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Opportunities for improving animal welfare in rodent models of epilepsy and seizures☆

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HIGHLIGHTS

- Report of an expert Working Group to identify opportunities for refining rodent models of epilepsy.
- Based upon a survey of epilepsy community, literature review and expert opinion.
- Background information and recommendations provided to improve animal welfare.
- Practical guidance on refinement opportunities (e.g. induction, recordings, perioperative care).

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ABSTRACT

Animal models of epilepsy and seizures, mostly involving mice and rats, are used to understand the pathophysiology of the different forms of epilepsy and their comorbidities, to identify biomarkers, and to discover new antiepileptic drugs and treatments for comorbidities. Such models represent an important area for application of the 3Rs (replacement, reduction and refinement of animal use). This report provides background information and recommendations aimed at minimising pain, suffering and distress in rodent models of epilepsy and seizures in order to improve animal welfare and optimise the quality of studies in this area. The report includes practical guidance on principles of choosing a model, induction procedures, *in vivo* recordings, perioperative care, welfare assessment, humane endpoints, social housing, environmental enrichment, reporting of studies and data sharing. In addition, some model-specific welfare considerations are discussed, and data gaps and areas for further research are identified. The guidance is 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,

☆ Report of a Working Group of the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs).

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and the expert opinion and practical experience of the members of a Working Group convened by the United Kingdom's National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs).

Contents

1. Introduction

1.1. Background

Epilepsy (see Glossary) is one of the most common serious neurological diseases. It affects an estimated 1% of the population, which equates to about 60 million individuals worldwide (England et al., 2012). Epilepsy patients experience debilitating *seizures* (see Glossary) associated with abnormal electrical activity in the brain. Seizures typically last tens of seconds to a few minutes, and usually are separated by prolonged *interictal* periods. Epilepsy does more than cause seizures, however: depending on the type of epilepsy it may be associated with increased mortality (Sillanpää and Shinnar, 2010), transient postictal behavioural changes and a range of comorbidities including cognitive impairments (Inostroza et al., 2011), anxiety (Mazarati et al., 2009; Epps and Weinshenker, 2013) and systemic effects. At least 15 different types of seizures and 30 epilepsy syndromes have been identified across the lifespan (Berg et al., 2010). The diversity of clinical epilepsies has led to classification by the International League Against Epilepsy (Berg and Cross, 2010; Berg and Scheffer, 2011).

There remain many unresolved clinical issues in epilepsy (Baulac and Pitkänen, 2008; Galanopoulou et al., 2012). First, none of the anti-epileptic drugs in clinical use can prevent development of epilepsy in cases where the precipitating epileptogenic event is identifiable. Second, pharmacological therapy remains unsatisfactory: one third of the patients treated with antiepileptic drugs continue to experience seizures. In patients in whom seizures are well controlled, drugs may exert debilitating side effects and, in some cases, refractoriness to their therapeutic effects may develop. Third, disease-modifying therapies are needed: antiepileptic drugs do not prevent the progression of the disease, and there is a lack of therapies that can ameliorate or prevent the associated cognitive, neurological and psychiatric comorbidities, or the epilepsy-related mortality.

There is an increased demand for animal models that adequately recapitulate human epilepsy, to further understand the mechanisms underlying *epileptogenesis* in the different forms of epilepsy (Löscher and Brandt, 2010) and to develop therapies to prevent the epileptogenic process, better treat comorbidities and treat drug resistant epilepsy (Baulac and Pitkänen, 2008; Brooks-Kayal et al., 2013). However, animal models of epilepsy and seizures, mostly involving mice and rats, have the potential to cause pain, suffering, distress and lasting harm[1] to the animals involved. This can be as a consequence of the method of induction of the epilepsy syndrome, comorbidities and associated animal husbandry practices, acute or chronic post-ictal sequelae and instrumentation, and procedures for monitoring epileptiform activity. Hence such models are typically classified in legislation (e.g. the European Directive on the protection of animals used for scientific purposes (2010/63/EU))

[1] Herein referred to as harms.

as moderate or severe procedures; similar regulations apply elsewhere. There is therefore a need for clear guidance on their use and refinement, in order to minimise any suffering, which is important for ethical, scientific, and legal reasons.

1.2. Working group aims and scope

The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) is a scientific organisation established by the United Kingdom (UK) Government in 2004 to lead the discovery and application of new technologies and approaches to replace, reduce and refine the use of animals for scientific purposes. In 2013 the NC3Rs convened an expert Working Group with the following terms of reference:

1. To review and summarise current use of mammalian models of epilepsy.
2. To identify the animal welfare issues.
3. To recommend opportunities for refinement.
4. To assess and balance the benefits of epilepsy research against the harms to the animals involved.
5. To publish the deliberations of the Working Group in a peer-reviewed paper and promote the group's recommendations within the international epilepsy research community.

Membership of the Working Group comprised neuroscientists, neurologists, epileptologists, neuropathologists, neurosurgeons, animal welfare scientists and veterinarians from academia and the pharmaceutical industry, with experience covering a range of epilepsy research areas, including chronic and acute animal models.

It was not within the scope of the Working Group to identify opportunities for replacement of animal models of epilepsy; these have been discussed and published previously (Cunliffe et al., 2014). Nor did the Working Group investigate and assess the predictive value of the various animal models, since this overlaps with the goal of the Joint International League Against Epilepsy (ILAE) and American Epilepsy Society (AES) Translational Research Task Force to optimise and accelerate preclinical anti-epileptic therapy (Galanopoulou et al., 2013; Simonato et al., 2014). Most studies in animal seizure/epilepsy models are in post-weaned animals and there is a natural bias towards these models. The Working Group recognises the impact that age can have on welfare issues and where there is evidence or accepted best practice in pre-weaned animals, it has been included.

1.3. Report audience and contents

The Working Group's report provides background information and recommendations to give researchers, veterinarians, animal care staff, regulators, and local ethical or animal care and use committees the tools to refine the use of animal models of epilepsy and seizures. The focus is rodent models, but many of the principles for refinement apply to other species. General principles are given in Section 3. More detailed refinements for commonly used models are covered in Section 4. We note that many major epilepsy groups already work to high ethical standards; our aim is to promote best practice as widely as possible.

1.4. Methodology

The foundation of the Working Group's report was a literature search conducted using the PubMed and Go3Rs databases and combinations of the keywords listed in Table 1. These keywords were also used to screen for information in the book *Models of Seizures and Epilepsy* (Pitkänen et al., 2006). Titles and abstracts of the papers retrieved from the search were reviewed for relevance. Those not relevant to the aims of the Working Group were excluded. Full text copies of 142 articles published between 1970 and 2014 were obtained and screened for information on adverse effects and refinements in animal models of epilepsy. The quality of the reported studies was assessed according to the criteria in Table 2. A separate, smaller literature search was conducted to examine reporting of the use of analgesia in studies utilising rodent models of epilepsy (see Section 3.4).

A qualitative survey was conducted amongst researchers working with animal models of epilepsy identified from publications in the field. A questionnaire was emailed to 322 researchers in 28 countries. Researchers were invited to participate anonymously in the survey and asked to answer 12 questions (see Appendix 1), with the aim to identify which mammalian models are used in epilepsy research and to define best practice. The survey excluded animals used for the generation of *in vitro* models of epilepsy and seizures. There were three periods of data collection (July 2013, November 2013 and March 2014). A total of 60 survey responses covering a broad range of animal models were returned for analysis. This is a satisfactory response rate for a qualitative survey. The survey responses represented a wide geographical distribution with responses from 20 countries in Europe, North America, South America, Asia and Australia.

In addition to the survey, between February and April 2014, KL conducted interviews with the Named Veterinary Surgeons (NVS) and Named Animal Care and Welfare Officers (NACWO) at four UK universities with research groups using rodent models of epilepsy.

The Working Group's recommendations were graded according to the levels of evidence defined in Table 2, following the approach previously adopted by Prescott et al. (2010). Recommendations were graded (A–D) according to the highest level of evidence (I–IV). Where there is direct supporting evidence, the individual reference and level of evidence is indicated within the recommendation.

Table 1
Keywords used in the literature search relating to possible adverse effects and refinements in animal models of epilepsy.

Epilepsy terms Convulsion; epilepsy; seizure; status epilepticus	
Possible adverse effects	Possible refinements
Aggression; anxiety; dehydration; excessive; fighting; infection; locomotor activity; pain; mortality; weight loss	Analgesia; antibacterial; antibiotics; enrichment; group housing; heating; fluids; food; post-operative care; refinement

Table 2
Levels of evidence and grades of recommendation. Table based upon a scheme proposed previously (Prescott et al., 2010) and adapted from the National Institute for Health and Care Excellence (NICE) guidelines (Eccles and Mason, 2001).

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	
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	
III	Case reports, case series	C
IV	Expert opinion, formal consensus	D

1.5. Limitations of methodology

In general, there is a paucity of published information on both the animal welfare implications of animal models of epilepsy and opportunities for their refinement. Where evidence was lacking, the Working Group based its recommendations on responses from the survey and the expert opinion and practical experience of its members (Level IV evidence).

2. Animal models used in epilepsy research

The survey enabled the Working Group to obtain an overview of the current areas of epilepsy research, the types of animal model used and their limitations.

2.1. Area of epilepsy research

To ensure the results of the survey were representative of the epilepsy research community, researchers were asked in which area(s) of epilepsy research they worked; areas were divided into acute seizures, focal epilepsy, generalised epilepsy, *status epilepticus* (SE) (see Glossary) and genetic models with epilepsy as part of the phenotype. Survey responses were found to be broadly representative of current epilepsy research areas reported in the published literature (up to May 2015), with the exception of a few areas which were under-represented (e.g. tonic seizures) or over-represented (e.g. generalised tonic–clonic seizures and limbic epilepsy) (Fig. 1, Appendix 2).

2.2. Species and models used

Researchers were asked which animal model(s) they currently used and have previously used. A trend towards increased use of mice was identified; increasing from 33% of total reports of animals previously used to 49% of animals currently used (Fig. 2). This is likely to be a reflection of the growing increase in availability of genetically-modified mouse models; almost 400 genes associated with seizure phenotypes in mice are listed on the Jackson Laboratory database (http://www.jax.org/).

A trend towards decreased use of non-human primate models was also identified. Published non-human primate models include the macaque model of mesial TLE with kainic acid injection (Chen et al., 2013), the marmoset model of TLE with pilocarpine injection (Perez-Mendes et al., 2011) and naturally-occurring epileptic baboons (Szabó et al., 2012). Given the serious ethical and animal welfare issues raised by the use of non-human primates[2] in invasive research, their use should be limited to cases where there is strong justification on scientific and/or medical grounds (Prescott, 2010; Bateson et al., 2011).

An overview of the range of animal models reported in the survey, categorised into acute seizure models, chronic models with high propensity for induced seizures or epileptogenesis, and chronic models of epilepsy, is presented in Table 3 (classification devised by Simonato et al., 2014).

Where researchers had ceased to use a particular rodent model, they were asked the reasons for this. In 57% of cases this was due to a change in strategic direction, in 14% due to financial reasons, and in 11% due to lack of translation to the clinic. Other explanations provided included the severity of the model (7%) and its subsequent burden on animal wellbeing, high mortality (3%) and high levels of variability in the model (1%).

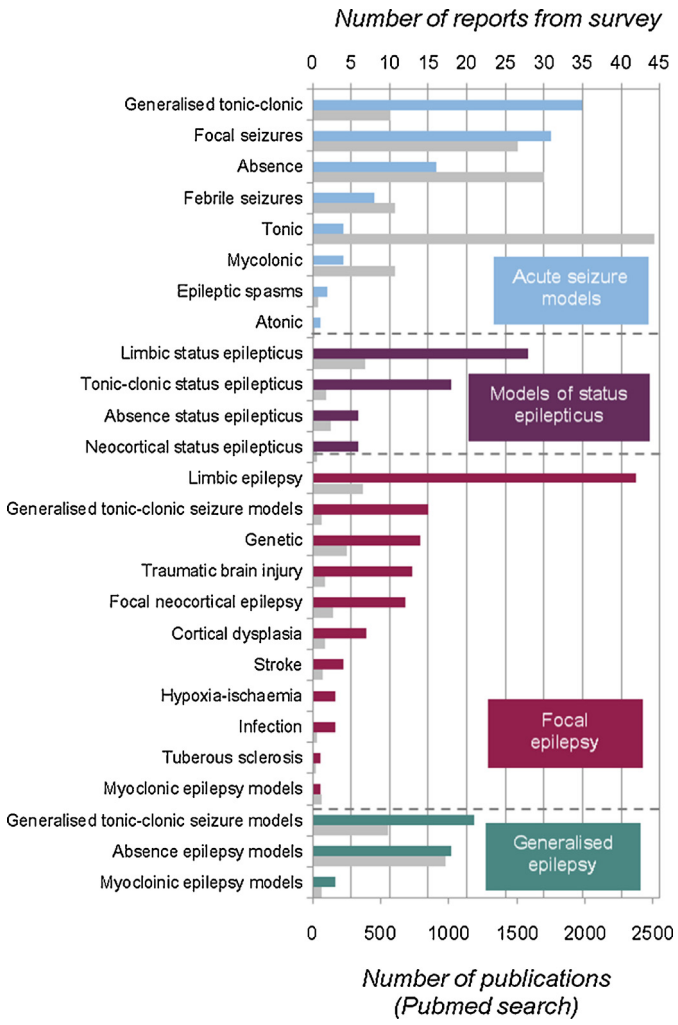

Fig. 1. Areas of epilepsy research represented in the survey. Coloured bars are representative of the number of respondents in the survey conducting research in each area of epilepsy research: acute seizure models (blue), models of status epilepticus (purple), focal epilepsy (red), and generalised epilepsy (green). Grey bars are representative of the number of publications in the scientific literature based upon a PubMed search conducted using search terms detailed in Appendix 2. (The search methods used may result in an overestimation of the number of publications representing a seizure type. For example, search results for "tonic" may result in publications, which the primary aim of the study was not to investigate tonic seizures but "tonic" may have been used in the abstract to describe features of other types of (e.g. generalized tonic–clonic or focal tonic–clonic)). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Researchers were asked the age of animals used. The age range varied according to the model; for example, genetic models were commonly used at very young ages (postnatal day 0 to postnatal day 10). Commonly used induced models, such as kainic acid and pilocarpine, varied from using young animals (postnatal day 10) to adults. The variation in ages reflects the different aspects of epilepsy and seizures being researched and technical considerations (e.g. ease of patch clamping or calcium imaging).

2.3. Choice of animal model

Researchers were asked what factors they considered were limitations on the choice of animal model (summarised in Fig. 3). High mortality rates and variability between animals were the most common limitations, along with high financial cost. Note that many of the limitations are related and have the potential to be addressed

[2] For advice on refining non-human primate use and care, see www.nc3rs.org.uk/welfare-non-human-primates.

Table 3
Animal models reported in survey (table format adapted from Simonato et al., 2014).

Acute seizure models		Chronic models of high propensity for induced seizures or epileptogenesis	
Electrical	Chemical	Genetic	Induced
6 Hz simulation Maximal Electroshock Seizure (MES)	Gamma-hydroxybutyrate (GHB) Pentylenetetrazole (PTZ)	D2R knockout En2 knockout	Kindling (corneal, hippocampal, amygdaloidal, PTZ) Hypoxia and/or ischemia

Models of epilepsy			
Genetic	Induced		
	Electrical	Chemical	CNS insult
5-HT2c knockout DBA/2 mice Genetic Absence Epilepsy Rats from Strasbourg (GAERS) Genetic benign familial neonatal-infantile seizures (BFNI) Genetic generalised epilepsy with febrile seizures (GEFS) Kcna1-null mouse Lethargic LGI1 knockout NaV 1.1 knockout Stargazer Scn1a knockin Sv2a knockout Tottering TGFβ and TGFβR transgenic Wistar Albino Glaxo Rats from Rijswijk (WAG/RiJ)	Amygdala stimulation Perforant path stimulation	Cobalt cortical Kainic acid (intraamygdala, intrahippocampal, intraperitoneal, subcutaneous) Lithium-pilocarpine Pilocarpine Penicillin Tetanus toxin	Albumin Blood brain barrier disruption Fluid percussion (traumatic brain injury) Hypoxia Hypoxic-ischemic encephalopathy Maternal teratogen model of autism and epilepsy Stroke

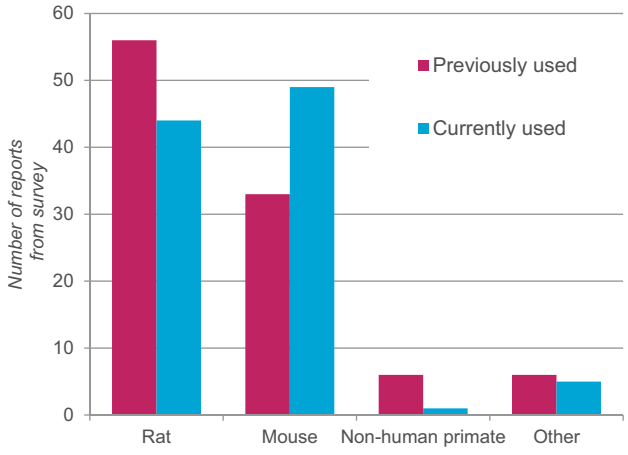

Fig. 2. Species of epilepsy models reported in the survey. Other species includes naturally-occurring epilepsy in cats, dogs, pigeons and pigs.

by taking advantage of the refinement opportunities highlighted in this report.

Researchers were asked "Do you think the limitations of animal models are restricting progress in epilepsy research?" 48% of researchers considered that they were restricting progress, 27% considered that they were not, and 25% were unsure. One reason given for the former view was difficulty in recapitulating specific aspects of the human condition. Re-assessment of translational approaches is currently being undertaken by the ILAE/AES Translational Research Task Force (Galanopoulou et al., 2013; Simonato et al., 2014).

3. General principles for refinement

The literature search, survey and interviews identified a range of refinement opportunities which are described below, supplemented by the expert opinion and professional experience of the Working Group members.

3.1. Considerations in the choice of animal model

The choice of model will depend on the type of epilepsy being modelled, the scientific question being asked, and the need to minimise animal suffering and numbers (Pitkänen et al., 2006; Grone and Baraban, 2015). Models should represent key features of the corresponding disease, but should not necessarily strive to be identical. For instance, testing methods to control seizures may need a

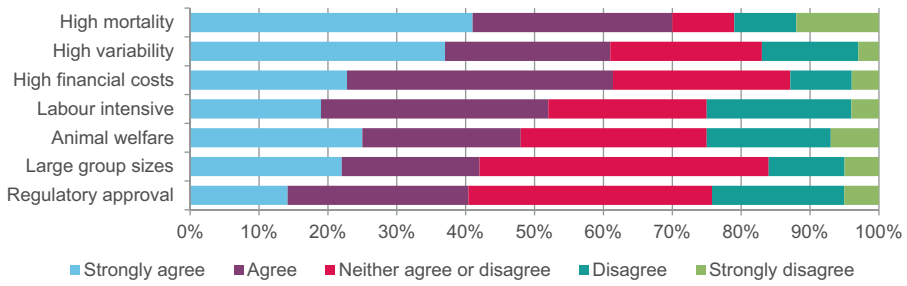

Fig. 3. Limitations on choice of animal model. Reasons provided by survey respondents in response to Question 9. "Do you consider the following to be limitations on your choice of animal model?".

higher rate of seizures than that which occurs in the typical clinical disease, because testing a decrease from extremely low rates is impractical in the current scientific settings and timelines.

Models can be distinguished between acute and chronic, focal and generalised, acquired and genetic. Acute animal models use chemical, electrical or physical stimulation to trigger epileptic activity and have been extensively used to discover new drugs but they have not led to the development of drugs that target either the generation or the maintenance of the epileptic state. Acute animal models model symptomatic seizures in normal brain, while chronic epilepsy results from pathological structural and functional changes in the brain. Chronic models have been recommended for many kinds of epilepsy research (Stables et al., 2003) including screening systems (e.g. NIH Anticonvulsant Screening Program (www.ninds.nih.gov/research/asp/index.htm) and target validation or proof-of-pharmacology for mechanisms that cannot be tested with acute seizure models (i.e. anti-inflammatory therapies) (Löscher et al., 2013).

Behavioural comorbidities observed in the interictal period (e.g. cognitive impairments, hyperactivity and depression (Barkmeier and Loeb, 2009; Tabb et al., 2007)) are an integral part of the epilepsy syndrome and legitimate subjects for investigation. However they may also impact on the animals' welfare, in which case researchers and ethical oversight committees must carefully weigh the potential research benefits and the harms to the animals. A roadmap for rational selection, implementation and interpretation of behavioural assays in animal models of epilepsy has previously been defined (Heinrichs and Seyfried, 2006).

One of the major welfare concerns for those working with animal models of epilepsy is the experience of the animal during and between seizures. It can be difficult to appreciate the experience of animals during seizures; the only surrogate is the experience of epilepsy patients. It is important to realise that the experience of witnesses can be very different from that of the patients (e.g. convulsions can be very distressing to witness, but since the person having the convulsion has markedly reduce levels of consciousness, they experience no distress during the seizure, Englot et al., 2010). Observations in rats have shown depressed cortical function during focal seizures (Englot et al., 2008); refinement of the choice of model solely on the frequency or intensity of seizure activity may not be appropriate. The priority should be to objectively assess the experience of the animal and think critically about what is the relevant model for the specific research question and the clinical relevance. Increasingly more sophisticated approaches to assessing adverse effects (e.g. pain, distress, anxiety) in laboratory rodents are being developed and research teams should be adopted when possible (e.g. by searching the literature and NC3Rs website www.nc3rs.org.uk).

Assessment of animal welfare, and balancing harms to animals against the potential benefits of the research, must address the whole epilepsy syndrome, not just one of the clinical signs. The periods between seizures are essential for animals to maintain themselves in good condition and allow recovery from seizures. The recurring episodic nature of epilepsy, and the repeated protocols that may be used to induce the epileptic state, means that the frequency of adverse effects, as well as their intensity and duration, must be included in the harm-benefit analysis. A judgment needs to be made about what is acceptable in terms of the type, duration, intensity and frequency of seizures, recovery time and the level of suffering following the initiation of seizures.

Recommendations:

1. 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).
2. Assessment of the harms to animals and balancing these against the potential benefits of the research, should take account of the lifetime experience of the animals and the whole epilepsy syndrome (not just seizures). The greater the animal welfare cost, the greater the strength of justification needed in terms of scientific and/or medical benefit (Grade D).

Choice of strain: Rodent models are used for studying the cellular and neural network mechanisms underlying epilepsy. Rodent strains can differ in seizure susceptibility (McKhann et al., 2003; McLin and Steward, 2006; Frankel, 2009; Schauwecker, 2011), effects of antiepileptic drugs (Leclercq and Kaminski, 2014), as well as the consequences of seizure activity (Schauwecker, 2011). For example, the use of C57BL/6 is hampered by its low sensitivity to seizure induction (Müller et al., 2009; Bankstahl et al., 2012); a problem addressed by back-crossing with other strains. Mouse genetic backgrounds play a crucial role in genetically-modified phenotypes and the susceptibility of these strains to seizures and neuropathological consequences (Schauwecker, 2011). It is, thus, important to ensure that the genetic background is controlled to avoid 'genetic' drift and appropriate wild-type littermate controls are generated from the same colony.

Choice of breeder: When obtaining animals from commercial breeders, the choice of the breeder is a critical factor. It was recently demonstrated that adult female Wistar rats from different breeders vary in anxiety-like behaviour, seizure susceptibility and epileptogenesis in the kindling model of temporal lobe epilepsy (Honndorf et al., 2011). A higher mortality rate after pilocarpine injection was observed in C57BL/6 mice depending on the supplier (Borges et al., 2003). Decisions about appropriate commercial colonies used for biomedical research should, therefore, be taken with care.

Choice of age: Seizure susceptibility and manifestation of epilepsies can be age-dependent. Some types of seizures and epilepsies occur in neonates and infants and are not present in adults (Mareš, 2012; Wasterlain et al., 2013) or vice versa (Sperber et al., 1999). Since brain function alters during development (e.g. neurotransmission, neuronal properties and connectivity (Galanopoulou and Moshe, 2011), the age of animals is likely to affect many factors such as sensitivity to chemoconvulsants (Wozniak et al., 1991) and kindling (Cilio et al., 2003), seizure latency and intensity (Pierson and Swann, 1988; Thompson et al., 1991), mortality (Blair et al., 2009) and behavioural, pathophysiological and pharmacological responses to anticonvulsant (Stafstrom et al., 1993; Shetty et al., 2012; Mareš, 2014).

Choice of sex: Gender differences are emerging amongst some types of epilepsies (Galanopoulou, 2014). Females are more susceptible to epilepsies such as juvenile myoclonic epilepsy (Camfield and Camfield, 2009) and childhood absence epilepsy (Panayiotopoulos, 2007) whilst males are more susceptible to West or Dravet syndrome and childhood epilepsy with centrotemporal spikes (Panayiotopoulos, 2007).

Sex hormones may also influence the timing and frequency of certain seizures, as occurs in catamenial epilepsy (Koppel and Harden, 2014). In animal models involving immature and adult animals, sex hormones and neurosteroids affect signalling pathways and brain regions regulating seizure initiation and maintenance (Scharfman and MacLusky, 2014). The expression of function of numerous signalling pathways and the anatomy, connectivity or function of brain networks involved in seizure control also exhibit sex differences that may affect the susceptibility to seizures, their consequences or response to drugs (Giorgi et al., 2014; Akman et al., 2015). Antiepileptic drug targets as well as pharmacokinetic and adverse effects of antiepileptic drugs may be affected by gender (Perucca et al., 2014; Pitkänen et al., 2014).

Most preclinical studies are performed on adult males (Pitkänen et al., 2014), possibly due to the confounding contributions of the oestrus cycle on seizure susceptibility (Scharfman et al., 2005); but there are plans to address the imbalance of sex across biomedical research (Clayton and Collins, 2014).

Recommendations:

3. Variations in the strain, genetic background, source, age and sex of animals can influence seizure susceptibility and mortality (e.g. Grade B for strain; Schauwecker, 2011, Level II; Grade A for age: Thompson et al., 1991, Level I, Pierson and Swann, 1988, Level I+; Grade A for sex; Scharfman and MacLusky, 2014, Level II; Grade A for source; Borges et al., 2003, Level I). The variability should be taken into consideration when designing and conducting studies and adequate measures taken to reduce experimental bias. The strain, source, age and sex of animals used in studies should be consistent, and reported in publications.
4. If using genetically-modified mice, the 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; Bourdi et al., 2011, Level I).
5. Given the evidence for sex-specific effects on epileptogenesis, 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; Scharfman et al., 2005, Level I).

3.2. *Induction procedures*

Procedures leading to the induction of seizures and/or epilepsy should be tailored to reach the scientific endpoint effectively whilst limiting suffering and mortality. The survey reflects the importance of the induction period, which showed of the 31 respondents reporting high mortality as an adverse effect, 90% (28/31) reported high mortality during the induction phase.

The status-inducing agent, its dose, its route of administration and SE duration all can affect both mortality and reliability of progression to chronic epilepsy, as discussed in more detail in Curia et al. (2008). It is important to determine a balance between minimising mortality and inducing the chronic epilepsy model. More detail on refinement of specific induction methods is given in Section 4, Model-specific refinements.

Long-term epilepsy studies investigating the efficacy of anti-epileptic drugs on spontaneous seizures may require long-term continuous administration of drug compounds. The choice of delivery method should be chosen to allow effective drug concentration levels (Löscher, 2007), whilst taking into consideration the potential additional stress to the animal. The advantages and disadvantages of different routes of administration of anti-epileptic drugs in rodents is summarised in Löscher, 2007. Refinement of the administration protocol, for example, administration of drug orally in food or water provides a non-invasive, less stressful alternative to repeated intraperitoneal injections and is more relevant to the human situation (Grabenstatter et al., 2007; Ali et al., 2012).

For general advice on refining procedures for the administration of substances to laboratory animals (e.g. consideration of the choice of route, dosing volume and frequency, and physiochemical properties of the substance) see Morton et al. (2001) and the NC3Rs-funded Procedures with Care website[3]. Use of appropriate and skilled handling techniques is essential to avoid anxiety and stress responses in the animals, which can lead to defensive aggression, difficulties in performing subsequent procedures and unwanted variation in experimental data. Handling mice by the tail induces aversion and high anxiety, to which mice do not readily habituate, and generally should be avoided (Hurst and West, 2010; Gouveia and Hurst, 2013).

Recommendations:

6. 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).
7. Research personnel should be adequately trained and competent in the manual skills for appropriate handling and restraint of animals for the administration of substances. Picking up mice by the tail should be avoided as this induces aversion and high anxiety; animals should be picked up by a non-aversive method (e.g. handling tunnels or cupping) (Grade A; Gouveia and Hurst, 2013), Level I+).

3.3. In vivo *recordings*

The diagnostic feature of epilepsy is recurring seizures which, according to the ILAE definition, are due to abnormally excessive and/or synchronous neuronal activity in the brain (Fisher et al., 2005). Seizures are often associated with motor signs, which provide end points for classical acute drug screening models such as the high dose pentylenetetrazole (PTZ) and the maximal electroshock (MES) models, and for many acute measurements of seizure susceptibility. Detecting spontaneous seizures in chronic models needs long term recording. Several approaches are used, either alone or in combination. Video recording or other measurements such as micro-electro-mechanical systems (MEMS), can detect movements associated with seizures (Sunderam et al., 2007). Automated observational systems (e.g. Van de Weerd et al., 2001) can be used to analyse the behavioural profile of animals following seizure induction (Riljak et al., 2014) and methods are currently being developed to allow automated recordings in the home cage environment[4]. Electrophysiological recording provides more direct detection of seizures, and is particularly important for those seizures with no or minimal motor consequences; video-EEG monitoring should be used to identify seizures with no motor symptoms (Arcieri et al., 2014). In addition to these means of monitoring seizure activity, intracerebral cannulation for drug delivery or sampling, as well as optical fibres for optogenetic illumination, which can be coupled with recording and stimulation ("optrodes") can be used to monitor and influence seizure activity. The considerations described below apply to these techniques.

The main welfare issues to be taken into consideration for chronic electrophysiological recording are the experimental setup (tethered systems or radiotelemetry), electrode properties (e.g. physical dimensions, location and logistics of device), surgical implantation of electrodes (e.g. infection, post-operative recovery) and maintenance of chronic electrodes (e.g. freedom of movement, cage size). A detailed account of the technology is beyond the scope of the paper but the reader is referred to Weiergraber et al. (2005).

Experimental setup: Data transmission can be achieved using either umbilical cables (tethers) or radiotelemetry. Tethered systems constrain movement. Torque on the headmount can be minimised by the use of slip rings (swivel commutators), reducing the risk of injury to the animal. The combined weight of the headmount, plug and cable can be managed by carefully adjusting the

[3] Procedures with Care offers practical advice on the manual skills required for administration of substances to mice and rats, including high-definition instructional videos: www.procedureswithcare.org.uk.

[4] NC3Rs funded CRACK IT challenge 'Rodent Little Brother' https://www.crackit.org.uk/challenge-7-rodent-little-brother.

length of the tethering cable, or by use of a counterweight or spring to adjust the cable to follow movements of the head. Careful attention to both vertical and rotational forces can allow continuous recording for weeks (Doheny et al., 2002).

Radiotelemetry avoids tethering wires and therefore allows animals to move freely, which has benefits for behavioural studies, animal welfare and can simplify longer-term recording (Bastlund et al., 2004; Weiergraber et al., 2005; Williams et al., 2006). The development of such devices should include evaluation of their impact on the viability of adjacent tissues. Battery life is a key factor, particularly for studies on seizure frequency or on disease progression. Recording from early post-natal animals is more challenging (Zayachkivsky et al., 2013).

Device dimensions and locations: The physical size and weight of devices can impact on the welfare of the animal. Devices should be as light as possible and positioned appropriately to allow the animal free movement to perform its normal behaviours, particularly to eat, drink and groom (Morton et al., 2003). It is difficult to define an exact weight limit. The experience of the Working Group is that devices of 5–10% of bodyweight appear to be tolerated, although there are no published data to support this.

Devices can be mounted in the locations listed. In general, implanted devices should be positioned away from the incision (i.e. not directly under the sutures).

- **Head mounted**—This is most common with tethered systems and some radiotelemetry systems.
- **Subcutaneous**—If radiotelemetry devices are small enough they can be implanted subcutaneously with wires tunnelled to the recording sites. Their size, weight and location should not place excessive strain on the skin or musculoskeletal system. Differences in skin properties (e.g. between rat and mouse, different parts of the body) can determine whether devices will remain in the pockets created for them, or whether they need tethering, for example with a permanent suture.
- **Intraperitoneal**—Some devices are too big to mount subcutaneously and are implanted inside the abdomen. Care is needed on the locations of devices and the paths of wires to the recording sites to avoid restricting natural movements and to avoid mechanical damage to adjacent tissues.
- **Harnesses**—Some systems use species-specific harnesses or saddles to carry external batteries or even the entire device (Ewing et al., 2013). They should be appropriate to the age/size/species of the experimental animal under study and permit free movement with frequent checks made for evidence of rubbing or discomfort.

Surgical implantation of electrodes: In almost all cases electrophysiological recording requires preparatory surgery to implant or attach electrodes, connectors, telemeters, cannulae and other devices. Electrodes can be implanted into brain structures using stereotaxic surgery, or onto the cortical surface using either a skull-screw electrode or a wire cemented into a burr hole. Large numbers of electrodes can be incorporated into ultrathin and flexible electrode arrays (Wu et al., 2008; Viventi et al., 2010; Park et al., 2014).

Good surgical practice, with proper attention to antibiotic prophylaxis, full aseptic technique (see video tutorials on the NC3Rs-funded Procedures With Care website[4]), pain management, maintenance of body temperature, replenishment of fluids lost under anaesthesia and effective post-operative care are required (see Section 3.4) (Fornari et al., 2012). In some cases surgery can be extensive, requiring careful planning and execution of both the operation and subsequent post-operative care.

Whatever the electrode assembly, it needs to be securely anchored in place. Loss of the head stage was reported in the survey as a defined humane endpoint (see Section 3.5). Typically electrodes will be fixed to the skull using cement (e.g. dental acrylic or glass ionomer) and anchoring skull screws. In some cases skull screws are not feasible (e.g. in the thin skulls of neonatal rats), in which case, careful matching of the shape of the device to the slight curvature of the skull allows the use of cyanoacrylate glues to anchor radiotelemetry devices (Zayachkivsky et al., 2013).

Animals with head-mounted devices may require additional housing space and it may be prudent to block access to low food hoppers, or to avoid wire lids with gaps that can trap projections from the head-mount. Choices of housing should also consider the risk of damage to head-mounted items (see Section 3.8 Enrichment). The latest individually ventilated cage (IVC) systems include double height cages from which the shelf can be removed to minimise the risk of head implants being caught in the cage or lid.

Recommendations:

8. The experimental setup should be maximally effective in delivering the research objectives while prioritising animal welfare and minimising interference with behaviour, especially in behavioural studies where the instrumentation for seizure recording may impede movement and significantly alter behaviour (Grade D).
9. Wherever possible, radiotelemetry should be used in preference to tethered systems for chronic electrophysiological recordings (Grade D).
10. 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 (e.g. eating, drinking, grooming and rearing) (Grade C; Morton et al., 2003; Hawkins et al., 2004, Level III).
11. 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) (see recommendation 15).

3.4. Perioperative care

Several models of epilepsy require surgical procedures to be undertaken for intracranial electrode implantation and stimulation (see Section 3.3) and/or stereotaxic injection of seizure-inducing compounds (Bouilleret et al., 1999; Lévesque and Avoli, 2013). The care provided before, during and after surgical procedures (perioperative care) is critical for minimising pain and discomfort, and improving study outcomes.

To understand current practices, evidence was gathered from the survey and literature search. The survey asked researchers how they avoid and minimise adverse effects (Fig. 4). The most common

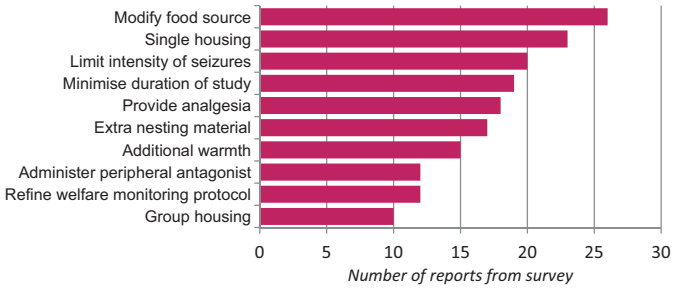

Fig. 4. Refinement of adverse effects during induction and/or maintenance of experimental epilepsy. Steps taken to control and refine adverse effects in response to the question: "How do you control and refine adverse effects?".

control measure was modification of the food source provided to animals to encourage eating and prevent weight loss, for example softened standard food pellets, gels and treats, which are often placed on the cage floor. In addition to modifications detailed in Fig. 4, researchers also reported administration of subcutaneous fluids following surgery, application of topical and systemic antibiotics to prevent infection, extra care when handling seizure-prone animals, and the use of skilled and knowledgeable animal care staff, with good communication between them, veterinarians, and researchers.

Animals should be given time to recover from surgery before a study commences and provided with necessary resources, for example, heating blanket or a temperature-controlled warm cabinet (avoid the use of heat lamps) to prevent hypothermia until there is evidence of recovery (McIntyre, 2005; Zhao and Holmes, 2005; Graber and Prince, 2005).

Analgesia and anaesthetics: Question 5a of the survey asked researchers: "What adverse effects do you observe at induction and/or during maintenance of experimental epilepsy?" Pain-related behaviour was reported in 3/44 (7%) of responses and all such cases were associated with chemically-induced epilepsy during the induction process. No pain-related behaviours were reported to be observed during maintenance of epileptic animals. Use of analgesia was reported in 18/51 (35%) responses to question 5b "How do you control and refine adverse effects?"; however this may reflect under-reporting of standard practice.

Pain during seizures is a rare phenomenon in people with epilepsy (Young and Blume, 1983) and it is thought to have a similarly low incidence in animal models of seizures. Postictal nociceptive thresholds have been assessed in experimentally-induced epileptic seizures in animals using thermal nociceptive tests (tail flick, plantar, hot plate) showing a postictal anti-nociceptive effect observed for 30–120 min after seizures (Caldecott-Hazard and Liebeskind, 1982; Caldecott-Hazard et al., 1982; Coimbra et al., 2001; Freitas et al., 2005; Mareš and Rokyta, 2009). Longer-term anti-nociceptive effects have not been defined. Although it appears pain associated with seizures is minimal and infrequent, there is, as for all surgical procedures, the potential for animals to experience pain. Protocols for identifying and alleviating pain around this time are therefore required.

To estimate current practice in analgesic administration in animal models of epilepsy undergoing surgical procedures, a small literature review was carried out. Peer-reviewed scientific papers reporting kainic acid-induced seizures published between 2012 and 2014 were included in the analysis. A total of 20 papers were screened for inclusion eligibility (i.e. if the paper described surgical procedures); eight papers were excluded because no surgical procedures were involved. Of the 12 papers included in the analysis (Guo and Kuang, 1993; Inostroza et al., 2012; Bernard et al., 2013; Chung et al., 2013; Xu et al., 2013; Dugladze et al., 2013; Harhausen et al., 2013; Huang and van Luijtelaar, 2013; Jimenez-Pacheco et al., 2013; Li et al., 2013; Qiao et al., 2013; Simeone et al., 2014), 11/12 (92%) reported the use of anaesthetics, but only 3/12 (25%) reported the use of analgesics. The low level of reporting of analgesia is consistent with previous reports in rodents undergoing experimental surgical procedures (Richardson and Flecknell, 2005; Stokes et al., 2009)); however this may reflect under-reporting. Until the ARRIVE guidelines[5] are routinely and fully implemented when reporting animal studies, it will remain difficult to ascertain whether pain is being managed appropriately.

[5] Led by the NC3Rs, the ARRIVE guidelines (www.nc3rs.org.uk/ARRIVE) were developed to improve the reporting of animal research, maximise the information published and minimise unnecessary animal use. (Kilkenny et al., 2010).

Anaesthesia and analgesia should be used to alleviate pain associated with invasive procedures such as stereotaxic delivery of convulsants and electrode implantation. In other surgical contexts analgesia has been shown to aid recovery (Hayes et al., 2000; Shavit et al., 2005). The anaesthetic and analgesic formulation, dose and route of administration should follow advice from the veterinarian, and should be reported in published papers. Anaesthetic agents should be chosen carefully due to their potential effects on the physiology of the animal (Tremoleda et al., 2012). For example, pilocarpine-induced SE rats show an enhanced response to general anaesthesia (pentobarbital, halothane and propofol), prolonged loss of tail-pinch response and loss of righting reflex. In contrast, the amygdala kindling model does not show enhanced response to general anaesthesia (Long et al., 2009).

There are indications that certain analgesic drugs (e.g. neuroleptics and opioids) have an effect on seizure susceptibility and expression (Czuczwar and Frey, 1986; Hashem and Frey, 1988). Pharmacokinetic and pharmacodynamic factors need to be considered with co-administration of drugs. If this is a problem in the specific model being produced, then alternative agents such as *NSAIDs* (see Glossary) combined with local anaesthetic block using a combination of lidocaine and bupivacaine can be considered. Care must be taken with local anaesthetics as leakage onto the cortex can cause seizures. The use of analgesics may be required under legislation in some countries unless there is robust scientific justification. Withholding of analgesia following an invasive procedure must be justified to the *AWERB/IACUC* (see Glossary).

The Mouse Grimace Scale (Langford et al., 2010; Leach et al., 2012) and Rat Grimace Scale (Sotocinal et al., 2011; Oliver et al., 2014) have been shown to be rapid, reliable and effective methods for assessing post-operative pain. An additional question about use of grimace scales "Do you use the Mouse Grimace Scale (MGS) or the Rat Grimace Scale (RGS) to assess pain?" was added to the survey. Only four respondents (13%) used the grimace scales to assess pain in mice and rats.

Infection: Inflammation due to infection can interfere with seizure susceptibility and infections have been shown to cause higher seizure susceptibility in audiogenic and electroshock-induced seizures (Flandera et al., 1973). Prenatal immune challenges can also cause an increase in seizure susceptibility (Yin et al., 2013). In the survey, only two survey respondents reported post-operative infection; one as a criterion for the humane endpoint and the other as a complication of surgery, which was avoided by using topical antibiotic application. Infections should be avoided by using best practices, including aseptic technique during surgical procedures. However, maintaining strict asepsis during complex implant procedures can be challenging, and as an adjunct to this, prophylactic antibiotic treatment should be given if appropriate on the advice of the veterinarian. The choice of antibiotics requires careful consideration; evidence suggests the tetracycline-class antibiotics (e.g. minocycline, doxycycline and tetracycline) have anti-apoptotic and anti-inflammatory effects (Abraham et al., 2012; Wang et al., 2012).

Food and fluids: Following induction, the feeding behaviour of animals may become disrupted. For example, animals injected with tetanus toxin display intermittent attacks of 'paroxysmal eating' lasting a minute or two, where animals consume their food pellets with excessive vigour (Mellanby et al., 1999). Modification of food source was the most frequently reported method to minimise adverse effects (weight loss) mentioned in the survey. Several studies have reported using modified food, including enhanced dietary glucose to accelerate weight gain following SE (Jedrzejko and Persinger, 2001). Hand-feeding animals with fruit juices mixed with sweetened milk or mashed food pellets can also be beneficial for animals in a poor condition following seizure induction (McIntyre; Persinger et al., 1988).

Recommendations:

12. Animals should be allowed sufficient time to recover following surgical procedures using anaesthesia, before subsequent recordings/measurements are taken (Grade A; Culley et al., 2004, Level I+).
13. Steps should be taken to identify, assess and alleviate pain following procedures requiring surgery. Appropriate pain relief should be provided and reported in publications. Choice of analgesic should be made with care based on veterinary advice and taking into account evidence of potential interference with the science (Grade C; Tremoleda et al., 2012, Level III).
14. Topical antibiotics should be used for simple surgical procedures and prophylactic antibiotics used for implantation procedures if appropriate, based on veterinary advice (Grade D).
15. A modified food source should be provided to encourage eating and prevent weight loss following surgery and/or seizure induction (Grade A; Jedrzejko and Persinger, 2001, Level I). This should be introduced prior to surgery to ensure familiarisation and consumption. Food and drink should be accessible from the floor of the cage (Grade D).

3.5. *Welfare assessment*

The creation of chronic disease conditions, including rodent models of epilepsy, requires continuous welfare assessment. The severity and frequency of the adverse effects will depend upon the model of epilepsy. Genetic models may show early failure to thrive and excess neonatal and juvenile mortality. Other models of epileptogenesis in previously normal animals may also present welfare issues unique to the induction method (e.g. electrical stimulation or chemical treatments). Once the epileptic state has been produced, on-going adverse effects related to the seizure activity must be taken into account. In addition to providing an immediate assessment of the animal's state of health and welfare, the information gained from welfare assessments enables compliance with the legal requirements in many countries for prospective severity classification of the experimental procedures and subsequent retrospective assessment of severity experienced by the animals (see Annex VIII of Directive 2010/63/EU). The severity classification can be assigned according to clinical signs observed during the assessment (Baumans et al., 1994). This can then be used to determine the appropriate action regarding the continuation of a study with due allowance for safeguarding animal welfare. The assessment should, whenever possible, use data from adverse effects observed in the animal model, rather than by simple extrapolation from man.

The survey showed adverse effects observed at induction include high mortality, weight loss and, in some cases, pain-related behaviour. During the maintenance phase the most commonly reported adverse effects were behavioural abnormalities, such as aggression and hyper-reactivity (Fig. 5). Management of aggression can result in animals being singly-housed, further impacting on their welfare (see Section 3.7 Social housing). Evidence from the literature search shows that the number of seizures and seizure-induced neuronal damage is correlated with the severity of aggression in animal models of epilepsy (Desjardins and Persinger, 1995; Persinger, 1996; Huang et al., 2012). Some methods to induce seizures, such as kindling, can result in long-lasting changes in emotional behaviour (Franke and Kittner, 2001), which have been shown to be defensive in nature (Kalynchuk et al., 1999). However, some comorbidities associated with clinical epilepsy (e.g. aggression) are an integral part of the disease and may be the object of the research.

Animal welfare assessments must be conducted to assess the state of wellbeing and health of each individual animal. Where

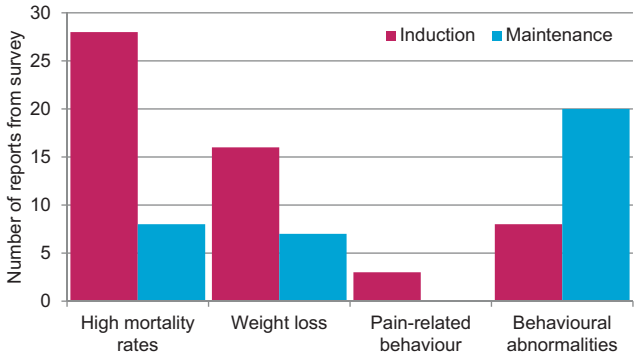

Fig. 5. Adverse effects during induction and maintenance in response to the question: "What adverse effects do you observe at induction and/or during maintenance of the experimental epilepsy?".

experimental procedures unavoidably impact on the health of the animal, which includes many disease models, appropriate welfare assessments will enable the impact of the procedures to be recognised and action taken to minimise harm by intervention/treatment and the application of humane endpoints (see Section 3.7).

Effective and regular monitoring of welfare is best facilitated by the use of score sheets (Jones et al., 1999; Morton, 1999, 2000; Hawkins, 2014). The survey showed 21 respondents (38% of the total) reported using a scoring system. Using a formalised scoring system has the advantage of encouraging a systematic, structured examination of the animal, and helps ensure a consistent evaluation at different time points, and by different assessors. Scores sheets also provide a useful basis for communication between researchers and animal care staff. How to best tailor the most appropriate scoring system for each establishment, species, project and group of personnel has been reviewed in detail by Hawkins et al. (2011).

Examination of the animal's physical appearance and behaviour is essential for the conduct of welfare assessment. Routine observations should be performed on a daily basis, and this is a legal requirement under some jurisdictions, although specific reports are typically only made if there is concern over the health of the animal. A more detailed welfare assessment scoring system should be considered, particularly if new models are being introduced, or if a particular model is known to present significant comorbidities. Criteria used for scoring systems that were described by respondents in the survey included body weight, fur condition, piloerection, colour of skin, aggressive behaviour, social interactions, ocular keratitis, *ataxia*, tremor, *ptosis*, *straub tail*, righting reflex, *mydriasis*, *catalepsy* and mortality rate (see Glossary). In addition, Table 4 provides a list of general welfare assessment criteria for laboratory rodents. Assessments should be made both by observation of the animal in an undisturbed state, and then by more detailed inspection involving, if necessary, removal from its cage. Care is needed, however, where handling may provoke hyperreactivity and compromise, rather than promote animal well-being.

Due to the specific nature of animal models of epilepsy and their associated adverse effects, a number of scoring systems have been developed to capture the seizure type observed. The Racine Scale (Racine, 1972) was originally developed to classify seizures induced by electrical stimulation to the hippocampus or amygdala in rodents. The modified Racine scale was introduced for PTZ chemical induction (Lüttjohann et al., 2009) and describes a wider range of motor seizures, possibly due to the convulsant reaching more parts of the brain than the focal stimulation of amygdala kindling. It is important to realise that these scales are not necessarily generalisable to other seizure models and immature animals. Proposals have been made to quantify animal suffering based on seizure scoring (Wolfensohn et al., 2013) but the Working Group considers

Table 4
Welfare assessment criteria. Examination of an animal's physical appearance and behaviour is essential for the conduct of the welfare assessment. Table adapted from AHWLA (Assessing the Health and Welfare of Laboratory Animals) www.ahwla.org.uk and based upon experience from the Working Group. Positive welfare indicators (grey) and negative welfare indicators (white) are categorised into three distinct areas: the cage environment, animal behaviour and the physical appearance of the animal.

Category	Indicators
The cage environment	Evidence of eating and drinking
	Evidence of fresh faeces and urine
	Evidence of nest building and use / a good quality nest (mice)
	Any blood staining of the cage sides or bedding
Animal behaviour	Alert to external stimuli
	Interested in surroundings (e.g. use of enrichment items)
	Normal interactions with handlers (e.g. not overly aggressive or overly passive)
	Normal interactions with other animals (e.g. no increase in aggression or anxiety behaviour, such marked escape responses or hiding)
	Isolated or withdrawn from other animals in the social group
	Abnormal posture (e.g. hunched posture, tilted head)
	Abnormal movements (e.g. abnormal gait, uncoordinated movement, lack of movement in the cage or on the bench)
Physical appearance of the animal	Good body condition (i.e. not overconditioned or underconditioned as defined in Ullman-Culleré & Foltz 1999
	Appropriate body weight (i.e. within normal range for age-matched controls; no significant weight loss or increase)
	Mucous membranes pink and moist
	Eyes clear and bright; free from discharge or prophyrin staining (rat) indicative of stress or disease; not sunken, dull or closed/semi-closed
	Nose free from discharge
	Mouth (including teeth and tongue) free from injury or abnormalities (e.g. malocclusion/overgrown teeth, salivation)
	Tail and anal genital area free from injury and discharge/soiling
	Normal skin and limbs (e.g. free from physical injury, lack of skin tenting = dehydration)
	Poor coat condition (e.g. unkempt due to lack of grooming, greasy, faecal or urine stained, piloerection, hair loss)
	Abnormal facial expressions, indicative of pain (e.g. grimace score of 1 or 2 using the rat (Sotocinal et al. 2011) and mouse (Langford et al. 2010) grimace scales

this to be of limited value due to poor association of seizure score with animal suffering.

Recommendations:

16. 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).
17. 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).

3.6. *Humane endpoints*

Humane endpoints can be defined as a set of predetermined physiological and/or behavioural signs that define the point at which an animal will be removed from an experimental study (e.g. by humane killing) before it experiences unacceptable harm whilst still meeting the experimental objectives. The implementation of humane endpoints is a major means of refinement (Morton, 1999; Hendriksen et al., 2011; Ashall, 2014). Identification of humane endpoints needs to take into account clinical aspects of the disease being modelled, which arguably can be harmful while being legitimate subjects for study; in such cases, care needs to be taken to balance harms and potential benefits.

Clearly defined scientific objectives are pivotal to help determine the earliest experimental and humane endpoints. The most effective biological indicators that denote success or failure of an experiment and which precede any unjustifiable suffering of an animal, should be obtained prior to the start of the experiment and reviewed regularly by a team of animal care staff, the scientists and the veterinarian (Ashall, 2014; Hawkins, 2014). Successful implementation of humane endpoints relies on training and teamwork (Hau, 1999; Hawkins et al., 2011). To ensure animal suffering is kept to an absolute minimum, the predetermined biological indicators of suffering and poor welfare need to be detected as quickly as possible and acted up on efficiently, for example by providing veterinary care such as pain relief or, if necessary, euthanasia of the affected animal.

Few published epilepsy studies address the issue of humane endpoints (Lüttjohann et al., 2009). Therefore the survey was used to assess when and how humane endpoints are used in animal models of epilepsy. Humane endpoints were used by 39/44 (67%) respondents to question 6 “Do you use a defined humane endpoint?”; with the most common humane endpoint criteria being significant body weight loss (ranging from 10% to 25% weight loss from pre-induction starting weight), signs of distress (including porphyria staining, poor grooming, difficulty breathing), opening of wounds or loss of an implant, wound infection, and prolonged repetitive seizures. Following a very prolonged seizure (SE) or post-operatively (pain), animals can have difficulties drinking or eating, which can result in a significant decrease in body weight due to dehydration or reduced food intake.

The results of the survey reflect that the single most objective clinical sign is change in body weight (Van Vlissingen et al., 1999). Body weight is easy to measure and record but is a relatively non-specific welfare indicator. Both an abnormal increase and a substantial decrease in body weight can be associated with serious distress and suffering. The rate of change, as well as the absolute change, can be of importance.

In epilepsy models sudden increases in body weights can be observed, associated with unrelated illnesses such as the development of neoplasms and fluid retention. Seizures can result in body fluid loss and this can produce short-term changes in weight.

Careful monitoring of body weight therefore needs to be carried out regularly (see Section 3.5) and diet amended as necessary. If an animal does not eat or drink normally and as a result has a significant body weight loss, euthanasia should be considered.

Body weight measurements should not only be compared to the previous measurements, but also plotted in a chart and compared to the normal growth curve for that species and strain. This will enable assessment of whether the animals are growing normally; condition scoring can also be used for this purpose (Ullman-Culleré and Foltz, 1999; Hickman and Swan, 2010). For example, reduction in weight gain or an absolute loss in body weight can be used to determine a humane endpoint (Jones et al., 1999). Use of weight as the sole criterion for application of a humane endpoint is often inappropriate (Chapman et al., 2013); in addition to body weight loss, other "signs of distress" should be identified, for example; appearance (coat condition, posture and mobility/gait), clinical signs (respiration, salivation, tremors, prostration), unprovoked behaviour (socialisation, vocalisation) and response to stimulus (provoked behaviour) (Jones et al., 1999).

Defined endpoints need to be tailored specifically to the epilepsy model used. The type, duration, intensity and frequency of seizures, recovery time and the level of suffering following the initiation of a seizure (if not spontaneous recurrent seizures) considered to be acceptable will differ with each experimental model and needs to be defined. For example, if SE cannot be stopped by pharmacological intervention within the defined time, the animal should be euthanised. If SE is not the expected outcome of the model, it should be avoided and controlled if it occurs unexpectedly.

Recommendations:

18. 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; Morton, 1999, Level III). This should take into consideration the current legal framework, *scientific*, *justifiable* and *unpredicted endpoints* (see Glossary) and the results of welfare assessments.

3.7. Social housing

Social interactions are important contributors to the welfare of rodents, provided that the group composition is appropriate for the species, age, sex and strain. Social housing is the recommended default housing configuration under legislation on protection of animals used in science (European Union, 2010; National Research Council, 2011; Home Office, 2014). Individual housing has been shown to be stressful for rodents, giving rise to behavioural and physiological abnormalities (Hatch et al., 1963; Brain, 1975; Haseman et al., 1994; Hurst et al., 1997; Võikar et al., 2005; Meijer et al., 2006; Kalliokoski et al., 2014), and is therefore recognised as a harm and regulated under some jurisdictions. Housing of single-sex groups of male and female rats does not usually pose problems. Socially housed male mice, however, may show territorial behaviour, aggression and fighting, depending on strain, previous experience and cage enrichment (Van Loo et al., 2001). Nonetheless, there is evidence that subordinate male mice prefer company to being housed individually, even if their companion is dominant (Van Loo et al., 2001). Provision of visual barriers and refuges that allow animals to withdraw out of sight when a threat occurs can reduce aggression.

The issue of group or single housing for rodents used in epilepsy studies is complex. Whilst group housing should be best practice, the removal of individual animals for surgery or behavioural testing can result in the disruption of an established social hierarchy (Ferrari et al., 1998; Bartolomucci et al., 2001, 2004; Arndt et al., 2009), whilst the appearance of disturbed behaviour (aggressiveness or passivity) in experimental animals (e.g. animals displaying overt seizures (Mellanby et al., 1981) or that are instrumented) may provoke hostile responses from cage-mates. Companion rats have been seen to bite rats that are experiencing seizures and may cause injury, or they may bite exposed devices including head-mounted guide cannulae, electrophysiological connections and telemeters; in either of these cases, the animals should be separated. Early after major surgery a companion may eat appealing foods faster than the recovering rat and delay restoration of weight. On rare occasions apparently neutral behavioural contact between rats has triggered seizures, so that removing the companion may be beneficial for the epileptic rat. The tendency for epileptic rats to be submissive may affect the social hierarchy in group housing which could create confounds for some kinds of experiments (Castelhano et al., 2013), particularly for those investigating behavioural comorbidities.

Several studies support the hypothesis that social housing accelerates post-surgical recovery in rodent disease models (Baran et al., 2010; Jirkof, 2015). The survey asked how animals were housed during an experiment (after induction) and the results showed that instrumented animals were more likely to be singly housed (36/49, 73% of total) compared to non-instrumented animals (16/49, 33% of total) (Fig. 6). Rodents are often housed individually after surgical procedures because they may disturb each other's incisions, but this is a rare occurrence and may be addressed by use of subcuticular sutures. The experience of some members of the Working Group is that a familiar companion animal helps accelerate recovery of animals that have undergone prolonged and/or complex surgery, but that the companion animal may need to be removed if fighting occurs. Group housing needs careful monitoring, ideally with video recording, to detect adverse effects that may be detrimental to the welfare of the epileptic animal or to the reliability of the experimental results. The possibility exists to divide cages with a grid-like barrier that allows odours and some physical contact whilst reducing the risk of serious injury and allowing retreat to safety (Van Loo et al., 2007; Boggiano et al., 2008). Whilst this seems like a suitable compromise, space issues arise if larger rodents such as rats are confined to one half of a standard cage (Boggiano et al., 2008) and increased levels of stress were reported in mice separated by a grid

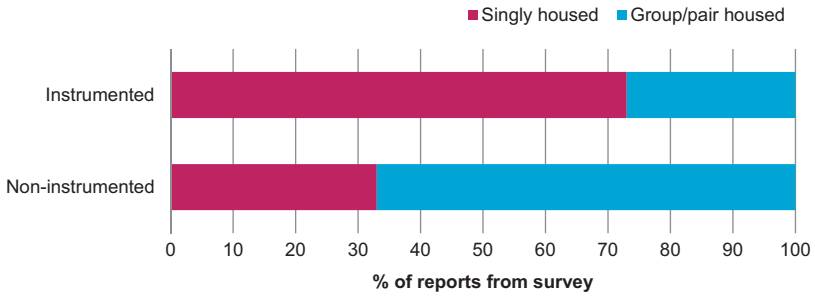

Fig. 6. Social housing of instrumented and non-instrumented animals in response to the question: "How are the animals housed during an experiment?".

leading the authors to conclude that even individual housing may be more suitable than separation by a grid (Van Loo et al., 2007).

In summary, the balance of pros and cons in social housing is very delicate. The incidence of complications of group housing varies greatly between models, and possibly between strains of experimental animals. A companion animal allows for natural social interactions beneficial for welfare and seems to accelerate recovery from more difficult surgeries, but it is a delicate balance. This was reflected in the survey, which showed respondents housed animals individually (23/56, 41% of total responses) or in groups (10/56, 18% of total responses) as refinement measures (Fig. 4). We conclude that group housing is desirable in some, but not all, work on experimental epilepsy, and that each case should be carefully evaluated, weighing the welfare benefits and costs against single housing. When individual housing is inevitable due to excessive aggressive behaviour, the presence of enrichment objects such as nesting material can mitigate some of the negative consequences of social isolation (Belz et al., 2003; Van Loo et al., 2004).

Recommendations:

19. Mice and rats should be socially housed unless there are compelling scientific or animal health reasons for single housing. Each case should be carefully evaluated; weighing the welfare benefits and costs (Grade A; Võikar et al., 2005; Kalliokoski et al., 2014, Level I).
20. 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).
21. 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; Mellanby et al., 1981, Level I).

3.8. Environmental enrichment

Most rodents used in epilepsy studies spend the majority of their time in their cages, so refinement of housing and husbandry is a key issue. Providing an environment which meets the animals' species-specific behavioural and physiological needs is important for their wellbeing (Olsson and Dahlborn, 2002; Baumans, 2005; Hubrecht and Kirkwood, 2010). Despite many generations of breeding in captivity, laboratory rodents are still motivated to perform behaviours seen in their wild counterparts (Berdoy, 2003). Barren, restrictive and socially-deprived housing conditions can interfere with brain development and cause abnormal behaviours, such as stereotypies and barbering. Animals from refined housing conditions are expected to be physiologically and psychologically more stable and might therefore be considered superior animal models, ensuring more generalisable results (Poole, 1997; Martin et al., 2010; Kulesskaya et al., 2011). Structuring the cage with nesting material, nest boxes, tubes, partitions and chew blocks allows for the performance of natural behaviours, such as nest building, hiding, exploration, foraging, gnawing and resting and provides the animals with an element of choice, complexity and control (Sherwin and Nicol, 1997; Manser et al., 1998b; Latham and Mason, 2004; Baumans, 2010). Environmental enrichments can also provide useful indicators for severity assessments, as animals with poor welfare are less likely to utilise enrichment objects and to carry out normal behaviours such as nest building (Hawkins et al., 2011). Preference tests and consumer demand studies have been used to determine the strength of motivation for specific resources. For example, Manser et al. (1996) showed that rats were motivated to lift a door weighing 83% of their bodyweight to rest on a solid floor rather than a grid floor; Collier et al. (1990) showed that rats will continue to press a bar to open a door to gain access to a nest box even when required to do so 20 times per entry; rats prefer chewable over non-chewable objects and show a clear preference for dark nest boxes (Manser et al., 1998a). Alternatives to standard housing systems that better cater for the behavioural needs of rodents are commercially available (e.g. elevated cage tops and multi-level cages that allow exploration, climbing and rearing).

The concept that environmental enrichment improves cognition and well-being in rodents has long been established and a wealth of literature demonstrates that environmental enrichment alters synaptic plasticity, brain (and neuronal) morphology, neurogenesis, neurochemistry and gene expression (Lewis, 2004). Environmental enrichment has been shown to modify pathology in rodent models of neurodegenerative disease (Hannan, 2013) and the influence of environmental enrichment specifically in the context of epilepsy and epileptogenesis has recently been reviewed (Kotloski and Sutula, 2014). Some salient points are highlighted below.

Many studies have demonstrated that environmental enrichment has a dramatic effect on seizures and their comorbidities, but the effects of environmental enrichment are model-dependent. In the kainic acid model of limbic SE, environmental enrichment prior to the SE is neuroprotective, increases neurogenesis, and increases seizure threshold (Young et al., 1999). Similarly, in a genetic model of temporal lobe epilepsy secondary to a missense mutation of the sodium channel NaV1.2 gene (*Scn2a*), environmental enrichment from birth greatly reduced spontaneous seizures and neuronal damage (Manno et al., 2011), whilst mice with a mutation in the presynaptic protein bassoon experienced shorter tonic–clonic seizures and a preservation of synaptic transmission and plasticity (long-term potentiation, paired-pulse facilitation), dendritic spine density and apical dendrite length in area CA1 of the hippocampus (Morelli et al., 2014). Environmental enrichment after pilocarpine-induced limbic SE has different effects as there seems to be no effect on EEG or neuronal damage, but cognitive performance is still enhanced and neurogenesis increases (Faverjon et al., 2002; Rutten et al., 2002). Environmental enrichment has also been shown to delay epileptogenesis and increase neurogenesis in rats subjected to kindling (Auvergne et al., 2002). Also, in a model of atypical absences secondary to injection of the cholesterol biosynthesis inhibitor AY-9944, environmental enrichment had no effect on seizure activity, although it did reverse the behavioural phenotype in these mice (Stewart et al., 2012). These results contrast somewhat with results obtained in generalised epilepsies. In genetic models of absence seizures, environmental enrichment increases the length and frequency of runs of spike-wave discharges, but not the number of animals exhibiting them (Schridde and van Luijtelaar, 2004).

A potential confounder in all these studies is that environmental enrichment protocols differ. In some cases, cages contain toys, running wheel, tunnels and food reward (or edible treats), but in some studies the objects are regularly changed and there is considerable variation in the number of objects in the cage. As a result of this variation, one research group has developed a standardised environmental enrichment using an enriched cage, that contains a maze, which separates food and water bottle compartments and which offers the potential for increased exercise, multiple activities, cognitive stimulation and the opportunity of changing the maze, so introducing novelty (Fares et al., 2013).

Overall, it appears that the effects of environmental enrichment are complex but seem to lessen the cognitive impact of prolonged seizures and epilepsy. This has potential disadvantages, in for example drug screening, where reduced seizure frequency or intensity may require additional animals to be used to gain statistical power, although the effects on seizure threshold appear to be epilepsy-type dependent. Providing standard enriched cages (e.g. Fares et al., 2013) may go some way to preventing variation

in experimental results between laboratories, but may have the disadvantage of promoting a particular view of what constitutes appropriate environmental enrichment. Instead, an appreciation of naturalistic behaviour and attempts at permitting their display in the laboratory environment may provide the enrichment necessary to observe consistent effects of environmental enrichment, including on the survival, weight gain and general welfare of rodents following SE.

Enriching the environment of experimental rodents has been shown to reduce the impact of both induced and genetic models of epilepsy and may therefore increase the number of animals needed to achieve statistical power. Despite this, the preference should be to provide an enriched environment using the minimum number of animals consistent with the scientific objective.

Recommendations:

22. Environmental enrichment should be provided to allow animals to express naturalistic behaviours unless there is a justified reason to withhold it (Grade A; Koh et al., 2007, Level I+).
23. Environmental enrichment should be consistent in the home cage to reduce variability (Grade A; Fares et al., 2013, Level I+). Enrichment protocols should be described carefully and detailed in published manuscripts to allow others to adopt a similar approach and reduce inter-laboratory variations.

3.9. Reporting and data sharing

A significant proportion of *in vivo* research publications fail to report key details (e.g. animal characteristics, methods to reduce subjective bias and statistical analysis) (Rice et al., 2008; Kilkenny et al., 2009; Vesterinen et al., 2010). During the process of conducting a literature search for this report, it was difficult to identify adverse events and opportunities for refinement in epilepsy studies due to the lack of reporting in this area. Use of the ARRIVE guidelines can help to ensure animal studies in the epilepsy research field are reported comprehensively; improving their interpretation and replication (to build upon the existing knowledge base) and enhancing the feasibility of systematic reviews and retrospective analysis of the preclinical literature. In addition, the use of more descriptive, standardised wording for recording adverse effects should lead to capture of more accurate and meaningful information, allowing improved decision making in respect to the severity of the adverse effects. The survey asked researchers if they use the ARRIVE guidelines when reporting animal research; 16/60 respondents (27%) used the guidelines, 12/60 respondents (20%) did not, and 30/60 (50%) were unaware of the guidelines. Endorsement of the ARRIVE guidelines by journals publishing animal studies in epilepsy would help to increase their use.

The survey asked researchers "Do you participate in data sharing? (e.g. Code Analysis Repository & Modelling for e-Neuroscience (CARMEN) (http://www.carmen.org.uk/) and the NIH-funded International Epilepsy Electrophysiology Portal)". Only 7/60 respondents (12%) reported doing so. The ILAE/AES Translational Research Task Force is currently generating common data elements (CDE) and guidelines for preclinical epilepsy research. These will include CDE modules for generation of epilepsy models, pre- and postoperative care, monitoring of seizure susceptibility and seizures, behaviour and cognition, video-EEG monitoring in young rodents, and imaging (Galanopoulou et al., 2013). These resources are expected to greatly facilitate harmonisation of experimental procedures between laboratories (e.g. in preclinical pharmacological or biomarker studies) and standardisation of the collection and recording of experimental details, resulting in more reliable data comparison and data sharing between laboratories.

Publication bias is a recognised problem in preclinical and clinical research, with potential impacts on scientific progress using animal models (Sena et al., 2010). The survey asked researchers how often they reported negative data; 13/56 respondents (23%) always reported negative data, 24/56 (43%) sometimes did so, 9/56 (16%) rarely did so, and 10/56 (18%) never did so. Of those who published negative results, 50% published in peer-reviewed journals (although this was reported to be challenging), posters and oral presentations.

Recommendations:

24. 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).
25. 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).
26. 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; Sena et al., 2010, Level I+).

4. Model-specific welfare considerations

Induction methods include systemic injections (e.g. pilocarpine, kainic acid), intracerebral injections (e.g. kainic acid, tetanus toxin) and electrical stimulation (both kindling and self-sustaining SE). Variations in the details of each induction method can have major impacts on the frequency, duration and severity of the resulting seizures. The optimal range for each of these features of the chronic epilepsies depends on the purpose of the research. At one extreme, basic research on the process of epileptogenesis may benefit from a long latent period and low seizure frequency, although progression to spontaneous seizures should be confirmed in each experimental cohort. Translational research on treatment, and basic research on seizure mechanisms, generally requires more frequent seizures: designing experiments with the power to detect reductions in seizure frequency becomes easier when seizures are frequent.

The Working Group has developed a framework (Table 5) for the identification of potential adverse effects associated with epilepsy models and the available refinement opportunities, based on the approach taken by a European Commission expert working group on severity assessment (http://ec.europa.eu/environment/chemicals/lab_animals/pdf/examples.pdf). Many of the adverse effects observed in animal models of epilepsy are not exclusively prevalent in epilepsy models; therefore the framework is divided into (a) generic adverse effects and (b) epilepsy-specific adverse effects.

A comprehensive review of all epilepsy models surveyed (Table 3) is beyond the scope of this report. However, some model-specific adverse effects are identified below with suggestions for refinement. It is recommended that researchers identify and review refinement opportunities for each epilepsy model using the framework provided (Table 5).

Recommendations:

27. The refinement opportunities framework (Table 5) 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; EU Severity Assessment Framework, Level IV).

Table 5
Model specific refinements.

(a) Generic adverse effects and refinements

Procedures: What are the animals subjected to?	Potential adverse effects/co-morbidities	Risk	Possible refinements to reduce suffering	Control measures/Humane endpoints
Transient interference with the brain (e.g. stereotaxic injection, lesioning)	Death due to anaesthesia	Low	Informed choice of anaesthetic (seek veterinary advice) Trained and competent staff. Provide further training of staff if deaths exceed defined minimal levels Animal in optimum physiological condition prior to anaesthesia Close supervision of animals Maintenance of body temperature	
	Infection	Low with good practice	Sterile procedures to minimise risk of contamination Administer antibiotics	Animal euthanised if clinical signs of infection are present and cannot be controlled
	Post-surgery pain	Low with good practice	Perioperative analgesia taking full account of pharmacokinetics Monitor for signs of pain	Animal euthanised if clinical signs of pain cannot be ameliorated
	Failure of sutures	Low to moderate	Clinical scoring system to assess welfare Skilled surgeon. Provide training for staff if failures repeat in several animals	To be assessed by veterinarian and repaired under anaesthesia. Animals will be humanely euthanised if more than one failure occurs
	Dehydration	Low with good practice	Hydration with fluid replacement *via* intraperitoneal, oral or subcutaneous route Glucose saline injection and moist and/or appealing food	Monitor weight, body condition and fluid consumption. Kill animal if it cannot maintain good hydration after defined period of recovery
Implantation of devices (e.g. electrodes, telemetry, cannulae, microdialysis probes, optogenetics)	Interference with specific brain systems	Low	Avoid implantations that could interfere with primary sensory and motor areas unless justified by careful balancing of benefits and harms	Ethical review
	Inflammatory and other tissue reactions to foreign bodies	Low with appropriate materials	Choose biocompatible materials Use (histo)pathology to determine whether reactions are excessive	Kill if inflammatory reactions are evident in the living animal
	Interference with behaviour after recovery	Low with appropriate design	Devices to be of size, weight and location to allow animals to maintain themselves in good condition and perform natural behaviours (e.g. eating, grooming, rearing)	
Use of previously implanted devices	Damage to head stage	Low with appropriate design and housing	Counterbalanced swivel commutator used for connections in order to minimise trauma Animals housed in special cages to avoid head stage being trapped or knocked inside the cage Use cage enrichment which reduces the chances of damage to the head stage	Animals to be euthanised immediately if head stage is lost
	Loosening of devices	Moderate	Take care to attach head-mounted devices with robust methods (e.g. cement, skull screws) Assess integrity of device regularly	Consider whether repair is possible. Kill animal if not

Table 5 (*continued*)

(b) Epilepsy specific adverse effects and refinements

Procedures: What are the animals subjected to?	Potential adverse effects/co-morbidities	Risk	Possible refinements to reduce suffering	Control measures/Humane endpoints
Intracerebral injection of epileptogenic agent	Adverse reaction to agent (batch dependent)	Low	Assay new batches of agent if possible. Test new batches of agent on small numbers of animals and adjust dose to achieve required frequency and classes of seizures	Animal euthanised immediately if unacceptable adverse effects develop
Induction by SE	Prolonged SE induced by stimulation or systemic injection/s of epileptogenic agent	High but can be managed by appropriate control measures	Anticonvulsants to be given if self-sustaining SE continues beyond preset limit (e.g. 3 h)	
	Individual variation in sensitivity to systemically injected epileptogenic agents	Moderate	Consider administering agent by repeated small doses until SE starts. More sensitive animals will require fewer doses and thus will avoid risk of overdose (Hellier et al., 1998)	
	SE induced by electrical stimulation	Moderate	Stimulation to be stopped if animals display "explosive" running/jumping behaviour that could result in injury to animal or head stage	
	Depressed consciousness after SE	High but can be managed by appropriate care	Maintain body temperature and hydration and nurse animals until they are walking and drinking	
Traumatic brain injury	Craniotomy		Use of artificial bone	Animal euthanised if signs of increased intracranial pressure (e.g. somatosensory hypersensitivity, breathing problems)
	Hemiparesis		Close supervision of animals Maintenance of temperature, hydration, feeding	
Development of spontaneous seizures	Loss of weight or general condition	Low for most models	Regular assessment of animal condition by the care and scientific teams using bespoke score sheets	Animals to be fed a mashed diet
	Increased stress and anxiety	Moderate	Provide environmental enrichment to reduce stress (e.g. rodent castle for animals with head stages)	
	Hyper-reactivity/aggression	Moderate	Avoid startling or surprising the animals (e.g. loud noises or sudden movements of humans). Ensure the animals are placed in a quiet room with minimal disturbance Avoid unnecessary handling of animals (e.g. plan cage changes around the hypersensitive period upon seizure onset) Have the same experimenter/technician handling the animal regularly If possible, group house animals	
	Wild running or jumping: risk of injury	Low	Design enclosure to minimise risks of damaging collisions	
	Persistent seizures with difficulty drinking/eating	Very low for most models	Treat with anticonvulsant (intraperitoneal injection of diazepam)	Animals euthanised if there is no response from treatment within 15 min
Administration of pharmaceutical substances, viral vectors, labelling substances	Depressed consciousness and cardiorespiratory depression	Low to high, depending on the substance	Maintain body temperature Hydration with fluid replacement *via* intraperitoneal, oral or subcutaneous route Nursed until walking and drinking	
Repeated behavioural assessment (motor, cognitive motivational) Not restricted to epilepsy models	Risk of animal falling during Rotarod assessment	High	Provide padding to break the fall	
	Loss of bodyweight due to withholding of feed.	High	Provide animals with a special palatable diet (gels, nuts) Weigh animals daily (twice daily)?	Bodyweight below 85% of the weight at entry to protocol or below 85% of the weight that is normal for species, strain, sex and age

4.1. Models dependent on initial SE

Most common methods of inducing experimental models of temporal lobe epilepsy use an initial period of SE, which carries significant risks of mortality (e.g. early versions of these models having mortality rates of up to 60%) (Curia et al., 2008). Much effort has been devoted to reducing mortality during and immediately after SE. One common refinement is to control the duration of SE, or at least the convulsive consequences of SE, using appropriate anti-convulsant drugs; reducing the duration of SE has been shown to reduce mortality (Curia et al., 2008). Another is to deliver additional care following SE to ensure that animals maintain sufficient intake of food and water, and maintain their body temperature; adopting appropriate welfare protocols has been shown to increase survival up to 70% (Longo et al., 2003).

Pilocarpine-induced SE: The pilocarpine model is a widely used rodent model of temporal lobe epilepsy (Curia et al., 2008). Many modifications of the model exist which have been driven not only by the aims of the experiment but also by problems such as high-mortality rates and the reliability of seizure induction. It is therefore a good example of how models can be refined. The efficacy and mortality of the pilocarpine model has been reviewed in some detail and various research groups have modified protocols by using different doses of pilocarpine (Longo et al., 2003), titrating pilocarpine to the individual seizure threshold by giving several low doses, pre-treatment procedures, animal strain and species (Curia et al., 2008). The pilocarpine model (at 300–400 mg/kg) is associated with mortality rates around 30–55% for male Wistar rats, but can be lower for Sprague-Dawley rats (Turski et al., 1983, 1989; Cavalheiro et al., 1991; Liu et al., 1994; Esclapez et al., 1999; Curia et al., 2008). Peripheral cholinergic effects (pilocarpine causes changes in cardiovascular function, excessive salivation, diarrhoea and dehydration) can be antagonised by subcutaneous administration of 1 mg/kg methyl scopolamine 30 min before injection of pilocarpine. The side-effects of anticholinergic agents can include drying of the mucus membranes, and reduction in tear formation; use of ocular lubricants (e.g. "liquid-tears") can reduce the impact of the latter problem. Increasing general hydration state by fluid therapy (by mouth or parenterally) can also be beneficial. When implementing these interventions it may be important to apply them to control groups also. After a defined duration of SE (time period should be adjusted to research aim and protocol, e.g. 40–60 min) administration of 8–20 mg/kg diazepam with or without ketamine (Martin and Kapur, 2008) can be used to decrease the severity of behavioural seizures, reduce seizure severity, and reduce mortality rates. Glucose depots can be injected subcutaneously 1 h following diazepam treatment to help animals to recover faster from SE. This can be further refined by titrating pilocarpine to the individual seizure threshold by giving several low doses of pilocarpine, which has been shown to also efficiently produce SE and subsequently chronic epilepsy with lower mortality rates than the aforementioned pilocarpine models (Glien et al., 2001). The mortality with this approach can be further improved when rodents are given a central muscle relaxant (xylazine) reducing the severity of the convulsions but not affecting electrographic seizures (Yang et al., 2006). Mortality and suffering can be further reduced by optimising perioperative care.

Kainic acid-induced SE: Systemic kainic acid induces SE and can be associated with high mortality in adults. Mortality in adult rats has been reduced by administering kainic acid in several small doses, repeated every 30–60 min, until each rat reached SE (Hellier and Dudek, 2005). This approach reduced mortality to 15% while achieving spontaneous seizures in 98% of animals. Tailoring the dose to individual rats avoids the most sensitive rats being at risk of death and the least not developing chronic epilepsy. Experimental induction methods may result in a sub-population of animals that do not show an obvious epileptic phenotype.

Electrical stimulation-induced SE: SE can also be induced by electrical stimulation of the brain. As with other models dependent on initial SE, the duration of a self-sustaining SE can be terminated by drugs. If SE is not self-sustaining then stimulation can be terminated, both to control duration and limit "explosive" running or jumping that could result in injury to the animal or damage to the head-stage.

4.2. Models not involving SE

Chronic epilepsy can be induced by methods that do not cause SE. One is tetanus toxin injected into the hippocampus or neocortex, both of which result in spontaneous seizures, usually after a latent period of a few days to a week or two. Dose matters: 1000 minimum lethal doses (MLD) proved lethal to 7/10 rats (Bagetta et al., 1990), while 6–12 MLD had no mortality and induced reliable epileptic seizures (Mellanby et al., 1977). One complication is that toxicity can vary between batches, with both production and transport having an impact; duration and conditions of storage also may lead to loss of toxicity. Ideally the whole toxin should be assayed before use, but local regulations may not permit *in vivo* assays.

Traumatic brain injury (TBI): In humans the risk of epilepsy after TBI depends on its severity, ranging from about 4% (mild TBI) to 53% (penetrating injury) (Annegers et al., 1998). Over the past 40 years, a large number of models of TBI have been developed to investigate the pathologies caused by TBI and mechanisms of consequent recovery (Xiong et al., 2013). Only recently, intensive EEG monitoring studies have been conducted to determine if animals with TBI also develop epilepsy. The most commonly investigated models are controlled cortical impact (CCI) and fluid-percussion injury (FPI). Depending on the injury severity and genetic background, epilepsy develops in up to 50% of animals, even though some investigators have reported occurrence of spontaneous seizures in 100% of animals (D'Ambrosio et al., 2004; Pitkänen and Immonen, 2014). Acute (<48 h) mortality depends on the injury severity, (e.g. 30% after severe TBI), relating to increased intracranial pressure. Even though acute seizures can occur in >30% of animals, seizure- or SE-related mortality have not been reported at acute post-TBI phase. Compared to SE models, the frequency of late (>1 week post-TBI) spontaneous seizures is much lower. Acute post-injury care should focus on treatment of possible skin irritations or infections around craniotomy, follow-up of weight, fluid balance (animals can have severe hemiparesis during the first two weeks post-injury) and maintenance of body temperature. When animals are implanted with electrodes, the same follow-up procedures apply as in other models (Table 5).

Absence models: The most commonly used models of absence seizures are the polygenic inbred Genetic Absence Epilepsy Rats from Strasbourg (GAERS) (Danober et al., 1998), Wistar Albino Glaxo/Rijswijk (WAG) rats, (Van Luijtelaar and Zobeiri, 2014) and the spontaneous monogenic mutant mice stargazer, tottering and lethargic (Maheshwari and Noebels, 2014). Many mouse strains are listed in the Jackson Laboratory database as showing an absence phenotype, and genetic manipulations of voltage- and transmitter-gated channels have been reported to lead to the expression of spike-and-wave discharges (Maheshwari and Noebels, 2014). In contrast to human absence, however, both rodent models have a higher frequency of spike-and-wave discharges and seizures persist for their entire life span. Absence seizures carry minimal adverse effects and are thus in general kept under standard husbandry conditions; nor have they been reported to express any adverse behavioural signs of suffering even after 1 or 2 years with absences. In many cases, data is unavailable on the co-expression of the behavioural components (i.e. immobility, vibrissae twitching, *etc.*) of absence seizures in rodents. Spontaneous mutant mouse models may carry additional behavioural phenotypes (e.g. mild

to moderate ataxia) which may become an issue, in particular after implantation of intracranial electrodes or other intracerebral devices. Increased anxiety and depression-like behaviour have been reported in WAG and GAERS rats, suggesting the presence of comorbidity in these rat absence models (Jones et al., 2008; Van Luijtelaar, 2011; Epps and Weinshenker, 2013).

Genetic models: Increasingly, genetically-modified animals are used in epilepsy research (Fig. 1) and the number of identified human epileptogenic mutations and genes is rising at an accelerated pace (Guerrini et al., 2014). Besides spontaneous mutant animals identified by phenotypic screening, genetic modifications can be generated by transgenesis, chemical mutagenesis or gene targeting (Mantegazza et al., 2010). Transgenic models may not reproduce real pathophysiological conditions as accurately as other models (even if there are exceptions, e.g. Peters et al., 2005), but they are relatively easy to generate and not very expensive, allowing small-scale screening of mutants. Chemical mutagenesis can be used within a phenotype-driven strategy (forward genetics) in order to generate animal models of epilepsy; this generates a large number of mutant mice that are screened and excluded if they do not show an appropriate phenotype. Gene targeting is the best available method for accurately reproducing genetic conditions, pathological conditions and phenotypes of human disease. Recent gene targeting techniques allow the introduction of specific mutations in different species at reduced cost and time. Refinement opportunities for the generation, management and care of genetically-modified animals have been reviewed elsewhere (Robinson et al., 2004). In addition to these, specific refinements related to breeding strategies and genotyping should be considered.

5. Discussion and conclusions

Animal models play a key role in epilepsy research but their use is associated with considerable welfare cost to the animals involved. As the field of epilepsy research evolves and moves towards studies of epileptogenesis, potentially more animals will be used. The 3Rs principles should be considered during the design, conduct and reporting of *in vivo* epilepsy research projects. Where animals are used, the onus is on the researcher to minimise avoidable harm, for scientific and ethical reasons. The Working Group has identified a number of opportunities for refinement of the use of rodent models of epilepsy and seizures. Implementation of the recommendations will not only minimise animal suffering but also can potentially improve the quality of scientific data derived from the animals, therefore maximising animal use. Some adverse effects may be unavoidable; in such cases, assessment of the balance between the potential scientific and medical benefits of the research, and the likely harms to the animals involved requires careful consideration; a higher welfare impact requires a higher level of justification.

5.1. 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. Funding opportunities are available to address these data gaps (e.g. NC3Rs funding schemes: www.nc3rs.org.uk/funding).

- 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 SE (see Section 3.1).
- Technological advancement of electrophysiological devices with increased biocompatibility and reliability to allow more data to be acquired per animal (see Section 3.3).
- Automated analysis tools to allow high throughput analysis of EEG data and maximise animal use (see Section 3.3). 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.
- 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.
- Increased understanding of the interactions of anaesthetic agents and common analgesics on seizure susceptibility and intensity so that more informed choices can be made (see Section 3.4).
- The use and relevance of seizure scoring systems with different animal models of epilepsy and for different age groups (see Section 3.5). The Racine's scale and its adaptations are commonly used but are not generalisable to all animal models of seizures and epilepsy.
- The effects on welfare of group housing animals prone to seizures, in particular instrumented animals (see Section 3.7).

A focused effort from the epilepsy research community to address these research objectives could extend and provide higher levels of evidence in support of the Working Group's recommendations and help to define contemporary best practice in this area of research.

5.2. Recommendations

A summary of the recommendations in the report is provided below:

1. 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).
2. Assessment of the harms to animals and balancing these against the potential benefits of the research, should take account of the lifetime experience of the animals and the whole epilepsy syndrome (not just seizures). The greater the animal welfare cost, the greater the strength of justification needed in terms of scientific and/or medical benefit (Grade D).
3. Variations in the strain, genetic background, source, age and sex of animals can influence seizure susceptibility and mortality (e.g. Grade B for strain; Schauwecker, 2011, Level II; Grade A for age: Thompson et al., 1991, Level I, Pierson and Swann, 1988, Level I+; Grade A for sex; Scharfman and MacLusky, 2014, Level II; Grade A for source; Borges et al., 2003, Level I). The variability should be taken into consideration when designing and conducting studies and adequate measures taken to reduce experimental bias. The strain, source, age and sex of animals used in studies should be consistent, and reported in publications.
4. If using genetically-modified mice, the 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; Bourdi et al., 2011, Level I).
5. Given the evidence for sex-specific effects on epileptogenesis, 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; Scharfman et al., 2005, Level I).
6. 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).
7. Research personnel should be adequately trained and competent in the manual skills for appropriate handling and restraint of animals for the administration of substances. Picking up mice by the tail should be avoided as this induces aversion and high anxiety; animals should be picked up by a non-aversive method (e.g. handling tunnels or cupping) (Grade A; Gouveia and Hurst, 2013, Level I+).
8. The experimental setup should be maximally effective in delivering the research objectives while prioritising animal welfare and minimising interference with behaviour, especially in behavioural studies where the instrumentation for seizure recording may impede movement and significantly alter behaviour (Grade D).
9. Wherever possible, radiotelemetry should be used in preference to tethered systems for chronic electrophysiological recordings (Grade D).
10. 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 (e.g. eating, drinking, grooming and rearing) (Grade C; Morton et al., 2003; Hawkins et al., 2004, Level III).
11. 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) (see recommendation 15).
12. Animals should be allowed sufficient time to recover following surgical procedures using anaesthesia, before subsequent recordings/measurements are taken (Grade A; Culley et al., 2004, Level I+).
13. Steps should be taken to identify, assess and alleviate pain following procedures requiring surgery. Appropriate pain relief should be provided and reported in publications. Choice of analgesic should be made with care based on veterinary advice and taking into account evidence of potential interference with the science (Grade C; Tremoleda et al., 2012, Level III).
14. Topical antibiotics should be used for simple surgical procedures and prophylactic antibiotics used for implantation procedures if appropriate, based on veterinary advice (Grade D).
15. A modified food source should be provided to encourage eating and prevent weight loss following surgery and/or seizure induction (Grade A; Jedrzejko and Persinger, 2001, Level I). This should be introduced prior to surgery to ensure familiarisation and consumption. Food and drink should be accessible from the floor of the cage (Grade D).
16. 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).
17. 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).
18. 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; Morton, 1999, Level III). This should take into consideration the current legal framework, *scientific*, *justifiable* and *unpredicted endpoints* (see Glossary) and the results of welfare assessments.
19. Mice and rats should be socially housed unless there are compelling scientific or animal health reasons for single housing. Each case should be carefully evaluated; weighing the welfare benefits and costs (Grade A; Võikar et al., 2005; Kalliokoski et al., 2014, Level I).
20. 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).
21. 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; Mellanby et al., 1981, Level I).
22. Environmental enrichment should be provided to allow animals to express naturalistic behaviours unless there is a justified reason to withhold it (Grade A; Koh et al., 2007, Level I+).
23. Environmental enrichment should be consistent in the home cage to reduce variability (Grade A; Fares et al., 2013, Level I+). Enrichment protocols should be described carefully and detailed in published manuscripts to allow others to adopt a similar approach and reduce inter-laboratory variations.
24. 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).
25. 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).
26. 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; Sena et al., 2010, Level I+).
27. The refinement opportunities framework (Table 5) 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; EU Severity Assessment Framework, Level IV).

Conflict of interest statement

None of the authors has any conflict of interest to disclose.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jneumeth.2015.09.007.

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Glossary

This glossary provides definitions of terms covered in the report in context of animal models of epilepsy and seizures. **Ataxia**: Lack of ability to coordinate muscle movements.

AWERB (Animal Welfare and Ethical Review Body): Committee responsible for ethical review of animal research projects and local oversight of animal use in the United Kingdom.

Catalepsy: Muscular rigidity and fixity of posture.

Comorbidity: Presence of one or more additional disorders co-occurring with primary disease.

Epilepsy: Conceptual definition: a disorder of the brain characterised by an enduring predisposition to generate unprovoked epileptic seizures and by the neurobiological, cognitive, psychological, and social consequences of this condition (Fisher et al., 2005). Operational definition: (i) at least two unprovoked (or reflex) seizures occurring more than 24 h apart; (ii) one unprovoked (or reflex) seizure

and a probability of further seizures similar to the general recurrence risk after two unprovoked seizures (at least 60%) occurring over the next 10 years; and (iii) diagnosis of an epilepsy syndrome (Fisher et al., 2014).

Epileptogenesis: The development and extension of tissue capable of generating spontaneous seizures, resulting in (a) the development of an epileptic condition and/or (b) progression of epilepsy after the condition is established. Recently a Working Group of the International League Against Epilepsy revised the terminology related to the term epileptogenesis and provided recommendations for conducting anti-epileptogenesis studies (Pitkänen et al., 2013).

IACUC (Institutional Animal Care and Use Committee): Committee responsible for ethical review of research protocols and evaluation of institution animal care and use in the United States and other counties.

Interictal: The period between seizures that is characteristic of an epilepsy disorder.

Justifiable endpoint: Pain or suffering beyond this point would be unacceptable and not increase the scientific yield. This is based on a harm/benefit justification and needs to be identified and defined for each experiment between scientists, the local ethical or animal care and use committee and regulators. If an experiment causes any pain or suffering, the benefits, which might be achieved by the experiment, need to outweigh the level of expected suffering.

Mydriasis: Dilation of the pupil.

NSAIDs (non-steroidal anti-inflammatory drugs): A class of analgesics that act by inhibiting enzymes associated with prostaglandin and leukotriene production, and so reduce pain and inflammation.

Ocular keratitis: Inflammation in the cornea.

Ptosis: Drooping or falling of the eyelid.

Scientific endpoint: The point when the objective of the experiment has been achieved and any suffering beyond this point should be avoided, as there is no further scientific gain (Morton, 1999). The scientific endpoint needs to be ethically justifiable and falls within the limits of permissible suffering (Ashall, 2014).

Seizure: The transient occurrence of signs due to abnormal excessive or synchronous neuronal activity in the brain (Fisher et al., 2005).

Status epilepticus: A prolonged seizure usually lasting more than 30 min. The duration of SE should be reported in publications.

Straub tail: Abnormal posture and rigidity of the tail.

Unexpected endpoint: Either suffering which is more severe than anticipated at the outset of the experiment or unexpected because of an unrelated illness, accident or non-anticipated adverse effect. This can occur following an unrelated illness, accident or through unexpected adverse effects of the experiment, these are not considered in the cost/benefit justification (Ashall, 2014).