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Review Article

Refining rodent models of spinal cord injury

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ABSTRACT

This report was produced by an Expert Working Group (EWG) consisting of UK-based researchers, veterinarians and regulators of animal experiments with specialist knowledge of the use of animal models of spinal cord injury (SCI). It aims to facilitate the implementation of the Three Rs (Replacement, Reduction and Refinement), with an emphasis on refinement. Specific animal welfare issues were identified and discussed, and practical measures proposed, with the aim of reducing animal use and suffering, reducing experimental variability, and increasing translatability within this critically important research field.

1. Action points

The report includes a number of recommendations to improve animal welfare in, and translation of, preclinical models of SCI, many of which are highlighted in Table 1. These recommendations represent some key points that were raised and discussed during meetings of the EWG and during the preparation of this paper.

2. Introduction

SCI is a devastating neurological condition which impacts on the lives of many people worldwide. For example, around 1200 people in the UK and 17,000 in the US are paralysed each year, with a worldwide estimated 27 million people living with the condition (Centre, N, 2019; GBD 2016 Traumatic Brain Injury and Spinal Cord Injury Collaborators, 2019). SCI can lead not only to loss of sensory and motor function, but also to other significant problems such as sexual, bladder and bowel dysfunction, infections, chronic pain and cardiac and respiratory issues. Current treatment options for SCI in humans are limited and mainly focus on adaptive and rehabilitative therapies as well as the management of secondary complications (Ahuja et al., 2017; Rogers and Todd, 2016). While these approaches are important, there is an urgent need

Abbreviations: EWG, Expert Working Group; SCI, Spinal Cord Injury; NSAID, Non-Steroidal Anti-Inflammatory Drug; IT, Immediate treatment required; IE, Immediate Euthanasia; BAR, Bright, Alert, Responsive; QAR, Quiet, Alert, Responsive

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Table 1
Recommendations for good practice in SCI research.

	The Working Group makes the following recommendations:
1	Where cervical level injuries are necessary, injuries should be confined to one side or use a mild/moderate insult where possible.
2	Give careful consideration to the balance between translatability and harms to the animal, via discussion with animal technologists, veterinarians and the local ethics or animal care and use committee.
3	The SCI research community should share knowledge on effective strategies to avoid adverse effects, including those listed in Table 2b, through scientific meetings, publications and online resources.
4	Model selection should always be based on causing the least harm to the animals whilst still enabling the scientific question to be answered.
5	Provide environmental enrichment tailored to the animal's level of disability and evaluated where necessary; provide information on enrichment in publications.
6	Tailor anaesthetic protocols to the species, strain and model, in collaboration with a laboratory animal veterinarian.
7	Adequate analgesia should always be given in circumstances where animals may experience pain. Compelling scientific evidence must be provided to withhold pain relief.
8	Do not administer antibiotics routinely, but ensure they are provided if necessary.
9	Consider whether male and female animals can both be used as subjects in a given study. Bladder expression is easier in female rodents, and the risk of complications is reduced when compared to males, but using only females introduces a sex bias. This issue should be discussed with the ethics committee on a case by case basis.
10	Humane endpoints should be defined a priori (and revised periodically in the light of ongoing expertise). Advice can be sought from the ethics committee.
11	Ensure that a structured, objective monitoring system has been tailored to each protocol, and that this is reviewed as necessary to reduce the risk of important indicators being overlooked.
12	Plan and publish SCI research in accordance with good practice guidelines e.g. PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence), ARRIVE (Animal Research: Reporting of In Vivo Experiments), GSPC (Gold Standard Gold Standard Publication checklist).
13	Consider working with national or international organisations or funding bodies to help develop/support training opportunities for new SCI researchers to develop the necessary skills to perform SCI models competently and reproducibly.

Several key issues were identified during the expert working group (EWG) discussions and some recommendations were made, these are included here and highlighted in the text.

for the development of condition-modifying or regenerative therapies that will restore function and improve quality of life for patients.

Pre-clinical research into SCI is currently dominated by the use of animal models. In an attempt to recapitulate many of the clinical features of SCI, these models can involve causing significant injury, with consequent functional losses to the animal, and have the potential to induce high levels of suffering. This leads to strong ethical imperatives to ensure robust implementation of the Three Rs (Replacement, Reduction and Refinement) and to maximise the translational validity of animal models of SCI. As it is also widely acknowledged that higher standards of animal welfare go hand in hand with better science, this EWG was established to identify welfare issues associated with modelling SCI in animals and set out practical refinements to improve both animal welfare and scientific quality (Poole, 1997; Baumans, 2005; Lloyd et al., 2008; Wurbel, 2001; Everds et al., 2013; Hall et al., 2015).

Whilst SCI has been studied in a wide range of species including rats, mice, cats, dogs, mini-pigs and non-human primates, this report will focus on mice and rats (hereafter referred to as '*rodents*' in the report) since these species feature predominantly in the pre-clinical SCI literature. The EWG comprises UK-based researchers, veterinarians and regulators of animal experiments with expertise in SCI research, but this document is intended to be applicable worldwide, as the requirement to minimise pain, suffering and distress is central to international legislation and guidance regarding animal experiments, e.g. in the UK, EU, USA, Canada, Australia and China (Home Office, 2014; European Commission, 2010; Committee for the Update of the Guide for the Care and Use of Laboratory Animals; National Research Council, 2010; Canadian Council on Animal Care (1993; revised), 2017; National Health and Medical Research Council, 2013).

The EWG has set out to provide guidance for both established SCI researchers and those new to the field, with respect to (i) selecting the most suitable model to use in their research and, if animals are used, (ii) improving welfare and reducing suffering, and (iii) implementing practical steps to improve rigour and translatability, to ensure that benefits of animal use are optimised.

3. General considerations for preclinical studies of spinal cord injury

SCI that occurs as a result of a traumatic event (such as a motor vehicle accident, fall, sporting injury or act of violence) is a multifactorial process involving a complex series of molecular and cellular events. The initial traumatic injury causes local cellular damage which is followed by secondary reactive processes, including ischaemia, inflammation, oedema, cell death, axonal degeneration, gliosis and formation of scar tissue (Ahuja et al., 2017; Liverman et al., 2005; Verhaagen and McDonald, 2012). The complexity of this secondary injury pathology is currently not possible to recapitulate in in vitro model systems and typically requires the use of experimental animals. As with many areas of pre-clinical research, SCI research faces some translational challenges. A number of the key issues regarding translation of SCI research have been summarised by Curt (Curt, 2012). All models, by definition, have limitations and these need to be considered and acknowledged.

3.1. *In silico and in vitro approaches*

Reliable in silico models for screening new potential therapeutics for SCI, whilst being highly desirable, are not yet available due to the lack of a comprehensive knowledge of the molecular and cellular mechanisms involved in SCI. However, in silico simulations are under development, and may be useful to model certain aspects of SCI (e.g. cervical spine loading during sporting activities) or mechanisms of action of treatment (e.g. design of neuromodulation protocols), in order to influence clinical and preclinical study design (Capogrosso et al., 2013; Cazzola et al., 2017; Moraud et al., 2016; Woolfe et al., 2007; Clermont et al., 2004; Vodovotz and Billiar, 2013). A next step could be the use of in silico technology (e.g. non-invasive 24/7 monitoring of welfare coupled with machine learning/artificial intelligence approaches) to better predict humane experimental endpoints, which can help to reduce animal use and suffering.

There is more progress with regard to the development and use of in vitro models to help understand the complex cellular mechanisms involved in SCI (and subsequent changes) and to help identify novel therapeutic targets. For example, in vitro technology, including using multiple cell types and three-dimensional structures, can have significant value (Slovinska et al., 2016; Mladinic and Nistry, 2013; Abu-Rub, and A., and Pandit, A., 2010; Jeong et al., 2011; Shrirao et al., 2018). These developments are in part due to ethical considerations and the complexity and cost of in vivo models. They have also arisen because in vitro systems permit detailed and controlled examination of specific mechanisms, including in human cells. In addition, in vitro model systems permit screening of novel pharmacological tools and potential therapies prior to testing in animal models, helping to reduce

and avoid animal use. It is outside the scope of this report to list all of the currently published in vitro models of SCI; many are discussed in the reviews cited above and in some recent papers (Shahriary et al., 2019; Bijland et al., 2019).

Despite this progress, complete replacement of animal models of SCI is unfortunately not currently possible. It should also be noted that some in vitro work may also raise ethical and welfare issues if animals are directly or indirectly used for the model system (e.g. where primary cell cultures are generated from animal tissues), or animal serum is required for cell or tissue culture; non-animal-derived defined alternatives are often available.

3.2. In vivo approaches

Where animal use can be justified on scientific grounds (e.g. following the harm-benefit analysis required under many national regulations), the choice of animal model should be guided not only by the scientific objectives, but also by animal welfare considerations, with the aim of minimising or avoiding suffering. The EWG views this as an integral part of the process of selection of the best scientific approach. The following sections describe issues relating to selecting animal models of SCI, including points to consider and recommendations from the EWG.

3.2.1. In vivo model selection

There are different ways to model SCI in animals and all approaches have intrinsic strengths and weaknesses (Kjell and Olson, 2016; Kwon et al., 2002; Zhang et al., 2014; Cheriyan et al., 2014). Broadly speaking, in vivo SCI models are characterised by the spinal level where the injury takes place (e.g. sacral, lumbar, thoracic, cervical) and the nature of the injury or lesion (e.g. contusion, compression, transection, hemisection). Model selection will depend on what aspect(s) of SCI is/are being investigated. The reviews referenced above (and others) provide information on the pros and cons of current SCI animal models from a translational perspective and the EWG does not wish to replicate this information here. However, at the time of writing there is comparatively little discussion in the literature of the animal welfare implications associated with different models – yet this is an essential consideration, bearing in mind that model selection should be determined by the likelihood of providing robust, translatable data whilst causing the minimum pain, suffering, distress or lasting harm (or severity[1]).

The overall severity of an SCI model, with respect to the impact on the animal, depends not only on the lesion type but also on the segment of the cord that is lesioned and the severity of the injury at that level. In general, lesions higher up the spine carry a higher welfare burden and, from an ethical perspective, should require robust justification. Complete transection at a cervical level is rarely performed because of the impact on the animal - this causes permanent paralysis in all four limbs, profound autonomic and cardiac deficits and difficult post-operative care (Kjell and Olson, 2016; Lujan et al., 2018), whereas moderate or mild bilateral contusion of the C5/6 cervical spinal cord results in transient paresis of all four limbs and with appropriate postoperative care can be managed effectively (James et al., 2015; Burnside et al., 2018). Although most clinical injuries occur in the cervical region (Kang et al., 2018), this results in a higher animal welfare burden than similar lesions lower in the spine. Many experiments demonstrating basic principles do not need to use cervical injury models, e.g. for studying tissue responses to injury, or the regenerative potential of a new therapeutic. For these studies lower level (e.g. thoracic) lesions can be sufficient. Cervical level injuries are typically used for more advanced pre-clinical evaluations, e.g. when efficacy of a therapy has previously been demonstrated in lower level injuries, or for assessing specific functions controlled by the cervical cord such as respiratory function, upper limb and skilled hand function. For these studies cervical level injuries are necessary. However, wherever possible the experimenter should consider confining injuries at the cervical level to one side or use a mild/moderate insult.

Recommendation: Where cervical level injuries are necessary, injuries should be confined to one side or use a mild/moderate insult where possible.

Similarly, within one spinal region (particularly for cervical and thoracic), there will be different burdens depending on the injury level. For example, lesions in the high (e.g., T3) but not low (e.g., T12) thoracic region and above may induce autonomic dysreflexia, potentially affecting cardiovascular and respiratory function and the ability to fight infection (Prüss et al., 2017). It is common to perform mild, moderate or even severe contusions to the low (e.g., T9/10) thoracic spinal cord, causing disability to hindlimbs without affecting respiratory or upper limb function. The balance between translatability and harms to the animal requires careful consideration, and discussion with animal technologists, veterinarians and the local ethics or animal care and use committee can be helpful in the decision-making process at the project design stage.

Recommendation: Give careful consideration to the balance between translatability and harms to the animal, via discussion with animal technologists, veterinarians and the local ethics or animal care and use committee.

Strain selection is also an important factor to consider, as morphologic, sensory and motor differences have been shown to exist between commonly used strains of laboratory rat and mouse (Webb et al., 2003; Basso et al., 2006). Indeed, rat strain selection has been reported to influence the development of chronic central pain following SCI and substrain differences (Sprague-Dawley rats from three different breeders) have been identified in spontaneous locomotor recovery (Mills et al., 2001; Kjell et al., 2013). Therefore, it is important to be aware that species/strain/substrain selection may bias the interpretation of true efficacy of a therapeutic tested with a view to successful translation to patients.

With regard to dorsal root crush models, one EWG member has found that 'docile' strains of rat (e.g. Lewis and Sprague Dawley) have a lower risk of autotomy (denervation-induced self biting; M. Andrews, personal communication; January 2018). It is important to discuss strain selection with local experts (animal care staff, veterinarians and local ethics or animal care and use committees) as well as with experienced SCI researchers. Any welfare concerns that arise from the use of a specific species or strain, and other strategies to avoid adverse effects and reduce severity, should be communicated to other groups, to help them prevent avoidable harms.

Recommendation: The SCI research community should share knowledge on effective strategies to avoid adverse effects, including those listed in Table 2b, through scientific meetings, publications and online resources.

Ultimately, model choice should be decided on the basis that the least invasive (causes the least welfare impact on the individual animal) approach is used that enables the scientific question to be answered.

Key to this process is a clear a priori definition of the scientific question being asked coupled with a detailed understanding of the strengths and limitations of the various models that are available. The EWG recommend that, within the context of the experimental question, model selection should be based on the following principles:

(i) Selecting the *type* and *location* of lesion that has least welfare/

[1] UK and EU legislation regulating animal use includes severity categories ranging from non-recovery (procedures carried out under general anaesthetic without recovery) through mild and moderate to severe (similar categorisation systems are used in the USA and Canada). Injury intensity within SCI research can also be classified as mild, moderate or severe, but a 'mild' injury to the spinal cord with respect to the nature and degree of injury will almost certainly cause more than 'mild' suffering, as defined by legislation, to the animal.

physical impact,
(ii) Considering partial as opposed to complete lesions if possible,
(iii) Researching and consulting on species and strains that will recover best from the proposed protocol.

Recommendation: Model selection should always be based on causing the least harm to the animals whilst still enabling the scientific question to be answered.

4. Potential adverse effects and how these can be refined

As well as addressing and refining harms due to experimental procedures and their after-effects, a useful complementary approach to reducing suffering is to set out the whole life experience of the animal and consider how each potentially painful or distressing event could be refined. The overall impact should be a significant reduction in severity. The overarching principle of this approach is the '*accumulation of marginal gains*', in which each individual refinement may not make a significant difference in itself, but when implemented all together the effect may be that a procedure is considerably less severe to each animal (Lilley and Jennings, 2013).

Tables 2a and 2b set out potential adverse effects that may be experienced by animals used in SCI studies, with suggested ways of ameliorating pain or distress in line with the 'accumulation of marginal gains' principle. Table 2a lists general adverse effects associated with surgery and behavioural training, and Table 2b sets out model-specific adverse effects. The EWG understands that not all of the suggested refinements will be feasible within every project, and additional text to supplement the Tables is set out below.

4.1. Housing, husbandry and care

Ramsey and colleagues have published a set of proposals for the care of rats with high-thoracic SCI (T3 complete transection), based on many years of experience with this model (Ramsey et al., 2010). The EWG recommends this paper as an excellent guide to refining housing and care for rodents following high thoracic SCI, although the principles it describes are applicable to other severe SCI models. Specifically, they focus on the lifetime experience of the animals, including:

- Acclimatisation (at least one week if animals are shipped in from external suppliers);
- Habituation to the post-operative diet *before* surgery;
- Housing refinements, e.g. low-reaching water bottles, matting on the floor to help mobility, refuges and social housing;
- Refined post-operative care, including optimal analgesia and frequent cage change to avoid pressure sores.

Table 2a
General adverse effects and refinements.

Potential adverse effect	How this may be refined
Discomfort or distress due to capture, handling and restraint	Catch and restrain animals using the most refined approach for the species; for example, catching mice by cupping in the hands, or in their home cage tunnel, instead of by the tail. Catching in the hands, or tunnel, is less aversive and induces less anxiety (Gouveia and Hurst, 2017; Gouveia and Hurst, 2013; Hurst and West, 2010). Similarly, rats should never be picked up by the tail. Following surgery, ensure that extra care is taken when handling animals to minimise discomfort and ensure that further damage does not occur. Handling should be gentle and empathetic throughout the animals' lives, to reduce distress. Habituation to handling techniques, especially in rats, will reduce anxiety and stress.
Stress during behavioural assessment training	Habituate animals to handling and behavioural testing methods prior to SCI surgery. This is essential to reduce stress following surgery, and to ensure animals unable to learn the behavioural tests will not be operated on. The acclimatisation period will depend on the test and apparatus, as well as the species and strain of animal, and some individuals may require more training than others.
Pain and infection risk due to surgery	Research and use the most effective and least aversive anaesthetic agent that is compatible with the scientific objectives. Provide appropriate peri- and post-operative analgesia, using a suitable multi-modal protocol, e.g. carprofen/buprenorphine. Use a regulated (homeothermic) system to maintain the body temperature of the animal during procedures and recovery. Use aseptic techniques: the EWG recommends working to standards set out in the Laboratory Animal Science Association (LASA) guidance on aseptic surgical technique (Jennings and Berdoy, 2016); antibiotics should not be used routinely. Ensure optimal surgical approach, and handle tissues gently during surgery, so as to minimise unwanted tissue damage. Ensure that the surgeon is adequately trained and competent; record individual surgeon-related postoperative outcomes including animal behaviour observations and analgesia requirements. Review these 'benchmark' data regularly.
Discomfort or distress during recovery and initial post-operative period	Keep animals homeothermic during the initial surgical recovery period, and beyond if shown to be beneficial. Warmed, quiet and darkened recovery cabinets (e.g. with air controllably warmed to 26 °C) can be useful (Keijer et al., 2019). Less preferred are heated mats or lamps: care must be taken to ensure that temperature is adequately controlled and not too high, with heat uniformly distributed, and ensuring that animals are sufficiently mobile to be able to move away to a cooler zone. Avoid excessive handling for 24 h post-surgery, e.g. no more than twice daily. Regularly review postoperative monitoring protocols, including use of structured recording systems such as score sheets.
Hunger and/or thirst due to difficulty accessing food and water during the recovery period	Provide moistened standard chow in accessible containers on the cage floor and soft/liquid nutrition until animals can access food independently; hand feed in the interim if necessary (e.g. by syringe) (Ramsey et al., 2010). Take proactive measures to prevent dehydration, e.g. by using water bottles with long spouts and/or providing hydrogel. If necessary, rehydrate with sub-cutaneous injections of saline pre-warmed to body temperature. Monitor body weight and food/water intake daily during recovery until body weight returns to the pre-operative value. Pay attention to any deviation from expected values/rate of recovery, monitoring the rate of change and duration of any plateaus as indicators of the quality of recovery. Give supplementary fluids (e.g. subcutaneous saline) to counter weight loss, which is principally due to dehydration. For example, if a rat loses 10 g in body weight acutely, supplement with 10 ml prewarmed saline.

List of general adverse effects that animals may experience in a SCI study and approaches to ameliorate these.

Table 2b
SCI model-specific adverse effects and refinement.

Potential adverse effect	Model(s) where relevant	How this may be refined
Impaired bladder function	Transection Contusion	Perform manual bladder expression with sufficient regularity (e.g. twice a day) to reduce the risk of bladder infection and discomfort, and wash genitals twice daily.
Urine scalding/urinary tract infection	Transection Contusion	Even when animals can urinate normally, hind limb paralysis can cause problems. Use appropriate absorbent litter to reduce the incidence and impact of urine scalding, e.g. a sheet of 140 gsm white crepe paper on top of standard litter such as Lignocel premium hygienic animal bedding. Alternatively add fenestrated matting with bedding above (Ramsey et al., 2010).
Constipation	Transection Contusion	Monitor animals carefully to enable early recognition of changes in bowel function. Mineral oil can be added to food and fluids to aid bowel function. A gentle enema of warm saline may also be beneficial.
Impaired locomotion	Transection Contusion	Provide plastic mesh or corrugated card on the cage floor to help animals move around the cage (always in conjunction with appropriate litter and nesting material, as a grid floor without litter is not appropriate) (Ramsey et al., 2010).
Autotomy	Contusion Compression Hemisection Dorsal rhizotomy	Strain selection may reduce incidence of autotomy (e.g. in EWG experience, Hooded Lister rats tend to have higher risk than Sprague Dawley and Lewis). Ensure that monitoring is adequate to rapidly identify animals at risk so that humane endpoints can be implemented. Consider prophylactic treatment using amitriptyline for models where autotomy is common (Sotocinal et al., 2011; Seltzer et al., 1989).
Difficulty feeding in the acute post-injury phase	All surgical models - particularly injuries at the cervical level	Provide moistened standard chow, fluid rich fruit (e.g. grapes/melon) or liquid/semi solid nutrition, (e.g. baby food) in the cage, with hand feeding via syringe if necessary. Introduce supplementary food before surgery so that animals recognise and are prepared to take the post-surgery diet.
Problems with hydration	All surgical models	Facilitate access to water by providing water bottles with longer spouts or provide hydration gel (e.g. HydroGel). Administer subcutaneous saline if necessary.
Pain associated with the model, i.e. following recovery from surgery	All surgical models	Consider using pain face/grimace scales to help identify acute pain, as part of a tailored welfare assessment protocol (Descovich et al., 2017). Use appropriate analgesia (e.g. gabapentin) if neuropathic pain develops following surgery (Baastrup et al., 2018).

List of SCI model specific adverse effects and approaches to ameliorate these.

Besides implementing tailored husbandry refinements such as the above, environmental enrichment is important for animals used in SCI protocols and should be provided wherever possible. There are two main reasons for this.

First, it is commonly acknowledged that environmental enrichment improves animal welfare. For example, recent versions of legislation, guidelines and codes of practice relating to laboratory animal use emphasise the importance of a stimulating environment to encourage appropriate natural behaviours (European Commission, 2010; National Research Council, 2009; CIOMS/ICLASInternational Guiding Principles for Biomedical Research, 2012). These can be facilitated by including group housing for social animals and environmental enrichment such as nesting material, refuges and chew blocks for rodents.

Second, environmental enrichment may aid recovery, by stimulation of locomotor activity, in some animal models (Faralli et al., 2013; Vachon et al., 2013; Lankhorst et al., 2001). Indeed, it may be argued that enriched caging is more reflective of human clinical practice, where physiotherapy will be used to promote recovery for patients. Enrichment should be tailored to the animal's level of disability, evaluated where necessary (e.g. if an item is new, or there are questions as to benefit) and clearly reported in publications.

Recommendation: Provide environmental enrichment tailored to the animal's level of disability and evaluated where necessary; provide information on enrichment in publications.

4.2. Surgery

Anaesthetic protocols and agents should be chosen in collaboration with a laboratory animal veterinarian and tailored to the species (and possibly the strain) being used. The duration of anaesthesia should generally be minimised, to facilitate recovery; inhalational anaesthesia (e.g. isoflurane) is preferred over longer-lasting injectable anaesthetics by some laboratories for improved post-operative recovery after cervical contusion injury (L Moon and E Bradbury labs, personal communication; February 2019) or long surgeries. In addition, the choice of anaesthetic agent may be influenced by the surgical equipment being used to stabilize animals for surgery (masks for inhalation of volatile agents may be too large or cumbersome in some experimental set-ups; some EWG members use reversible injectable anaesthetics for this reason such as ketamine combined with medetomidine (Domitor) that can be reversed by *atipamezole (Antisedan*). Some gaseous agents are aversive to some species and strains, which can cause distress; the attending veterinarian should be able to advise on preferable gaseous anaesthetics if these are used.

Recommendation: Tailor anaesthetic protocols to the species, strain and model, in collaboration with a laboratory animal veterinarian.

During and following surgery, mice and rats should be kept warm in order to mitigate the risk of hypothermia which can slow recovery and is a major risk factor for post-surgical mortality (Pottie et al., 2007; Flecknell, 2009). Incubators with regulated warm air systems (e.g. 26 °C) designed for animal recovery are preferred over heating blankets placed under the cage (Keijer et al., 2019). Following recovery, animals should be returned to their stable pairs, or groups, unless there are compelling scientific or animal welfare reasons for single housing. In cases of single housing, additional enrichment or stimulation should be provided to the animals.

4.3. Analgesia

Provision of pain-relieving drugs should be the default position for all potentially painful experimental procedures, unless there is compelling scientific justification otherwise. This is a legal requirement in the EU and UK. SCI is clearly an example of an experimental paradigm that has the potential to cause significant pain for laboratory animals. There are two aspects to the pain: that induced by the surgical procedure, and that which may arise from the SCI itself. The EWG recommends that provision of analgesia should be the default position during and after surgical SCI, unless specific evidence for its exclusion can be given. Multi-model analgesia should be considered including opioid (e.g. buprenorphine), non-steroidal anti-inflammatory drugs

(NSAID; e.g. carprofen, meloxicam) and local anaesthetics (e.g. bupivicaine) (Redaelli et al., 2019). Analgesics for neuropathic pain such as gabapentin or pregabalin could also be provided; indeed, pregabalin has been shown to reduce mechanical hypersensitivity following contusion injury in rats (Baastrup et al., 2018). The attending veterinarian should be consulted when selecting the most appropriate analgesia regimen. Without analgesia, uncontrolled pain will cause increased variability between animals (Carbone, 2011; Carbone, 2017). Assessment of pain in SCI animals can be challenging, especially if postural signs are lost due to the injury. The recent development of pain-face/grimace scales in rats, mice and a range of other mammals may provide a useful tool, if properly applied, to allow the assessment of acute pain as part of an integrated welfare assessment process (Sotocinal et al., 2011; Langford et al., 2010; Descovich et al., 2017).

Recommendation: Adequate analgesia should always be given in circumstances where animals may experience pain. Compelling scientific evidence must be provided to withhold pain relief.

In studies using dorsal root injury (rhizotomy, crush, avulsion; where the risk of autotomy can be high), consideration should be given to prophylactic treatment with amitriptyline which can decrease self-mutilation (Abad et al., 1989; Navarro et al., 1994). Indeed, amitriptyline is typically given to humans after avulsion injury and arguably, animals should be treated in the same way as humans in order for a model to have face validity (although potential interactions of pain-relieving analgesics with any therapeutic intervention should be considered) (Bruxelle et al., 1988).

4.4. Antibiotics

Surgical asepsis should negate the need for antibiotic use in the immediate post-surgical period, unless foreign material implants are being used. However, in SCI surgery there is a risk of bladder infection if animals cannot urinate spontaneously. Antibiotic use should be carefully considered, since long-term use of broad-spectrum antibiotics in large numbers of animals could lead to the development of antibiotic resistant bacteria, which would have broader implications and may impact upon all housed animals within research facilities (van den Bogaard and Stobberingh, 2000). Antibiotics can also induce gut dysbiosis, and it has been shown that an improved microbiome leads to better recovery after SCI (Kigerl et al., 2016). Urinalysis strips are available that permit early detection of urinary tract infection (UTI) and could be used to prompt antibiotic use only when required (Paquignon et al., 1993; Siska et al., 2016). Also, antibiotics can cause a variety of side effects and potentially interfere with the pharmacokinetics of therapeutic agents under investigation (Morris, 1995). Anecdotally, bladder infections are much rarer in rats with 'moderate' cervical contusion than 'moderate' thoracic contusions and antibiotics do not need to be used routinely after cervical contusion in rats. The attending veterinarian should be able to advise on the most appropriate approach to deal with the risk of infection, while still being able to achieve the scientific outcome.

Recommendation: Do not administer antibiotics routinely, but ensure they are provided if necessary.

4.5. Bladder function

Depending on the nature, spinal level and severity of SCI injury, bladder function can be impaired. It is important to ensure that urine is voided adequately with sufficient frequency until spontaneous voiding is re-established. Normal micturition in rats requires spinal and supraspinal circuitry to mediate contraction of the bladder detrusor and co-ordinated activation of the external urethral sphincter (Pikov and Wrathall, 2001). In T3 complete transection models and moderate T9/10 contusions (but not bilateral C5/6 contusions), for example, the bladder needs to be manually expressed 3–4 times a day during the initial post-surgery period, reducing to twice a day in models where reflexive micturition returns (Ramsey et al., 2010). It should be noted that bladder function recovery can vary between species - for example, rats with T9/10 contusions typically recover bladder function after 1–2 weeks. In mice with the same injury bladder function never recovers and they need manual bladder expression throughout the duration of the study. One technique to achieve manual emptying is shown in a recent Journal of Visualised Experiments (JOVE) article (Krishna et al., 2013).

During our discussions, the EWG suggested that bladder expression was easier in female rodents, due to the relative ease of bladder expression and lower risk of bladder infections and other complications (e.g. urethral blockages) when compared to males. This does however, raise the issue of the introduction of sex bias into SCI pre-clinical research as well as a potential issue with translation since around 80% of new cases of SCI occur in males (Wald and Wu, 2010; National Spinal Cord Injury Statistical Center: Facts and Figures at a Glance, 2016). This issue (animal welfare considerations in potential conflict with translation) should be evaluated on a case by case basis.

Recommendation: Consider whether male and female animals can both be used as subjects in a given study. Bladder expression is easier in female rodents, and the risk of complications is reduced when compared to males, but using only females introduces a sex bias. This issue should be discussed with the ethics committee on a case by case basis.

4.6. Pilot studies

Pilot studies are a useful way to evaluate the welfare impact of a particular procedure or intervention where prior knowledge is lacking. In particular, pilot studies can be used to evaluate a refinement of an existing procedure or model, for example the use of an alternative surgical approach or home-cage based behavioural assessment. However, although animals subsequently used in a full study should benefit, pilot studies demonstrating that a particular refinement is effective also have the potential to cause suffering and will require additional animal use. They should therefore be subject to a harm-benefit assessment.

4.7. Humane endpoints

A '*humane endpoint*' can be defined as the point at which an animal's pain and/or distress is terminated, minimised or reduced, by taking actions such as killing the animal humanely, ending the procedure or giving treatment to alleviate suffering (see http://www.humane-endpoints.info). Humane endpoints should be specific and tailored to each study and will depend upon factors including the aims of the study and the stage at which sufficient data are obtained. The scientific endpoint may correspond to the humane (welfare) endpoint – i.e. there is no benefit to keeping the animal alive any longer and therefore the experiment should end, usually by humanely killing the animal; in the context of SCI this is usually for the purpose of completing histological or other tissue analyses. Sometimes a predetermined '*severity limit*' has been set, such that the adverse welfare impact of the experiment cannot ethically or legally exceed a predefined level on ethical grounds. In these cases, local ethics or animal care and use committees and project evaluators may have input into defining humane endpoints.

In all other cases, experiments using living animals should have endpoints set which are sufficient to achieve the scientific endpoint but represent the most humane way to do this. Suggested humane endpoints for rodent models of SCI are given in Table 3; note that specific humane endpoints should be defined for each model, taking into account species/strain considerations.

The EWG believes that the use of early humane endpoints is an ethical obligation when using animals in SCI research and recommends that a dedicated welfare assessment system is used to help achieve objective and refined humane end points (see section 6 and Table 3). This may be a structured, objective recording system or a list of clinical

Table 3
Humane endpoints.

Clinical sign / welfare issue	Threshold for humane endpoint
Autotomy	1 phalange removed from 2 or more digits or 2 or more phalanges of 1 digit removed or paw becomes swollen and painful.
Pain/distress	Any unexpected pain/distress (not within the normal experience of the model) that does not resolve when treated with appropriate analgesia. For example, more than one of the following signs of severe pain and distress persisting for 1 h following administration of additional analgesia: • copious, persistent oculo-nasal discharge • unprovoked vocalisation • marked piloerection/staring coat
Body weight	Weight loss of > 20% unless other adverse welfare indicators are absent *and* weight loss is expected to be temporary.
Food intake	> 70% reduction in food intake, despite proactive support/hand feeding, for more than 72 h.
Bladder function	Persistent inability to urinate in models where bladder function is expected to recover (e.g. following moderate thoracic contusion injury in rats, reflexive bladder emptying should be restored within 2 weeks post injury). Bladder rupture following manual expression. Bladder infection that does not respond to treatment.
Paralysis	In models where partial or full recovery of movement is expected, persistent paralysis/lack of mobility/inability to bear weight. (e.g. following bilateral moderate contusion injury, weight bearing should be restored within 2 weeks; in dorsal root crush models, only transient weakness that resolves within 7 days is expected; in severe unilateral spinal cord injuries persistent paralysis is expected on the affected side).
Lethargy/apathy	Animals that do not respond to handling.
Wound healing	Post-surgical wounds that do not heal within expected timelines. Wound dehiscence that does not resolve following a single attempt to repair the wound. Infection following surgery that does not respond to veterinary intervention.
Wound scratching	Severe scratching that persists and the skin lesion is not reduced in size by 50% by 5 days.

List of clinical signs/welfare issues that, depending on the nature of the SCI study, may represent thresholds for intervention in order to prevent unnecessary suffering or distress.

signs and interventions. These require careful design and staff training and familiarisation. Attentive monitoring should be employed, at a frequency that reflects the time course and severity of the model so that suffering can be minimised, and humane endpoints effectively implemented.

There is an inherent dilemma associated with defining and implementing humane endpoints in potentially severe procedures. A balance must be reached between reducing the suffering of an individual animal against the need to conduct the same procedure on an additional animal. This '*tension*' between the principles of refinement and reduction should be discussed by the ethics committee.

Recommendation: Humane endpoints should be defined a priori (and revised periodically in the light of ongoing expertise). Advice can be sought from the ethics committee.

Once a humane or scientific endpoint has been reached, it is essential to use the most humane killing method possible that is compatible with any postmortem tissue collection or histology requirements. It should be noted that there is considerable debate about the humaneness of some killing techniques (e.g. the use of CO_2), and the EWG recommends that researchers should keep up to date with the literature and consult animal technologists and veterinarians with respect to current approaches (European Commission, 2012).

5. Assessing animal wellbeing, pain, suffering or distress in SCI studies

In order to assess the welfare status of animals used in any scientific study, it is essential to understand what '*normal*' behaviour and baseline physiological parameters are for the species, strain, sex and life stage of the animal being studied, and possibly for the individual animal. This enables rapid recognition of indicators of discomfort, pain or distress associated with the model, as well as detection of any unforeseen adverse effects, so that all of these can be ameliorated.

Post-operative care is critical to successful management of animal welfare in SCI studies. We recommend that post-operative care is tailored for the species, strain and model being used, taking into account the natural history of the model –intensive post-operative care is particularly important for some severe injury models and requires a significant commitment and pro-active monitoring by the principal post-operative care givers. This will require a team approach, with input from the researcher(s), animal technologists, attending veterinarian and ethics or animal care and use committee (Hawkins et al., 2011; European Commission, 2013; European Commission, 2012). An example of such a plan is included in Table 4.

Some groups have developed structured, objective recording systems that identify specific potential clinical signs and assign each of them scores that reflect their severity. An example monitoring sheet is shown in Table 5. Whichever approach is used, it is critical that welfare assessment is tailored to the species, strain and model being used, that staff are empathetic and competent and that a clear action plan is in place to alleviate suffering if necessary (i.e. if a humane endpoint is approached).

Recommendation: Ensure that a structured, objective monitoring system has been tailored to each protocol, and that this is reviewed as necessary to reduce the risk of important indicators being overlooked.

6. Strategies to improve the rigour and translatability of SCI research

This section sets out some approaches to help augment the benefits associated with in vivo SCI research projects, which the EWG believes is especially important given the potential severity of some protocols.

6.1. Experimental design and the potential for reduction

It is rare for power calculations to be reported in SCI publications, as is currently also the case for most published animal research, although this may be improving (Watzlawick et al., 2016; Macleod et al., 2015; Cressey, 2016). This raises the possibility that experiments have previously been underpowered (using too few animals, wasting animals and producing unreliable data) or overpowered (using too many animals and causing avoidable suffering). Additionally, a recent study into the reporting standards of animal research in critical care journals indicated that 'ethical quality' (defined by the authors as the reported use of analgesia, anaesthesia, welfare assessment and methods of humane killing) was poor (Bara and Joffe, 2014). Clearly, all research should be conducted and reported in a manner that upholds high ethical and scientific standards. This is especially important in areas such as SCI research, where there is the potential for severe suffering of laboratory animals and a pressing clinical need. Good practice guidelines are available for planning and reporting studies that involve experimental animals, and this EWG recommends that SCI researchers adopt the

Table 4
Example of expected outcomes and post-operative care: Cervical Level Bilateral Contusion Injury in rats (Adapted from a Post-Operative care protocol provided by the Bradbury Laboratory).

Time	*Expected outcomes*	*Post-operative care*
0-24 h	• Animals are expected to be laterally recumbent and lack full ability to groom effectively. There will be partial paralysis to forelimbs and hindlimbs. • Animals will appear sedate and may display signs of pain/distress such as porphyrin secretion from the nose/eyes or vocalisation when touched.	• Following the return of animals to the cage from the recovery incubator, inspect them multiple times during the day and evening to ensure food and hydration gel is within easy reach. • The morning of the day following surgery: o As standard, administer 5 mg/kg Carprieve analgesic subcutaneously. This is normally adequate pain relief, but a stronger analgesic should be administered if required (Buprenorphine, 0.03 mg/kg, subcutaneously). o Deliver 5 ml saline subcutaneously. o Animals should be placed in freshly lined cages, with some soft enrichment bedding added into the cage, alongside non-standard dietary provisions. • Animals should be inspected again every 3 h on the first day following surgery, or more frequently if any animals are displaying signs of pain in case additional analgesic is required. • Food and hydration gel should be placed within reach. • In the evening on the day following surgery animals will be offered recovery gel/ peanut butter mixed with water from a syringe.
24 h-4 days	• Animals are expected to be laterally recumbent and lack full ability to groom effectively. There will be partial paralysis to forelimbs and hindlimbs. • Animals will appear sedate and may display signs of pain/distress such as porphyrin secretion from the nose/eyes or vocalisation when touched.	• At 48 h 5 mg/kg Carprieve analgesic should be administered. Analgesic beyond this will be administered in consultation with the NVS. • Animals may be bathed (and dried fully to prevent temperature loss) as necessary. • Hydration state should be ascertained via cutaneous pinch and saline delivered for 3 days subcutaneously as standard, and after this if required. • Every 3–4 h during the day, and evening (if required), animals will be monitored and food and hydration gel placed within easy reach of each animal. • Animals will be offered DietGel Boost/ peanut butter mixed with water from a syringe. • Body weight should be measured daily and recorded until animals gain, and begin to plateau, in weight. Any animal which loses > 20% preoperative weight is has reached an end-point and must be humanely euthanized via schedule 1 or perfusion for tissue collection and removed from experimental study. Any animals which lose 10% bodyweight will be intensively hand-fed every 4 h during the day and evening and supplementary fluids given orally or subcutaneously. • Typically animals will remain housed on absorbent cage lining, with increasing quantities of sawdust and enrichment bedding added daily and fresh cage lining applied where necessary.
4-7 days	• Condition of animals should improve during this period. Animals may appear laterally recumbent or sedate at rest but are able to locomote in the cage to access food and water. The ability to groom increases. There will be partial paralysis to forelimbs and hindlimbs. Full weight bearing is delayed until the 2nd week post surgery in most instances.	• Increasing quantities of sawdust and enrichment bedding should be added to the cage on a daily basis towards resumption of normal husbandry conditions. • Cages may be removed from the cage holding incubator chamber and returned to the holding room as deemed appropriate by the experimenter, unless there is cause for concern. • Food and hydration gel is added in-cage until animals can obviously reach hoppers and animals offered DietGel Boost/ peanut butter mixed with water from a syringe if bodyweight has not increased. • Animals should be inspected a minimum of twice daily.
7 days+	• Animals are able to locomote in the cage to access food and water and groom with much greater efficacy. There will be partial paralysis to forelimbs and hindlimbs. Full weight bearing is delayed until the 2nd week post surgery in most instances.	• Standard husbandry conditions are restored and animals returned to the normal cage-holding room. • Food and hydration gel is added in-cage until animals can easily reach hoppers and animals offered DietGel Boost/ peanut butter mixed with water from a syringe until bodyweight increases if required. • Throughout the experiment, animals will be monitored for potential adverse events listed in the Project License.

Model specific methods of welfare monitoring are important in order to identify welfare issues and apply appropriate care. This is an example of a post-operative care plan for animals following cervical level bilateral contusion injury.

principles of these guidelines when designing and reporting their studies (Smith et al., 2018; Landis et al., 2012; Hooijmans et al., 2010; Kilkenny et al., 2010; ILARGuidance for the Description of Animal Research in Scientific Publications, 2014).

Recommendation: Plan and publish SCI research in accordance with good practice guidelines e.g. PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence), ARRIVE (Animal Research: Reporting of In Vivo Experiments), GSPC (Gold Standard Gold Standard Publication checklist).

6.2. Training

Gaining the surgical skills to perform SCI reproducibly in animals is not trivial and the EWG recognises the importance of continuity of staff and the requirement for training opportunities for new/replacement staff in this field. Formal training courses are currently limited in number and include a USA university training course (Ohio [http://sci.osu.edu/]) and the European Neurotrauma School [https://www.spinal-research.org/neurotrauma-summer-school]. Most researchers are trained through cascade training (i.e. where the principal investigator receives training and then passes the training on to their colleagues and research staff). Lack of training provision is problematic with regard to reproducibility of SCI research and raises a challenging ethical issue; poorly trained researchers cannot perform experiments robustly, which may result in unreliable data, avoidable suffering and wasted animals. On the other hand, using live animals for training requires stringent ethical consideration and must be specifically justified

Table 5
Examples of indicators for welfare assessment scoring systems for SCI studies using mice or rats (adapted from score system provided by S. McErlane, UBC, Canada).

Health observations	Score 0	Score 1	Score 2	Score 3	Score 4	Score 5
Response	Continue monitoring as per protocol; provide supportive care based on clinical signs	Monitor at least once day; provide supportive care based on clinical signs	Monitor at least twice a day; provide supportive care based on clinical signs	Increase monitoring frequency to at least three times a day; provide supportive care based on clinical signs	Increase monitoring frequency to at least four times a day; provide extensive supportive care based on clinical signs	Immediate euthanasia unless Veterinarian has given approval to continue supportive care
Body Weight/Body condition scoring (BSC)	≥ Presurgical weight	↓weight by < 5%	↓weight by 5–9%	↓weight by 10–14%	↓weight by 15–19% (IT)	↓weight by ≥20% (IE)
Behavior: Activity Gait	Normal mobility above and contralateral to injury	BAR (Bright, Alert, Responsive)	QAR (Quiet, Alert, Responsive) Slightly slow moving; still interested in environment, social and alert	Less interested in the environment, interacts less with cage mates, disregards observer; when nudged, reluctantly moves away. Occasional abnormal gait e.g. limping or "tip-toed" gait on "normal" legs.	Isolated from cage mates, minimally active; does not readily move when cage disturbed. When nudged, reluctantly moves. Frequent limping or "tip-toed" gait on "normal" legs. (IT)	Immobile or hyperactive; not moving when nudged; animal cannot right itself. Dragging or non-weight bearing on "normal" legs (IE)
Appearance: Grooming/Fur Posture/ Porphyrin	Normal	Mild porphyrin around eyes but groomed away	Piloerection (< 10% of body); Mild porphyrin around eyes/nostrils; not groomed away	Piloerection (25% of body e.g. base of neck) and dull fur (not shiny or smooth). Slight hunching in back Moderate porphyrin around eyes and nostrils.	Piloerection (50% of body), fur matted and un-groomed. Severe hunching in back even when walking. Moderate porphyrin around eyes, nostrils and front legs. (IT)	Piloerection (> 75% of body), fur matted and un-groomed with other severe signs of illness. Porphyrin crusted around eyes, nostrils and front legs. (IE)
Incision Site/Skin	Normal	Small (estimated < 1 cm) seroma at incision site	Skin red but clean and dry. One or two sutures missing but skin still apposed.	Skin red, swollen, but dry. Missing sutures or wound slightly open but clean and dry Evidence of scratching creating lesion that is exposing deeper tissues or lesion < 0.5 cm Seroma around incision site (1–2 cm)	Skin/lesion open with wet discharge (blood, pus), signs of infection. Evidence of scratching creating lesion that is exposing deeper tissues or lesion > 0.5 cm	Skin severely damaged (infected, necrotic, open to muscle or bone). Evidence of severe self-mutilation. Constant scratching (even when disturbed) creating lesion that is > 1 cm. Large seroma (> 2 ml). Implant exposed.
Dehydration	Normal		Mildly sunken eyes (appear > 75% open).	Skin tent > 2 s (decrease in skin elasticity) represents 10–15% dehydration. Sunken eyes (appear half closed).	Skin tent > 5 s represents 15–20% dehydration. Completely closed or severely sunken eyes; tail feels square. Cool to touch. (IT)	Animal unresponsive and cold to touch. Severe skin tent (> 10 s). (IE)
Elimination: Feces Urine Bladder Urine scald	Normal	Soft Stools	Daily paste-like feces. Hydration normal. Bloody urine. Bladder regularly more than half full.	Daily watery diarrhea with occasional formed soft feces. Small area of fecal staining around anus or mild urine scalding (wet, reddish skin). Hydration normal. Bladder regularly requires expression.	Watery diarrhea or increased urine output resulting in dehydration. Large area of fecal staining on fur or urine scalding (wet, reddish skin). Bloody urine with clots; difficulty expressing bladder (IT)	Only watery or bloody diarrhea and increased urine output; unable to maintain hydration. Bladder cannot be expressed or bladder ruptured. Severe urine scald exposing underlying tissue or associated with severe swelling.(IE)
Respiration: Rate Rhythm Effort (especially for oral gavage)			Subtle change in rate or effort with activity but normal at rest. Regular rhythm.	Obvious change in rate or effort with activity and at rest (see chest expansion). Occasional irregular rhythm. Small amount of nasal discharge or sneezing.	Reduced rate (easy to count) at rest and when active. Irregular rhythm. Appears to require effort (head bobs or body moves with breathing). Noisy breathing; Nasal discharge affecting breathing. (IT)	Reduced rate (Rats < 75 bpm). Irregular rhythm. Increased effort to breathe (head bobbing) for more than 4 h; Gasping, struggling to breath, open mouth breathing. Skin cyanotic (blue). (IE)
Autotomy			Mild chewing on toes though no loss of tissue (mild redness or swelling)	Loss of toenails on 1 or more digits, some exposed raw tissue	Loss beyond 1st knuckle on < 2 digits	Loss beyond 1st knuckle on 2 or more digits. Loss of any one digit > 2nd knuckle. (IE)
Pain			Facial Grimace: Narrow or closed eyes, Flattening of bridge of nose, bulge on top of nose, cheek (between eye and whiskers) bulge, ears back or flat, whiskers pointing back or "standing out on end". Other: Muscle twitching or flinching, staggering, back stretch, abdominal writhing or pressing.			Persistent signs of pain that interfere with normal functions or cannot be alleviated. (IE)

Animal Monitoring Key: IT (Immediate treatment required) and IE (Immediate Euthanasia). A score of three in any category or a cumulative score of 6 or higher results in increased monitoring and supportive care (e.g. supplemental heat, subcutaneous fluids, additional analgesia gel food or food treats/moistened pellets on cage floor, etc. based on clinical signs).
Structured, objective recording systems can also be useful in order to identify clinical signs that may indicate adverse animal welfare. Systems such as this set out clear actions (e.g. increased monitoring, supportive care, veterinary treatment or euthanasia) for animal care staff.

under some regulatory frameworks. Clearly the optimal approach to balance these issues is to develop and validate training materials that deliver the learning objectives required for SCI model training without using animals, as far as is possible. The EWG recommends that such an approach is evaluated and required resources and materials are designed, developed and freely shared. An online repository, such as Open Data Commons for Spinal Cord Injury (ODC-SCI; https://scicrunch.org/odc-sci) could be a good place to store such resources or materials. Some online materials are available with respect to handling and injecting animals (e.g. http://www.procedureswithcare.org.uk/, https://flairelearning.com/). Some SCI relevant resources (e.g. surgical models, behavioural testing and neurophysiology) are available on the JOVE (Journal of Visualised Experiments) website (Lee et al., 2012; Kathe et al., 2014; Cheah et al., 2017; Brown and Martinez, 2019).

Recommendation: Consider working with national or international organisations or funding bodies to help develop/support training opportunities for new SCI researchers to develop the necessary skills to perform SCI models competently and reproducibly.

6.3. Maintaining and disseminating good practice

The EWG intended this report to be used as the basis of discussions as to how SCI animal models can be developed and refined within a given research group or establishment, keeping abreast with current good practice and stimulating ongoing improvement. The EWG recommends regular discussion with animal technologists and care staff, veterinarians and the local ethics or animal care and use committee as well as with other researchers working in the field. It is also worth consulting websites of national 3Rs centres e.g. NC3Rs in the UK, NORECOPA in Norway, ICAR3Rs in Germany and the North American 3Rs Collaborative in the USA. Publications should also include full details of experimental protocols including any details relevant to improving animal welfare (online in supplementary materials if necessary); attention to reporting standards mentioned earlier (in section 6.1) can help with this.

7. Future directions

7.1. Biomarker measurement

In drug discovery programs, two key pieces of information are needed regarding the pharmacology of new potential therapies: demonstration of target engagement and the free plasma concentration required to achieve the required target engagement. Where the proposed therapeutic mechanism is well characterised, model systems can be used that assess activity only on the mechanistic pathway of interest, and a disease model may not be required. For example, if a potential drug discovery target is relevant to neurotrauma in general, a nerve injury that has the potential for a lower welfare burden (e.g. optic nerve injury) can be used for drug screening purposes. This approach is often referred to as mechanistic modelling as opposed to disease modelling (Hunter, 2011). The mechanistic modelling approach to developing new therapies is obviously easier to apply to diseases in which the underlying pathophysiological mechanisms are well characterised.

In SCI, the key processes and pathways that underlie neurodegeneration, neuroinflammation, resolution and neuroplasticity, which are presumed but not proven to underlie the prognosis for patients, are unfortunately not yet adequately understood, despite significant research investment. Currently, SCI research is largely focussed on disease models with high face validity and mechanistic modelling largely reserved for target identification studies. There is a need for relevant, specific, translatable biomarkers of the different aspects of SCI which could enable pathway driven, mechanistic modelling in drug discovery. This could drive selection and further development of in vitro or in silico techniques to contribute to advancements in this field. Although some interesting work is being done in this important field the EWG agreed that this should be an area incorporated into future research (Saadoun and Papadopoulos, 2016; Kwon et al., 2017).

7.2. Bioinformatics and 'Big Data'

An interesting direction in the field of experimental SCI is the development of a worldwide database for translational SCI research, which will harness the power of analysis of '*big data*' to better understand the mechanisms underlying recovery across species, thus enhancing the potential of successful clinical translation (Ferguson et al., 2011; Nielson et al., 2014).

Such an approach has the potential to promote improved experimental design (including randomised block design, blinding and other measures to reduce bias) and, where appropriate, standardisation of SCI pre-clinical model selection and deployment. Full standardisation may be impossible given the inherent complexity and variability of surgical SCI, but efforts to optimise the translational impact of pre-clinical research should be explored from an ethical perspective, both to reduce potentially fruitless animal use and to improve patient benefit.

8. Conclusion

This report gives some guidance to help researchers apply the 3Rs to SCI research, with a focus on practical refinement approaches that can be used to reduce animal suffering. Applying replacement in SCI research can be challenging at present. However, there is always scope for applying the Rs of refinement and reduction, which are highly effective ways to reduce suffering and improve scientific quality. The authors hope that the recommendations in this document will be used and further developed and disseminated by researchers working in this field.

Author contributions

This manuscript represents the outcome of discussions from an expert working group (EWG) established by the RSPCA, as part of its work towards ending severe suffering for animals in research. EL, MRA, EJB, HE, PH, RMI, JK, ATM-T, LDFM, SP, JR, KR and PKY were all members of the EWG. The manuscript was written by EL and was edited and revised by the EWG.

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