maximise animal use. Telemetry is generally preferable to tethering for long-term recordings but this technology needs further development for greater miniaturisation, more channels and longer battery life.

Seizure monitoring

- Video analysis tools to assist with the monitoring and classification of spontaneous seizures, to provide important information about seizure frequency and type and to provide the context for the interpretation of data about co-morbidities and welfare issues.

Methodology

The Working Group's recommendations are based upon:

- A systematic review of the scientific literature.
- Survey of the international epilepsy research community
- Consultation with veterinarians and animal care and welfare officers.
- Expert opinion and practical experience of the members of the Working Group.

The Working Group's recommendations were graded according to the levels of evidence defined in Table 1. Recommendations were graded (A-D) according to the highest level of evidence (I-IV).

Figure 3. Limitations on choice of animal model.

Reasons provided by survey respondents in response to the question "Do you consider the following to be limitations on your choice of animal model?"

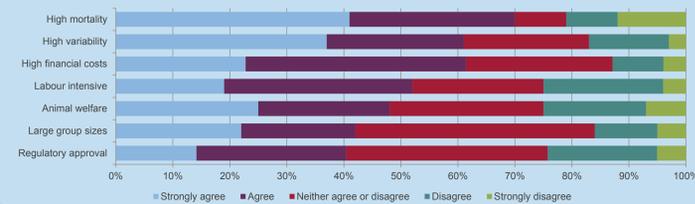


Figure 4. Species of epilepsy models reported in the survey.

Other species includes naturally-occurring epilepsy in cats, dogs, pigeons and pigs.

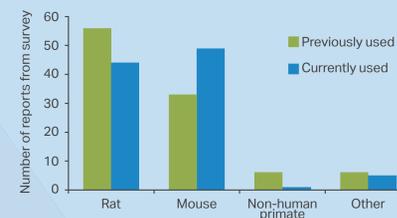


Figure 5. Refinement of adverse effects during induction and/or maintenance of experimental epilepsy.

Steps taken to control and refine adverse effects in response to the question: "How do you control and refine adverse effects?"

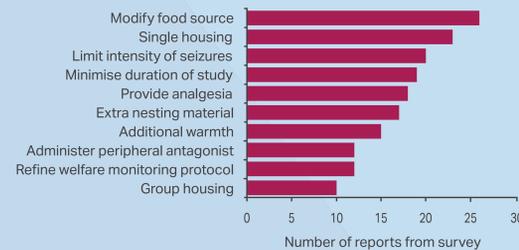


Table 1: Levels of evidence and grades of recommendation

Level of evidence	Type of evidence	Grade of recommendation
I+	Appropriately designed, controlled trials, with a low risk of bias (e.g. objective assessment of the data)	A
I	Appropriately designed, controlled trials	A
II+	Case-control or cohort studies, with a low risk of bias (e.g. objective assessment of the data)	B
II	Case-control or cohort studies	B
III	Case reports, case series	C
IV	Expert opinion, formal consensus	D

Reference

Lidster K, Jefferys JG, Blümcke et al., (2015). Opportunities for improving animal welfare in rodent models of epilepsy and seizures. *Journal of Neuroscience Methods*. In Press. doi:10.1016/j.jneumeth.2015.09.007

Recommendations

The refinement opportunities identified are described in detail in the report (Lidster et al., (2015).

A summary of the recommendations is provided below:

Choice of animal model

- A search of the scientific literature should be carried out to ensure the animal model chosen is scientifically relevant, the least severe model for the scientific purpose, and that any model-specific refinement opportunities are identified (Grade D).
- Assessment of the harms to animals and potential benefits of the research, should take account of the lifetime experience of the animals and the whole epilepsy syndrome (not just seizures) (Grade D).
- Variations in the strain, genetic background, source, age and sex of animals can influence seizure susceptibility and mortality and should be taken into consideration when designing, conducting and reporting studies (Grade A/B).
- Genetic background should be controlled for and appropriate littermate controls with the same genetic background should be used; for example, use age-matched wild-type littermates as controls (Grade A).
- Consideration should be given to using animals of both sexes. If females are used, the impact of the oestrus cycle on seizure susceptibility needs to be considered (Grade A).

Induction procedures

- Procedures leading to the induction of seizures and/or epilepsy should be tailored to reach the scientific objectives effectively whilst minimising harms and mortality (Grade D).
- Research personnel should be adequately trained and competent in the manual skills for appropriate handling and restraint of animals for the administration of substances (Grade A).



In vivo recordings

- The experimental setup should be maximally effective in delivering the research objectives while prioritising animal welfare and minimising interference with behaviour (Grade D).
- Wherever possible, radiotelemetry should be used in preference to tethered systems for chronic electrophysiological recordings (Grade D).
- Radiotelemetry devices should be as light as possible, consistent with the scientific objectives. Consideration should be given to the physiological conformation of the device and its potential impact on posture and natural behaviours (Grade C).
- Good surgical practice and aseptic technique should be used, with pain management, maintenance of body temperature, replenishment of fluids lost under anaesthesia and effective post-operative care and consideration of antibiotic prophylaxis (Grade D).

Perioperative care

- Animals should be allowed sufficient time to recover following surgical procedures using anaesthesia, before subsequent recordings/measurements are taken (Grade A).
- Steps should be taken to identify, assess and alleviate pain following procedures requiring surgery and appropriate pain relief should be provided based on veterinary advice (Grade C).
- Topical antibiotics should be used for simple surgical procedures and prophylactic antibiotics used for implantation procedures if appropriate based on veterinary advice (Grade D).
- A modified food source should be provided to encourage eating and prevent weight loss following surgery and/or seizure induction (Grade A). This should be introduced prior to surgery to ensure familiarization and consumption. Food and drink should be accessible from the floor of the cage (Grade D).

Relevant NC3Rs resources

Procedures with Care:
Practical advice on manual skills required for administration of substances
www.procedureswithcare.org.uk

ARRIVE guidelines
Improve reporting of animal research to ensure research is reproducible
www.nc3rs.org.uk/ARRIVE

Experimental Design Assistant (EDA)
An online tool to guide researchers through designing experiments involving the use of animals.
<http://www.nc3rs.org.uk/experimental-design-assistant-eda>

For further resources please see the resource hub on our website:
www.nc3rs.org.uk/our-resources

Welfare assessment

- Each animal model of epilepsy should be assessed and an appropriate welfare score sheet validated by both animal care staff and the principal investigator. The score sheet should define when action should be taken to minimise pain, suffering and/or distress by intervention/treatment and application of humane endpoints. Such scoring systems should incorporate both monitoring of actual model induction and monitoring of the resulting epileptic state (Grade D).
- Animal welfare assessments should be conducted at a frequency appropriate to the state of well-being and health of the individual animal; at least on a daily basis and multiple times per day in the immediate post-operative recovery period or following specific interventions (Grade D).



Humane endpoints

- A tailored approach should be adopted to assess, define and implement humane endpoints for each experiment in order to minimise harms, whilst allowing achievement of the scientific objectives (Grade C). This should take into consideration the current legal framework, scientific, justifiable and unpredictable endpoints and the results of welfare assessments.

Social housing

- Mice and rats should be socially housed unless there are compelling scientific or animal health reasons for single housing (Grade A).
- Animals should be paired or grouped prior to surgery to increase the social bond, thereby reducing the risk of adverse behaviour towards the operated or instrumented animal(s) (Grade D).
- Socially housed animals should be monitored to identify signs of aggressive behaviour and the consumption of supporting supplementary food intended for the experimental animal (Grade A).

Environmental enrichment

- Environmental enrichment should be provided to allow animals to express naturalistic behaviours unless there is a justified reason to withhold it (Grade A).
- Environmental enrichment should be consistent in the home cage to reduce variability (Grade A). Enrichment protocols should be described carefully and detailed in published manuscripts to reduce inter-laboratory variations



Reporting and data sharing

- Researchers should report their animal studies in accordance with the ARRIVE guidelines. Journals publishing epilepsy and seizure studies should: a) include the guidelines in their Instruction to Authors; b) require authors to submit an ARRIVE checklist with their manuscripts; and c) encourage editors to review the checklist (Grade D).
- Common Data Elements (CDE) should be prepared and used to help standardise the collection of data, including those relevant to animal welfare, and facilitate comparison of results (Grade D).
- Researchers should take advantage of opportunities to make all research studies regardless of their findings openly available to reduce publication bias in epilepsy research (Grade A).
- The refinement opportunities framework should be developed and used for each project as a tool for predicting, recognising and ameliorating suffering and assessing severity in the particular epilepsy model being used (Grade D).