RSPCA ANIMALS IN SCIENCE

Focus on Severe Suffering "Minimising Pain"

Summary Report

December 2024

Animals in Science Department

RSPCA 200

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Summary

Introduction

In November 2024, the RSPCA UK "Focus on Severe Suffering" event was held in collaboration with Newcastle University on the topic of "**Minimising Pain**". The meeting was divided into two sessions. The first session, **Refining Pain in Pain Research**, addressed reducing pain within pain research, i.e. within procedures that are undertaken to study pain. The second, **Refining Pain in Painful Procedures**, looked at reducing pain in research areas causing severe suffering, but where causing pain is not an objective. Discussions in both sessions addressed the use of analgesics, ethical review, training, and identifying those responsible for ensuring pain is minimised in animal research.

Short summary

Speakers presented the following case studies:

- A novel approach to spinal cord stimulation (SCS) in rats, using ECAP-controlled closed-loop stimulation to more accurately mimic human conditions and reduce pain severity.
- The importance of minimising pain in pain research, including peri-operative analgesia and exploring alternative approaches that avoid pain induction altogether, e.g. analysing changes in natural behaviors.
- Providing peri-operative analgesia with pregabalin in rodent models of chronic pain, which does not interfere with long-term outcomes - challenging the common practice of avoiding analgesia in such studies.
- A research pipeline that prioritises reducing animal suffering by using zebrafish larvae for early-stage drug screening, eliminating the need for rodents in these initial stages.
- A "bed to bench and back" approach to ensure responsible animal use and clinical relevance in central nervous system (CNS) injury research. This includes multimodal analgesia, comprehensive post-operative care, and automated home cage monitoring to minimise suffering and gather valuable data.
- A refined mouse model of myocardial infarction, using an ischaemia/reperfusion technique and improved pre-, intra-, and post-operative care to significantly reduce pain and improve welfare outcomes.
- A non-lethal model for snakebite antivenom testing reduces suffering in mice while providing more detailed and clinically relevant data.

The keynote presentation was on improving pain management in research animals by addressing gaps in pain assessment and analgesic use. Summaries of all the presentations are set out below, with key points from the two discussion sessions on 'how far can we go with reducing pain in animal research when it is the object of the study?' and 'perceived and actual barriers to providing analgesia in painful procedures'. This document also sets out some action points on the basis of the presentations and discussions.

Session 1: Refining Pain in Pain Research

This session focused on case studies in which severe suffering was reduced in pain research. Ilona Obara began by presenting her work on refining spinal cord stimulation (SCS) models. While SCS can treat neuropathic pain in clinical settings, its mechanisms remain unclear due to limitations in pre-clinical research. Commonly used rat 'models' differ significantly from humans; for instance, rats lack an epidural space, causing implanted leads to press directly on the spinal cord. Traditional pain assessments measure back muscle contractions in rats, but this would be considered intolerable for humans and likely represents overstimulation rather than a valid comparison. To address these issues, Ilona's team uses a novel approach using Evoked Compound Action Potentials (ECAP)-controlled closed-loop stimulation. This technique dynamically monitors and adjusts stimulation to consistently activate dorsal column fibres. In Ilona's studies, the pain threshold required for measurement is moderate, avoiding severe severity. This is the first in vivo use of ECAP recordings in freely behaving rats, showing effective analgesia and reduced hypersensitivity. Ilona continues to refine SCS models to enhance translatability and reduce animal suffering.

In the next presentation, Sara Hestehave explored the question of "is all pain necessary, when studying pain?". Sara highlighted that while the study of pain may necessitate inducing some level of pain, it must be limited to the absolute minimum necessary for achieving valid scientific outcomes. Traditionally, peri-operative analgesia has been avoided due to concerns it might interfere with pain models. However, Sara's research group has demonstrated that carefully chosen analgesics, such as buprenorphine, effectively manage acute pain in a number of models, including neuropathic pain, without affecting outcomes. As a further refinement, these can be administered non-invasively, e.g. mixed with Nutella®. Sara also pointed out that pain induction is not always necessary to study pain. For example, sensitivity changes, or changes in natural behaviours such as gait, climbing, or home cage activity, may offer more translationally relevant alternatives. She also suggested other specific refinements in pain research, like using lower adjuvant volumes in joint pain studies to reduce the risk of spread beyond the joint, which should reduce pain. Looking ahead, Sara highlighted a recently announced International Association for the Study of Pain (IASP) Presidential Task Force on guidelines for the use of laboratory animals in pain research. This task force will build on existing work by IASP members to set the highest ethical and experimental standards.

Next, Francesca Di Domenico demonstrated that analgesia can be administered in pain research without affecting pharmacological outcomes. With chronic pain affecting around 20% of the human population, there is a significant unmet need for new analgesics. However, many animal studies commonly avoid peri-operative analgesia to preserve pain-related behaviours. Through a literature review, Francesca revealed that only 5% of studies report the use of peri-operative analgesia in rodent models of chronic pain. To address this, Francesca refined standard practices by administering pregabalin peri-operatively in rodent models of spinal nerve ligation (SNL). Her findings demonstrated that pregabalin effectively reduced acute mechanical allodynia for up to three days post-surgery without affecting chronic pain behaviours, long-term electrophysiological outcomes or measurements of spinal neuronal activity. These results directly challenge the assumption that peri-operative analgesics confound the neurobiological outcomes of chronic pain studies.

In the final presentation of this session Matt Parker outlined a research pipeline aimed at reducing suffering in animal models and accelerating pain therapeutic development. Developing a single pain drug costs ~£2.5 billion, with a 99% failure rate, partly due to reliance on limited animal models. To address this, Eptiva Therapeutics combines big data analysis with innovative in vivo screening. Their approach uses in-silico bioinformatics to map proteins linked to pain phenotypes and validate targets based on FDA-approved mechanisms. Traditionally, the next step would involve using rodents, however, a new approach led by Matt will use larval zebrafish to test novel analgesic compounds. The larvae will be used to test compounds through light-dark response tests after exposure to pain-inducing chemicals, with and without treatment. Although zebrafish use is not regulated by the UK Animals (Scientific Procedures) Act 1986 until the larvae are 5 days post-fertilisation (dpf), there are concerns that more immature stages are able to experience pain and suffering. However, using the larvae eliminates the need for breeding, housing, and testing on rodents during the early stages of pre-clinical trials, which often result in failure. Additionally, zebrafish larvae will only be used for up to 4 dpf, a period during which they are believed to be less capable of experiencing pain than adult zebrafish or rodents.

Session 2: Refining Pain in Painful Procedures

This session began with Jordi Lopez-Tremoleda presenting on refining experimental and welfare practices in studies of central nervous system (CNS) injuries. Jordi advocated for a “bed to bench and back” approach which first identifies clinical needs, advances through laboratory and animal research, and integrates findings back into clinical applications. This builds on the “bench to bedside” approach, which helps to ensure responsible animal use which is tailored to address

specific clinical requirements and provide meaningful outcomes. Jordi also described his experimental paradigms for refining CNS injury models, including multimodal analgesia, assistance during surgery, comprehensive twice daily care post-surgery, support with thermoregulation and oxygen support during recovery, all of which minimises cumulative severity. Recent advances in automated home cage monitoring show increasing promise for both animal welfare and scientific data collection. Jordi's team has found that automated home cage analysis revealed subtle changes in animals post-CNS injury including changes in locomotion, such as reduced activity, and social behaviour changes including increased aggression and reduced social separation. Using deep learning technology, the team is now looking to analyse specific behaviours like nesting, burrowing, and climbing to better understand injury impacts. While predicting CNS injury severity based on behavioural data remains challenging, future efforts aim to refine these tools to differentiate between mild, moderate, and severe severity, enhancing both welfare assessments and data quality.

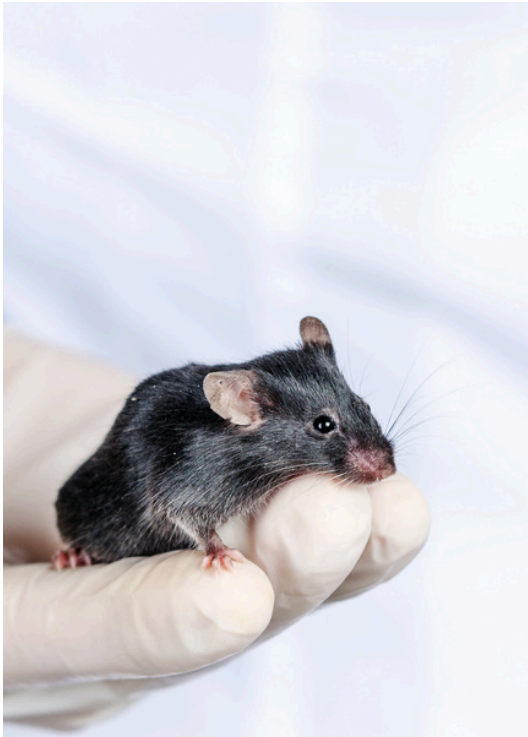
Next, Rachael Redgrave presented a refined mouse model of myocardial infarction (MI). Her team addressed welfare challenges by implementing several refinements, including the use of an ischaemia/reperfusion technique. This technique creates an infarction by temporarily reducing the blood flow for 60 minutes, and produces a smaller area of tissue damage compared to the permanent ligation method. While technically more challenging, it significantly reduces the pain experienced by the mice and also more closely mimics clinical scenarios. Additional refinements include improved pre-, intra-, and post-operative care protocols. Pre-operatively, mice are handled using low-stress techniques and warmed to minimise heat loss. During surgery, advanced anaesthesia and intra-operative analgesia are used, along with techniques to reduce tissue damage, such as the replacement of metal retractors with less traumatic suture materials and the use of pre-warmed saline to maintain tissue moisture. Post-operatively, buprenorphine is provided via injection and in jelly, with pain managed intensively during the critical 36-hour period. Animals are monitored closely with a cumulative scoring system that triggers veterinary intervention if needed. Recovery conditions have also been optimised. Mice are returned to socially housed, enriched recovery cages with litter from their home cage, supplemental oxygen, and palatable treats like peanut butter to mitigate stress and encourage appetite. Social housing and enrichment help reduce stress, promote natural behaviors, and improve post-operative recovery outcomes.

In the final presentation of this session, Amy Marriott highlighted the challenges and advancements in reducing severity for mice in snakebite antivenom assays. The traditional World Health Organisation-endorsed protocol involves injecting mice with a premixed lethal dose of venom and antivenom, assessing efficacy based on survival after 6 or 24 hours. While this method has played a critical role in testing lifesaving antivenoms for over 40 years, it raises significant ethical and scientific concerns. The protocol causes severe suffering in mice, mirroring symptoms seen in human snakebite victims, such as organ damage and paralysis.

However, scientifically, the premixed venom and antivenom do not replicate real-world envenoming scenarios, limiting insights into pharmacokinetics and risking overestimation of antivenom efficacy. This has led to catastrophic failures in some cases. To address these issues, a new non-lethal model is being developed. This approach uses minimal venom doses to induce measurable biomarker changes, such as blood fibrinogen levels and prothrombin time, which are clinically relevant to human cases. Combined with non-invasive monitoring of vital signs, this method provides detailed, rigorous data without severe suffering. Mice subjected to this procedure experience mild or moderate severity, with no outward signs of envenoming. This innovation marks a significant step toward reducing animal suffering while providing scientifically robust antivenom testing.

Keynote

Paul Flecknell's keynote presentation was focused on improving pain management in research animals by addressing gaps in pain assessment and analgesic use. He highlighted common pain assessment methods - clinical appearance, pain behaviours, and in-house score sheets - and their limitations. For example, one study found that while individual assessors could recognise severe pain from photographs, assessing mild to moderate pain remains challenging, underscoring the limitations of relying solely on appearance. Paul emphasised the importance of selecting the right analgesic, doses, and timing, referencing his latest textbook, Laboratory Animal Anaesthesia and Analgesia, as a resource for evidence-based guidance. He set out the benefits of pre-emptive analgesia and combining drugs from different classes to improve outcomes, while cautioning against potential side effects. He stressed that pain management protocols should always include a plan for alternative approaches if the initial treatment is ineffective. Researchers must assess efficacy with validated tools and adjust protocols as needed. Training was another key focus, with resources like the Research Animal Training platform recommended for pain management education. He concluded by calling for a proactive, informed approach to refining analgesic protocols, urging ongoing education, robust assessments, and a commitment to improving animal welfare in research.



Discussion sessions

Participants were split into groups for **discussion sessions**. The first session addressed "how far can we go with reducing pain in animal research when it is the object of the study?" and groups discussed the following three topics:

1. Provision of analgesia

The discussion focused on balancing pain relief with the scientific integrity of studies testing analgesics. Concerns were raised about the potential impact of analgesics on research outcomes, such as NSAIDs affecting immunology and opioids influencing behaviour. A significant challenge was shifting traditional practices and overcoming the mindset of "the way things have always been done." There was also a call for improved pain scoring systems, with automated technologies suggested. Practical issues like determining optimal analgesic administration were also discussed. Participants recommended that analgesia should be standard in animal studies, as increasing evidence shows it does not compromise scientific outcomes, and justification (with evidence) should be required for withholding it. Research should focus on ensuring analgesic compatibility with study aims and exploring alternative administration routes. Peer review should also require justification for witholding analgesia to maintain good practice and transparency.

2. Refinements beyond analgesics

Participants discussed refinements beyond analgesics to improve animal welfare. Key suggestions included improvements in general husbandry, focusing on the "3Hs" - housing, handling, and habituation. Social housing, providing hiding places, extra heat, and low-stress handling were emphasised, as well as post-surgery options like diet gels and heat boxes. Habituation to handling before studies and enriched environments tailored to specific species and studies were also recommended. Terminal anesthesia was proposed for procedures not requiring conscious animals, and standardised training and acclimatisation protocols were advocated to ensure consistent care. Challenges such as time, staffing, and funding were also acknowledged, underlining the need for careful planning and prioritisation to implement these refinements.

3. Ethical review

This discussion centered on ethical decision making, including balancing the scientific and societal benefits of studying pain against the responsibility to minimise animal suffering. Participants emphasised the importance of conducting a thorough and robust harm-benefit analysis, supported by evidence and well-defined hypotheses. Ethical reviews should consider species and life stage selection, optimum animal numbers, and the most suitable 'models' to achieve scientific objectives while minimising harm. Training for ethics, and/or animal care and use committee (e.g. the UK Animal Welfare and Ethical Review Body, AWERB) members and

open discussions during committee meetings were considered to be vital for addressing these issues, alongside exploring refinements informed by existing data. Collaboration across disciplines and rigorous proposal reviews by diverse experts were seen as essential for making informed decisions.

The second discussion session addressed "perceived and actual barriers to providing analgesia in painful procedures", including the following four topics:

1. Barriers to providing pain relief

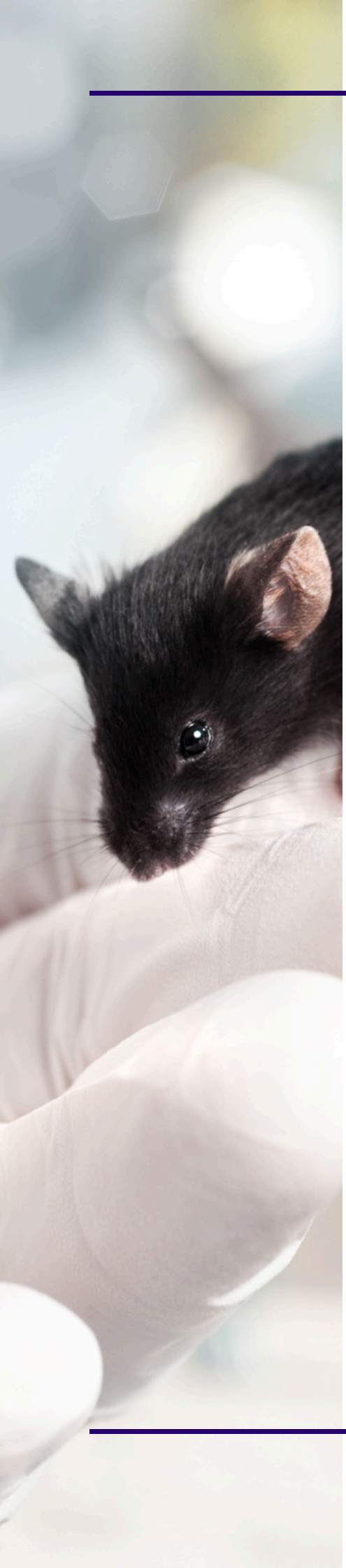
Groups discussed challenges to providing effective pain relief in research animals, identifying both practical and (scientific) cultural barriers. Resistance to change, concerns about reproducibility, and fears of altering models were noted as obstacles. Issues such as a lack of understanding about pain assessment, limited data on analgesics for specific models, and scepticism about animals' ability to feel pain were also highlighted. Practical challenges included choosing appropriate routes of administration and the potential impact of changing analgesic regimes on study comparability. Participants pointed out that veterinary medicine approaches pain relief differently, administering analgesics as standard, suggesting this could serve as a model for research practices. Solutions proposed included increased training around analgesia, involvement of the attending veterinarian, better pain monitoring, more frequent dosing if necessary to meet animals' needs, and improved pain score sheets. Participants stressed the importance of prioritising pain management, providing analgesia as standard.

2. Training and welfare assessment

Several issues were identified with current training practices, which often focus on theory or healthy animals, leaving researchers unprepared for practical interventions. Bias and subjectivity in welfare assessments also caused problems, which could be alleviated by using standardised approaches. A greater emphasis on training in animal biology, behaviour, and welfare was seen as essential to improve empathy and decision-making. Recommendations included investing in staff development, retaining experienced personnel, and enhancing core skills for animal technologists. The groups noted that training can be undervalued and inconsistent, suggesting integration with practical application with workshops to help bridge the gap. They also advocated for using guidelines such as PREPARE to help strengthen welfare assessments and embed good practice in general.

3. Challenges in identifying pain

Interpreting pain in animals can be challenging due to human bias and subjectivity, and individual variation between animals. Ensuring consistency across observers, who may be influenced by experience and personal perceptions, was identified as a key issue. Participants discussed the potential of technology, such as cameras, automatic data collection, and artificial intelligence (AI), to enhance pain assessments, for example by enabling nocturnal activity to be analysed or reducing human bias. However, concerns about financial cost, data management, and effective AI training were raised. High-quality training, independent scorers (blinded if possible), improved resources, and better dissemination of findings could all improve pain evaluation methods and their reproducibility.



Action points

Scientists

- Provide analgesia as standard practice in pain research, or protocols that will cause pain, unless there is compelling scientific justification for withholding it.
- Ensure that the chosen analgesic(s), and dosing regimes, are compatible with the study aims and select the administration route that is least stressful and most translatable (e.g. voluntary ingestion).
- Use terminal anesthesia for procedures that do not require conscious animals.
- Contribute to developing more accurate and objective pain scoring systems, potentially using automated technologies.
- Share findings and best practices to improve pain evaluation methods and their reproducibility. This could be in papers, posters and presentations, and directly with peers.
- Use PREPARE and ARRIVE to help implement good practice around project design and publication
- Support and empower animal technologists to play a key role in implementing welfare improvements.
- Engage in ongoing discussions about reducing suffering and help to promote a culture of continuous improvement.

Veterinarians and animal technologists

- Promote the "3Hs": housing, handling, and habituation.
- Advise on meeting animal's specific needs in pain research, e.g. social housing wherever possible, hiding places, extra heat, and low-stress handling, and help to implement these refinements.
- Advise on, and provide, post-surgical support such as diet gels, heat boxes, and tailored recovery environments.
- Ensure that all staff assessing welfare, and adverse effects, are adequately trained, to reduce bias and improve consistency in assessments.
- Be open to using cameras, automatic data collection, and AI to help enhance pain assessment and complement the expertise of empathetic human observers.

Action points

AWERBs and other animal ethics, or care and use, committees (e.g. AWBs, IACUCs)

- Ensure that staff receive appropriate training in animal biology, behaviour and welfare.
- Advise on integrating the practical application of welfare assessment protocols into training programmes, including workshops.
- Apply extra scrutiny to applications involving severe protocols, focusing on the harm-benefit analysis, experimental design and refinement.
- Ensure that researchers adhere to the PREPARE and ARRIVE guidelines.
- Encourage open dialogue between researchers, veterinarians, and animal technologists and care staff.

Regulators and competent authorities, e.g. the UK Home Office

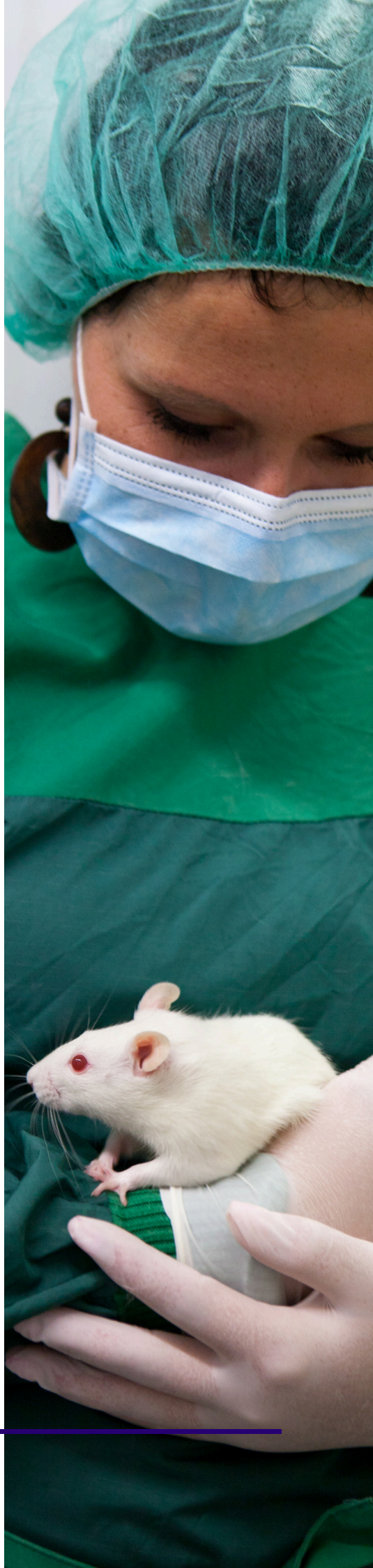
- Apply special scrutiny to project applications involving 'severe' protocols, as for AWERBs above.
- Conduct themed reviews of projects involving severe suffering, with a strategic aim to reduce the number of animals experiencing this.
- Regularly review and update guidance for the regulated community, to ensure these reflect current leading practices.

Funding bodies

- Prioritise funding for projects that fully implement all 3Rs, requiring rigorous justification for severe suffering.
- Ensure that funding decisions consider the ethical implications of the proposed research.
- Support initiatives that promote open access to research data, to facilitate reproducibility and prevent repetition.

Journal editors and reviewers

- Hold researchers accountable for the use of analgesia and pain management. Peer reviewers should insist on justification for withholding analgesia.
- Endorse the ARRIVE guidelines and insist that authors adhere to these. Require peer reviewers to check that manuscripts comply, and to return those that do not.
- Emphasise the importance of ethical considerations, including animal welfare, in the peer review process.



Note

The RSPCA is opposed to experiments that cause pain, suffering, distress and lasting harm to animals, and the Society's principal goal is replacement with non-animal methods. While animal use continues, we strive to help ensure the fullest possible implementation of the 3Rs, and robust ethical review that effectively challenges whether, and how, animals are used. The Focus on Severe Suffering initiative should be regarded in this context, and the RSPCA would like to acknowledge the strong support of the scientific community for the project. This has enabled a 67 % reduction in experimental procedures causing severe suffering in the UK since 2014 - for further information, see focusonseveresuffering.co.uk

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