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HUMANE SCIENCE

2018 ANZCCART Conference

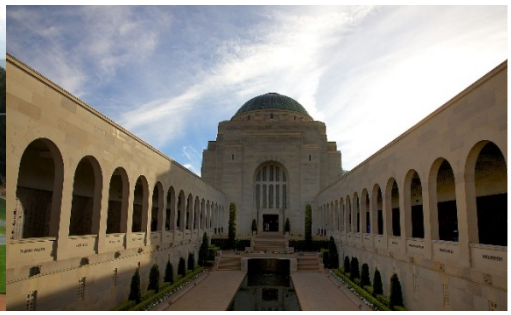
"Keeping it Relevant"

Conference Proceedings

Tuesday 24th to Thursday 26th July, 2018

Canberra, ACT





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2018 ANZCCART Conference Programme

Tuesday 24th July 2018

11.00am	**Conference Opening**
11.30am	**Geoff Dandie -** Can published guidelines help us to 'keep it relevant'?
12.00noon	**Clive May** - Large animal models of critical illness: a vital step in translating basic research into clinical applications
12.30 – 1.30pm	**Lunch**
Session Chair	*Geoff Dandie (CEO, ANZCCART)*
1.30pm	**Workshop 1 – Discussion Groups by AEC Membership Categories**
2.00pm	**Workshop 1 - continues**
2.30pm	**Workshop Reports to Delegates**
3.00 – 3.30pm	**Afternoon Tea Break**
Session Chair	*Arnja Dale (ANZCCART New Zealand Board Member)*
3.30pm	**Karina Burns -** Using 'The Code' to trace policy change in animal research practices in Australia 1969 - 2013
4.00pm	**Megan Fisher -** Aim high for high containment: Welfare in a high containment environment.
4.30pm	**Leigh Taylor** - The relevance of Category D members to the operations of every AEC
5.30pm – 7.30pm	**Cocktail Function**

Wednesday 20th July

Session Chair *Cathy Pitkin (ANZCCART Board Member)*

9.00am **Deirdre Burke** - Education and training programs: trans-Tasman convergence or divergence?

9.30am **Mary Bate** - Recent NHMRC initiatives to promote the 3Rs and research quality

10.00am **Gail Anderson** - Use of simulators in technical skills training to reduce animal use in teaching

10.30am Morning Tea

Session Chair *Marc Rands, (Executive Officer, ANZCCART New Zealand)*

11.00am **Margaret Rose** - Reliability of animal research data – something old, something new?

11.30am **Joy Verrrinder** - Can ethics be considered a science?

12.00noon **Linda Hearder** - Keeping it relevant in primary and secondary schools

12.30 – 1.30pm **Lunch**

Session Chair *Geoff Dandie (CEO, ANZCCART)*

1.30pm **Workshop 2 - Pseudo AEC Discussion Groups**

2.00pm **Workshop 2 - Mixed Group Discussions by Topics**

2.30pm **Workshop Reports to Delegates**

3.00pm **Afternoon Tea**

Session Chair *Jodi Salinsky (ANZCCART New Zealand Board Member)*

3.30pm **Amanda Buyan** - Replacing *in vivo* and animal testing with supercomputers to gain insight into effectiveness and specificity of medications for chronic pain and epilepsy

4.00pm **Stephen Fairweather** - Going small to make it big – How designing new drugs at the molecular level can help replace animal use in pharmacology

4.30pm **Arnja Dale** - AEC Training resources

5.00 pm End of formal sessions for day 2

6.30pm **Buses Depart Hyatt Hotel for Conference Dinner**

7.00pm **Conference Dinner** – Australian Institute of Sport

Thursday 21st July

Session Chair *Pete Hodgson (Board Chair, ANZCCART New Zealand)*
9.00am Tribute to the late John Schofield

9.30am **Malcolm France** - What do opinion polls really tell us about public attitudes to animal research?

10.00am **Melissa Lindeman** - Promoting behavioural change in people working with animals in research and teaching: Challenges and opportunities.

10.30am **Morning Tea**

Session Chair *Geoff Dandie (CEO, ANZCCART)*
11.00am **Panel Discussion & General Forum**

11.30am **Panel Discussion & General Forum**

12.00noon – 12.30pm **Panel Discussion & General Forum**

12.30 – 1.30pm **Lunch**

Session Chair *Richard Russell (Chair, ANZCCART Board)*
1.30pm **Tristan Reid** - Intraperitoneal telemetry devices: A new approach to temperature monitoring in experimental ferrets

2.00pm **Jodi Salinsky** - Welfare management of pigs undergoing gastrointestinal surgery

2.30pm **2019 ANZCCART Conference Announcement and Conference closure**

3.00pm **Afternoon Tea**

Presentations given

on

Tuesday 24th July

Can published guidelines help us to 'keep it relevant'?

Geoff Dandie

ANZCCART

Regular readers of ANZCCART News would hopefully have noticed an article we published in the last edition for 2017, because that article acknowledged ANZCCART's important decision to formally endorse both the PREPARE and ARRIVE Guidelines that have been published during recent years. The Board took this measured decision with a clear view and intent of ensuring that ANZCCART does whatever we can to help to encourage best practice and help to ensure that only the best and most justified experiments using animal gain approval.

The ARRIVE Guidelines (Animal Research: Reporting of *In Vivo* Experiments) guidelines are intended to improve the reporting of research using animals – maximising information published and minimising unnecessary studies. They were prepared by Carol Kilkenny, William J Browne, Innes C Cuthill, Michael Emerson and Douglas G Altman all from the UK. The ARRIVE Guidelines were originally published in *PLOS Biology* and were developed in consultation with the scientific community as part of an NC3Rs initiative to improve the standard of reporting of research using animals. The ARRIVE checklist is designed to be used when submitting manuscripts describing animal research and essentially aims to ensure that publications contain sufficient information to allow anyone to faithfully replicate that work.

While we see the ARRIVE Guidelines as an essential tool linked to the publication of results obtained from experiments that involve the use of animals (and all others), we felt that the PREPARE Guidelines might even be more relevant in the context of the AEC application and approval process. The PREPARE Guidelines were written by a group of experts (Adrian J Smith, R Eddie Clutton, Elliot Lilley, Kristine E Aa Hansen, Trond Brattelid), led by the Secretary of Norecopa. They were prepared as part of Norecopa's ongoing efforts to reduce waste and increase the reproducibility of animal research and testing. In the context of these guidelines, PREPARE stands for ***P**lanning **R**esearch and **E**xperimental **P**rocedures on **A**nimals: **R**ecommendations for **E**xcellence* and really focus on most of the same key aspects as our AEC application forms. PREPARE focuses on a large number of factors which, although they are seldom reported in scientific papers, can dramatically influence the validity and outcome of studies on animals, as well as the health and safety of all those concerned. They cover all stages of quality assurance, from the management of an animal facility or population to the individual procedures which form part of a study. The guidelines and checklist are based upon the authors' experience in running animal facilities, collaborating with researchers (including those working with farm animals, fish and wildlife), serving on advisory and regulatory committees, and planning their own animal studies. *PREPARE* should be seen as an *aide memoire* to remind scientists of all the topics which may be relevant when planning experiments.

ANZCCART's decision to endorse these guidelines was also in response to widespread concern about the quality, reproducibility and translatability of studies involving research animals.

Disclaimer: The opinions expressed in this presentation are those of the author and do not necessarily represent the views or policies of ANZCCART.

At last year's ANZCCART conference in Queenstown, we heard a lot about "social licence" and how to maintain it. I think we can all agree that this is an important consideration, but possibly the most important consideration when it comes to maintaining a social licence for the scientific use of animals would be ensuring that any such work is being done at the highest standards and is going to produce significant and relevant data. So, with numerous reports emerging in both the scientific literature and the broader media, indicating that research using animal models is producing spurious and / or misleading information, it is imperative that we all look closely at the work that is being done with animals so we can be sure that all such work is relevant and producing information that is sufficiently valuable to justify the use of animals.

When it came time for ANZCCART to look into this emerging lack of confidence in research data obtained from animal studies and the problems associated with this issue, we like a number of other like-minded organizations, decided that it was important for us to consider how such issues may have developed and what can be done to help address them.

One of the key concerns commonly described in publications is based around poor reproducibility between similar published experiments. Others cite examples of poor translatability from animal models to human healthcare. Some of the most compelling studies used to question the value of animal models have been underpinned by systematic reviews of animal based research and the applicability of the data obtained to human health and disease prevention and some of those studies have indeed indicated a very poor correlation between the two. Such studies really do raise significant questions about the validity of animal studies, but of course as an old researcher, I felt compelled to consider this from more than one perspective, so I set about trying to find equivalent systematic reviews that show the relevance (or not) of *in vitro* experimental models and / or computer models to human health and disease. Having initiated a series of literature searches, google searches and alike with great confidence of success, I was a little surprised and frankly disappointed to find none. This apparent lack of such studies is truly amazing as it difficult to see how anyone can claim that one type of experimental model is inferior without having clear and compelling data to show that other styles of model are significantly better. What was even stranger was the fact that there were a number of systematic reviews, including a Cochrane Library review (which generally regarded as the most influential source of such reviews) that showed preclinical animal studies do make a significant contribution to health care and more transparent translational medicine. So this is all potentially quite confusing.

Short of undertaking a systematic review of systematic reviews, perhaps we should assume that this apparent disparity between the results of the systematic reviews found by my search strategies reflects some change over time, which is a concept that may be supported by the literature itself. Alternatively, there may be some important, although not obvious, difference in the criteria used to define study parameters.

Considering the idea that these differences may in part, reflect a changing trend over time, it is worth noting differences between the way animal based studies are conducted today when compared to 30 years ago. Science done today may far more precise and carefully controlled that in the past, with a large proportion of rodent work now being done in genetically modified animals for example. We also have a greater understanding of the important interactions that exist between the various body systems and the environment in which we live. One clear example of this is a lot of the work that is being done currently that looks at the microbiome (e.g., the microbiological species living on and in our bodies) and the influences the microbiome can have over our immune system for example and our overall health and wellbeing. Similarly, it is becoming increasingly clear that the microbiome of animal species and even within an animal holding facility can be quite unique and very influential in many studies.

Certainly, when I started out as a junior research scientist quite a while ago now, there was a strong perception that animal models offered a good and true approximation of human disease and this was something the data actually seemed to support. Yet now, there still seems to be a correlation, albeit probably a variable one between experimental models and human disease, with some

anecdotal indications of 'dirty' animal models possibly being better in some studies at least, than the more highly refined and potentially artificial animal model species we currently favour. The so-called 'pet shop mice' studies of immune function that seem to more closely represent normal human immune function that many highly inbred or genetically manipulated mouse strains, potentially support this contention, but different species models may also closer approximate human systems that others as well. Again, drawing on my own past experience, during studies of the damage UV light can cause to the immune system that protects sunburnt skin in a sheep model. When another group eventually did a similar study in human volunteers, they found that the response and the kinetics of the response were almost identical in every way to what we had found in our sheep model.
While I am definitely not suggesting that we should ever consider abandoning modern, high biosecurity compliant, animal holding facilities in favour of going back to the bad old days of dirty, wild rodent infested animal facilities, I might suggest that if we are going to keep animal based research relevant, we may need to consider combining the more modern and exquisitely sensitive and precise models used in animal studies with similar experiments conducted in more conventional, less controlled animals. In essence, I am suggesting that using the most precise molecularly precise GM models as a guide to further experiments in matching conventional animals, or models that rely on the use of other species that more closely parallel humans in their diversity might be an ideal combination that engages the best of both worlds.

These are both very important considerations, but need not necessarily be the result of a single problem and so we might suggest that it is highly unlikely that a single solution will resolve all issues. ANZCCART did however undertake a review of the broader issue and this led to the Board taking some fairly clear but simple steps that might hopefully address some of the problems associated with reproducibility of experimental data. Two areas of potential concern to ANZCCART were the optimal design and execution of experiments as well as the accuracy and completeness of reporting the outcomes. In trying to help address both these key issues, Regular readers of ANZCART News would hopefully have noticed that we recently endorsed both the PREPARE and ARRIVE Guidelines. The aim of formally endorsing these guidelines is simply to encourage researchers to refer to these guidelines and use them to help plan the work they wish to undertake so that their work has the greatest possible chance of providing relevant, reproducible data.

ARRIVE Guidelines:

The ARRIVE Guidelines were prepared by Carole Killkenny and her colleagues in the UK, supported by the NC3Rs organization over there. They are all very experienced scientists with a good perspective of what needs to be included in a high quality manuscript. The great beauty of these guidelines is that there is nothing in them that is rocket science. It is all based around the kind of good sense, best practice information that we were all hopefully taught when first instructed about writing up laboratory reports as junior students.
These guidelines aim to Improve reporting of research using animals. To achieve this aim, they serve as a guide authors about what essential information should be included in a manuscript, but they are not absolutely prescriptive. The recognise the need to be flexible enough to accommodate reporting a wide range of research areas and experimental protocols. The ARRIVE guidelines also promote reproducible, transparent, accurate, comprehensive, concise, logically ordered, well written manuscripts and they aim to improve the communication of the research findings to the broader scientific community.
What they are NOT designed to do is promote uniformity, stifle creativity, or encourage authors to adhere rigidly to all items in the checklist. Some of the items may not apply to all studies, and some items can be presented as tables/figure legends or flow diagrams (e.g. the numbers of animals treated, assessed and analysed). Nor should they be seen as a guide for study design and conduct.

However, some items on the checklist, such as randomisation, blinding and using comparator groups, may be useful when planning experiments as their use will reduce the risk of bias and increase the robustness of the research.
When considering the types of research these guidelines can be applied to, they are probably most appropriate for comparative studies, where two or more groups of experimental animals are being compared; often one or more of the groups may be considered as a control. They apply also to studies comparing different drug doses, or, for example, where a single animal is used as its own control (within-subject experiment). That said, most of the recommendations also apply to studies that do not have a control group and they can generally be applied to any area of bioscience research where animals are used.
The guidelines are primarily designed to provide a checklist for those preparing or reviewing a manuscript intended for publication. However, they are also a very useful guide to the planning of any such work, including application to ethics committees and alike.

The ARRIVE Guidelines have been adopted by a number of highly reputable international journals and they have clearly indicated their intention to use them when reviewing manuscripts for publication. There have however, been some very real problems with enforcing the review process to be compliant. This is largely a result of unfamiliarity at this stage, but it also needs to be recognised that working to these guidelines will result in a significant increase in the workload / expectation placed on reviewers & editors and will therefore slightly increase production costs associated with compliance. So it is safe to assume that it will be a process ……

PREPARE Guidelines:

The PREPARE Guidelines have been specifically put together by Adrian Smith and colleagues at Norecopa as a companion to the ARRIVE Guidelines. They have been put together specifically to address key issues associated with the design of experiments that involve the use of animals so as to help optimise their use. They focus on a number of factors that are seldom reported in scientific papers, but can dramatically influence the validity and outcomes of studies on animals as well as the health and safety of all concerned.
The PREPARE Guidelines cover all stages of quality assurance, from the management of an animal facility or population, through to the individual procedures that are part of a study. In this regard, they essentially serve as a bit of a checklist of some of the essential considerations, based on the authors' experiences running animal facilities, serving on advisory and regulatory committees and planning animal studies. While they are not "yet another checklist to prove compliance", they are an aide memoire to remind scientists of all the topics which may be relevant when planning experiments. So while many of these topics do not need to be reported, they are vital for the success of the experiment, for health & safety, and for animal welfare.

Some of the key areas in which the PREPARE Guidelines aim to have an effect are:

A. Formulation of the study
    - Literature searches
        - This should allow for the formulation of a clear hypothesis with primary and secondary outcomes being defined.
        - Consider the use of systematic reviews
        - Decide on databases and information specialists to be consulted and construct search terms that will define literature searches.
        - Assess the relevance and the appropriateness of the species to be used, its biology and suitability to answer the experimental questions with the least suffering and its welfare needs.

        - Assess the reproducibility and translatability of the project

    - Legal issues
        - Consider how the research is affected by relevant legislation for animal research and other areas, e.g. animal transport, occupational health and safety.
        - Locate relevant local guidance documents (e.g. range of relevant NHMRC Guidelines documents, ANZCCART Guidelines, Institutional guidelines, etc.)

    - Ethical issues, harm – benefit assessment and humane endpoints
        - Construct a clear lay summary of the work to be undertaken and the potential benefits expected to come from it.
        - In dialogue with the Animal Ethics Committee (AEC) consider whether statements about this type of research have already been produced.
        - Address the 3Rs (Replacement, Reduction & Refinement) and the 3Ss (Good Science, God Sense & Good Sensibilities)
        - Consider pre-registration of your work and the publication of negative results.
        - Perform a Harm – Benefit assessment and justify any likely animal harm.
        - Discuss the learning objectives, if the animal use is for educational or training purposes.
        - Allocate a realistic and considered severity classification to the project.
        - Define objective, easily measurable and unequivocal humane endpoints.
        - Discuss the justification, if any, for death as an endpoint and fully explain why more acceptable endpoints cannot be employed.

    - Experimental design and statistical analysis
        - Consider pilot studies, statistical power and significance levels.
        - Define the experimental unit and decide upon animal numbers.
        - Choose methods of randomization, prevention of observer bias, and decide on exclusion and inclusion criteria.

B. Dialogue between scientists and the animal facility
    - Objectives and timescale, funding and division of labour
        - Arrange meetings with all relevant staff when early plans for the project exist.
        - Construct an appropriate timescale for the project, indicating the need for assistance with preparation, animal care, procedures and waste disposal / decontamination.
        - Discus and disclose all expected and potential costs.
        - Construct a detailed plan for division of labour and expenses at all stages of the study.

    - Facility evaluation
        - Conduct a physical inspection of the facilities, to evaluate building and equipment standards and needs.

- Discuss staffing levels at times of extra risk.

- Education and training
  - Assess the competence of staff members and the need for further education or training prior to the study.

- Health risks, waste disposal and decontamination
  - Perform a risk assessment, in collaboration with the animal facility, for all persons and animals affected directly or indirectly by the study.
  - Assess and if necessary produce, specific guidance for all stages of the project.
  - Discuss means for containment, decontamination, and disposal of all items in the study.

C. Quality control of the components of the study
- Test substances and procedures
  - Provide as much information as possible about test substances.
  - Consider the feasibility and validity of test procedures and the skills needed to perform them.

- Experimental animals
  - Decide upon the characteristics of the animals that are essential for the study and for reporting.
  - Avoid generation of surplus animals

- Quarantine and health monitoring
  - Discuss the animals' likely health status, any needs for transport, quarantine and isolation, health monitoring and consequences for the personnel.

- Housing and husbandry
  - Attend to the animals' specific instincts and needs, in collaboration with expert staff.
  - Discuss acclimatization, optimal housing conditions and procedures, environmental factors and any experimental limitations on these (e.g. food deprivation, solitary housing, etc.)

- Experimental procedures
  - Develop refined procedures for capture, immobilization, marking, and release or rehoming.
  - Develop refined procedures for substance administration, sampling, sedation and anaesthesia, surgery and other techniques.

- Humane killing, release, reuse or rehoming
  - Consult relevant legislation and guidelines well in advance of the study.
  - Define primary and emergency methods of humane killing.
  - Assess the competency of those who may have to perform those tasks.

- Necropsy
  - Construct a systematic plan for all stages of necropsy, including location, and identification of all animals and samples.

When considered in concert, these two guidelines documents offer a lot of very good advice about the preparation, planning, execution and reporting of experiments that rely on the use of animals. That advice, if followed diligently, should at least help to ensure that any published research findings represent work that has been conducted at a high standard and is accurately and completely reported in a way that should allow the study to be faithfully reproduced. However, working under the assumption (as I do) that perfection is more an aspiration than a reality, it is probably safe to assume that nothing will ever provide a guarantee of perfectly reproducible results from any experiment, but if there can be confidence in the accuracy of data and the quality of work that produced those data, then it should be seen as a good start and a worthwhile goal for everyone involved.

Large animal models of critical illness: a vital step in translating basic research into clinical applications

Clive N. May
Preclinical Critical Care Unit, Florey Institute of Neuroscience and Mental Health,
University of Melbourne, Parkville, Victoria.

Intensive care is a relatively new speciality that is increasingly important in view of the growing complexity of modern medical procedures. Intensive care offers a standard of monitoring, intervention and organ support for critically ill patients that cannot readily be provided in a general ward. Although numerous advances have been made in the treatment of critically ill patients more research is urgently needed. However, research on critically ill patients is difficult because their physiology is in a fragile state, they are highly monitored, have multiple intravenous lines, drains and catheters and they are usually ventilated and sedated. It is therefore necessary to use animal models to investigate the pathophysiology of critical illness to develop new therapeutics, biomarkers and devices ready for clinical testing in patients.

In the Preclinical Critical Care Unit at the Florey Institute we have developed large animal models of heart failure, septic shock, myocardial infarction and cardiopulmonary bypass and are collaborating on the development of a brain-machine interface. In our studies of septic shock, we have investigated the use of an endogenous hormone, angiotensin II, as a new vasopressor treatment. We have demonstrated that in an ovine model of sepsis angiotensin II effectively maintained blood pressure and improved renal function. Angiotensin II has recently been released as a novel drug to treat septic patients and has already proved to be lifesaving in those who did not respond to currently available drugs. In studies on the treatment of a heart attack, we have developed a novel drug that substantially reduces the damage to the heart after the blockage in a coronary artery is removed, the primary treatment following a heart attack. In an ovine model of myocardial ischaemia-reperfusion, occlusion followed by reperfusion of a coronary artery, we have shown that this drug reduces damage to the heart by 40%, which will reduce patient morbidity and slow the development of heart failure. This drug is currently in a Phase II clinical trial. Brain-machine interfaces translate neural information from the brain into commands that can control external devices such as a computer, wheelchair or a bionic limb. For implantation, current devices require a craniotomy, which has a number of risks. We have developed an electrode array on a stent, a Stentrode, which does not require a craniotomy for implantation. In normal non-paralysed sheep, we have developed an implantation technique by which the Stentrode is inserted via a peripheral vein into a blood vessel in the brain over the motor cortex.

We have shown that we can record neural signals from the surrounding neural tissue over many months. The aim is to implant a Stentrode in patients with motor neuron disease in 2019, to enable the patients to communicate via a computer screen. These studies demonstrate that using clinically relevant protocols and animal models that accurately mimic human disease, substantial advances can be made that lead to improved treatments for critically ill patients.

The speciality of intensive care medicine is relatively new, being developed as a consequence of the poliomyelitis epidemic in the 1950s. Intensive Care Units (ICU) cater to patients with severe and life-threatening illnesses and injuries, which require constant, intense monitoring and support from specialist staff, equipment and medications. Patients in ICU have fragile physiology and are offered a standard of monitoring, intervention and organ support that cannot be readily delivered in a general ward. Although numerous advances have been made in the monitoring and treatment of critically ill patients, more research is urgently needed, but research on critically ill patients, who are highly instrumented and monitored with support for multiple organ systems, is difficult. It is therefore necessary to use animal models of disease to understand the pathophysiology of critical illnesses and to develop improved treatments. Such studies pose ethical problems as realistic animal models of these diseases, that have a similar phenotype to the human disease, are essential if we are to make advances that translate to humans.

In the Preclinical Critical Care Unit at the Florey Institute we have developed large animal models of septic shock, myocardial infarction, heart failure and cardiopulmonary bypass and are collaborating on the development of a brain-machine interface. We have chosen to use large animals, sheep, as in many diseases, including sepsis, myocardial infarction and stroke, the success rate of translation of therapies developed in rodents has been very low. Where relevant, experiments are conducted on anaesthetised animals, but this is not always possible as anaesthesia has profound effects on sympathetic nerve activity to many organs that leads to changes in cardiovascular and renal function, thus confounding the findings. In this paper three projects are described in which we have used sheep as an experimental animal to develop improved treatments for sepsis and heart attack and to develop a brain-machine interface.

Sepsis is the overwhelming, life-threatening response to an infection that has reached the bloodstream. To fight the infection chemicals are released into the bloodstream that trigger inflammatory responses throughout the body that can cause a large decrease in blood pressure leading to organ failure and death. Sepsis is more common than generally realised by the public, one third of patients in ICU present with sepsis or develop sepsis while in ICU. Unfortunately, about a third of these patients die, approximately 5000/year, and concerningly, the incidence of sepsis is increasing. Current treatment for patients with sepsis is antimicrobial control followed by supportive treatments including fluid resuscitation and vasopressor drugs. Noradrenaline, an endogenous compound released from sympathetic nerves, which constricts blood vessels and thus increases blood pressure, is the most commonly used vasopressor drug. A major clinical problem

is that many septic patients become resistant to the effects of noradrenaline, which can result in large falls in blood pressure leading to reduced organ perfusion and multi-organ failure. There is therefore a need for alternative vasopressor drugs to treat septic patients that are resistant to the effects of noradrenaline.

Based on our studies of sepsis, we hypothesised that the endogenous hormone angiotensin II, which like noradrenaline is a potent vasoconstrictor, would improve blood pressure and kidney function in sepsis. We used a sheep model of sepsis induced by intravenous infusion of Escherichia coli, which induces a similar pattern of responses to those seen in human sepsis; decreased blood pressure, increased heart rate, rapid breathing, acute kidney injury and increased temperature (1). During experiments these animals are highly monitored 24 h/day and to reduce suffering animals are euthanised if the changes in blood pressure, heart rate, arterial PO_2, arterial lactate and body temperature exceed thresholds set by the Institute's Animal Ethics Committee. In this ovine model of sepsis, we demonstrated that treatment with angiotensin II restored blood pressure and improved kidney function, without any adverse effects on the heart, other organs, respiration, inflammation or metabolism (2, 5). Angiotensin II has now been developed as a drug to treat critically ill, hypotensive patients, it has recently been approved by the FDA and has already proved to be lifesaving in patients who did not respond to currently available drugs.

Heart attack remains a major cause of morbidity and mortality; over 50,000 Australians have a heart attack each year and over 8000 die. Following a heart attack the primary treatment is to revascularise the occluded coronary artery using thrombolysis, to breakdown blood clots, or percutaneous coronary intervention, to open the blocked artery. Paradoxically, this reperfusion of the infarcted area can itself induce injury to the cardiac tissue. In our studies we have developed a novel drug that substantially reduces the damage to the heart after the blockage in a coronary artery is removed. Studies were performed in anaesthetised sheep in which a coronary artery was occluded for 1-3 h and then reperfused, to mimic the clinical situation of a heart attack followed by treatment to revascularise the occluded blood vessel. The drug we have developed has multiple actions that we predict will reduce injury to the heart that occurs with reperfusion. In this ovine model of ischaemia/reperfusion, we have shown that our drug reduces damage to the heart by 40% (4, 6). Increasing the amount of viable cardiac muscle after a heart attack will reduce patient morbidity and slow the development of heart failure, which commonly occurs if there is a large amount of damage to the heart. This drug is currently in a Phase II clinical trial in Melbourne.

Brain-machine interfaces translate neural information from the brain into commands that can control external devices such as a computer, wheelchair or a bionic limb. Currently, the devices that give the best signals from the brain require a craniotomy and insertion of electrodes into the neural tissue, which has a number of risks including bleeding and infection. An additional problem that limits the use of implanted electrodes is that they are seen as 'foreign' by the brain and gliosis occurs around the electrodes so that much of the signal is lost over 6 months. To avoid these problems, we have developed an electrode array on a stent, a Stentrode, with the aim of implanting this in a cerebral vein to record neural activity from the neural tissue around the vein. In anaesthetised, healthy, non-paralysed sheep, we have developed a technique to implant the Stentrode is using a simple percutaneous procedure via the jugular vein and then deployment, using fluoroscopy, in a cerebral vein overlying the motor cortex, the area of the brain that controls movement. In studies in non-anaesthetised sheep with implanted Stentrodes, we have recorded signals from the surrounding neural tissue over many months. Importantly, the signal did not deteriorate with time and we have shown that implantation of the Stentrode in a cerebral vein does not block the blood vessel (3). The aim is to implant a Stentrode in patients with motor neuron disease in 2019, to enable the patients to communicate via a computer screen, and future plans are to implant the Stentrode in patients with spinal cord injury.

These studies demonstrate that using clinically relevant protocols and animal models that accurately mimic human disease, substantial advances can be made that lead to improved treatments for critically ill patients.

References

1. Langenberg C, Wan L, Egi M, May CN, and Bellomo R. Renal blood flow in experimental septic acute renal failure. *Kidney international* 69: 1996-2002, 2006.
2. Lankadeva YR, Kosaka J, Evans RG, Bellomo R, and May CN. Urinary Oxygenation as a Surrogate Measure of Medullary Oxygenation During Angiotensin II Therapy in Septic Acute Kidney Injury. *Critical care medicine* 46: e41-e48, 2018.
3. Oxley TJ, Opie NL, John SE, Rind GS, Ronayne SM, Wheeler TL, Judy JW, McDonald AJ, Dornom A, Lovell TJ, Steward C, Garrett DJ, Moffat BA, Lui EH, Yassi N, Campbell BC, Wong YT, Fox KE, Nurse ES, Bennett IE, Bauquier SH, Liyanage KA, van der Nagel NR, Perucca P, Ahnood A, Gill KP, Yan B, Churilov L, French CR, Desmond PM, Horne MK, Kiers L, Prawer S, Davis SM, Burkitt AN, Mitchell PJ, Grayden DB, May CN, and O'Brien TJ. Minimally invasive endovascular stent-electrode array for high-fidelity, chronic recordings of cortical neural activity. *Nature biotechnology* 34: 320-327, 2016.
4. Thomas CJ, Ng DC, Patsikatheodorou N, Limengka Y, Lee MW, Darby IA, Woodman OL, and May CN. Cardioprotection from ischaemia-reperfusion injury by a novel flavonol that reduces activation of p38 MAPK. *European journal of pharmacology* 658: 160-167, 2011.
5. Wan L, Langenberg C, Bellomo R, and May CN. Angiotensin II in experimental hyperdynamic sepsis. *Crit Care* 13: R190, 2009.
6. Wang S, Dusting GJ, May CN, and Woodman OL. 3',4'-Dihydroxyflavonol reduces infarct size and injury associated with myocardial ischaemia and reperfusion in sheep. *Br J Pharmacol* 142: 443-452, 2004.

Workshop 1 – Discussion Groups by AEC Membership Categories

Summary of Feedback to the Conference from each Group

One of the key aims of this session is to allow all delegates to meet with others at the conference, with whom they share a role on their AEC. Hopefully, meeting with all other delegates at the conference in the same AEC category as yourself will help you to network with your colleagues from around the region during the next few days and share experiences, wisdom and possibly a few problems in a way that obviously respects the confidentiality agreement you will have entered into when you joined your AEC, but still helps you resolve some of those issues.

Each group was instructed to begin by having members introduce themselves and then to discuss either one or more of the suggested topics or alternatively a topic considered by the group to be particularly relevant to AEC members of your category.

Suggestions for discussion were based on analysis of the following three topics:

1. How well you think the AEC system is working in your institution and overall
2. How well is ANZCCART serving you as:
   As a Category (whatever you are) member?
   As an AEC?
   As an Institution?
   As a community?
3. What can we (The AEC system / Each Institution / ANZCCART) realistically do better?

At the conclusion of time allocated for discussions, a representative from each group reported back to the conference and these are recoded below by AEC category.

Category A (Veterinarians) and AWO (Animal Welfare Officers)

Very large group that could have discussed these issues for far longer. Discussions focussed on a variety of topics as listed below, but a number drew current themes such as the issues associated with consistency of approach, decision making, techniques, etc.

- Differences between AWOs and vets in New Zealand and Australia
- AECs and vets are not necessarily following the same guidelines
- Differences in the allowed weight loss
- Euthanasia techniques such as CO_2 which is not consistent on the AECs
- Involvement in pilot studies as AECs are asking for vets to be included
- Lack of support from institutions
- Lack of openness translates to lack of institutional support and lack of social and community support
- Lack of training which is an opportunity for ANZCCART to facilitate with ANZLAA, some training in Australasia
- ANZCCART and ANZLAA jointly working on initiatives

- ANZCCART could post standardised SOP templates for people to adjust on the website
- Post hot topics on the website which are relevant to both Australia and New Zealand

Category B (Experienced Scientists with substantial recent experience with animal work)

It definitely would be good to have more Cat Bs and to have a more favourable representation at these meetings. Broad ranging discussions but tried to particularly focus on the following three main points.

- We expressed the importance of the Veterinarian or the AWO on the Committee and how essential it is to have their valuable input, very much in terms of going out to research groups and assessing researchers to see if they have the ability to undertake each of the techniques so that comes into the expertise report of each researcher. We very much value their opinion.

- Statistical experience - ensuring there are at least one or two members of the Committee who have some background in statistics. Some Cat B members have expressed that in some applications they have a tick box to mark if the statistician has been consulted and need to address the power calculation, what is the alpha power value you use ie is it 80% power and if it's anything greater than 80% power, you may need to justify that as it means you are using more animals. We need to flesh out the statistics more in meetings and have someone who has that experience and can look very closely at numbers and the expected outcome.
- Discussion on the time and length of the meetings as some are short and others are quite lengthy and expressed the importance of the chair in terms of facilitating discussion making sure everyone gets their opinion across. It is also the responsibility of the chair they move the discussion on as they feel appropriate, not suggesting we should cut the time short for anyone but just to make sure we are getting through meetings and the content in a reasonable amount of time while still addressing the important issues.

Category C (Members with a demonstrable commitment to the welfare of animals, who are independent of the institution)

The following issues were discussed:

- similar issues to Cat D such as the difficulty in getting Cat Cs, lack of training, lack of support from institutions to provide background to the ethics and access to that information. That varied within the group as some people received a lot of information and training while others didn't get much at all. There is a real need for us to have this information.
- the language in many of the project applications with the need for that language to be understood and the difficulty in convincing researchers it an important issue. One solution could be mentors in various schools to help those researchers.
- coming to grips to what extent we should allow an animal to suffer to get the scientific output and how you judge that.
- field research with the difficulty in interpreting bycatch and how to understand the amount of bycatch, how it is treated and how you assess that.
- GM mice breeding – the numbers are quite concerning, the amount of wastage occurring and how you can assist that.

- most of the animals are supplied by research facilities but some researchers do access animals from breeders outside the facility and what measures should be put in place to assess where they come from and the conditions they were bred in.
- members who are on several AECs said it is very difficult when you get a number of projects which are doing the same thing at different institutions and the difficulty in accessing information about the ramifications of the experiments and whether that is a wastage of animals, whether there needs to be greater communication and accepted by researchers the confidentiality that it brings.
- monitoring in field research as it is very difficult to get out into the field and to actually monitor the animal's treatment in the field as an AEC and to understand that.
- The value of ANZCCART - it's fantastic to meet other Cat C members and really great to share issues. They noticed there were not many Cat B members at the Conference and perhaps this could be something that is supported more by institutions.

Category D (Lay members who are independent of the institution and have never been involved with the scientific use of animals beyond their own undergraduate education)

Discussions within this group of delegates covered a wide range of topics that were both specifically relevant to them as well as a number that were more related to the work they do and the welfare of animals. Specific topics addressed by this group were:

- First question raised was AECs being publicised. We felt that the public from a D members point of view, didn't understand what the AEC did and whether it should be publicised more because we get questions about sheep exports, that has nothing do with the AEC and people think it does.
- The problem of members belonging to a number of committees. D categories can be on 2,3,4 and 5 AECs and that is a problem we felt with people set in their way. Some of the committees have 2 periods of 3 years, others have 3 periods of 3 years and others have unlimited.
- It's very hard to find D members, even if it's publicised as there aren't many volunteers and most of them are retirees. That is a big problem as a lot of retirees are not computer literate and they do not get any training.
- Not all members are volunteers and some get paid honorariums, eg travel expenses, paid parking, petrol allowance and some of those who aren't retired have to take annual leave which can be a real problem.
- How can you keep the enthusiasm up for category D members as they have an enormous amount of reading they don't understand and most find that they don't understand the lay summaries either. Conferences like ANZCCART give the members a lot of support and they get a lot information from them.

- Environmental enrichment for animals – things like toilet rolls, pvc tubing, ropes for rats and mice, birch trees for sheep, hammocks for bats, rabbits put them on the lawn
- We all agreed that D members are not all misunderstood and that we are valued

Category E (Members of the institutional animal care staff)

This group decided to limit their discussions to a few issues that were of specific relevance to animal care staff and their role on the AEC as well as the really important role they can and should be playing when it comes to the training of investigators. Specific discussion points were:

- A key issue is communication from the animal facility and back to the AEC. It varied between different states and whether the Cat E member was actually part of the quorum or not, however, we all found that even if there were issues we did have an avenue to explain that to the AEC and come up with different methods to fix our problems. Regardless of how different it was for all of us we actually knew there were ways to work together and depending on who you reported to it all ended up back to the AEC.
- Another thing we did find was the difference with the newly released Code in the push for training and compliance. They looked at how the training needs to be more into the animal facility, for researchers themselves and investigators and teachers, and what type of training they need and how we can prepare for compliance. With all the different institutes it would be nice to have some over-arching structure we could follow and we thought maybe ANZCCART could help with some sort of guidelines and how we might be able to achieve that in institutes.
- An issue with our own technicians was to find out what type of training they received before we could employ them. We noticed that different states did things differently – some had TAFE requirements, others employed people from a University background and we would really like something that specialises for animal technicians and laboratory-based type of training. It is something which seems to be lacking.
- An improvement we had found was communications between AWOs and Category E members. Most of us had noticed that throughout the years this has become a much bigger stronger link in a united front which has been fantastic.

AEC Chairpersons

It was noted that the Chairs present came from a range of backgrounds that related to Categories A, B and D, and so acknowledged that they were therefore not quorate, a reference to the occasional difficulty in ensuring that every meeting does meet that mark. Specific topics discussed included:

- Internal review – forced to provide every 4 years, risk of external auditor as they don't always complete the audit.
- What qualifies as an animal and standards across the country re fish etc
- Sharing SOPs – AWO drafts them and AECs accepts them – may be duplication so there is an opportunity for ANZCCART to combine them
- Observational studies – when do we start reporting on them
- Minimal risk forms help PIs putting in submissions
- AEC member security – important to maintain
- Citizen scientists - Bird watchers etc are more prevalent
- Challenges in competency assessments – manage by skills based register
- Future recruitments – it looks great on the resume

SEC Secretary / Executive Officers

Most of the discussions of this group related to the handling of specific and or common issues or problems that can effect AEC operations, essentially tending to focus a little on setting up mechanisms that might help the committee handle similar or related requests in a consistent manner. Specific topics included:

- How to handle tricky situation so it's good to have advisors on the Committee
- How to handle animals which are not recognised by your state
- How to make sure the skill level is good - in the field use videos, have people nearby to check, online training courses
- How to educate researchers on the 3Rs – animal ethics training as a requirement, pre-screening by executive officers, research officers assigned to faculties
- How do you inspect facilities outside the University – facility staff, AWOs to check or use videos, AECs to check
- It's struggle to get AWOs and Category C members – advertising on SEEK volunteer
- Does anyone have horse online systems?
- ANZCCART – common welfare legislation and reporting requirements, templates, provide training on the code, sharing online modules

Animal Welfare legislation Regulators (regional government)

Pretty much all the discussions among this group related to the broader topic of cross – border harmonization of processes and procedures relating to the administration of AEC operations. Specifically, they considered:

- Discussed research across states
- challenges in trying to manage and run AECs
- how to simplify the process
- adopting common project purposes and procedures
- unified national template for reporting
- have single AEC member nomination form which can be sent to multiple jurisdictions
- Opportunities to create standard form for applying for animal use licences
- Adopt consistent time frame for the duration of projects to be granted

Using 'The Code' to trace policy change in animal research practices in Australia 1969-2013

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This paper, as part of a broader analysis of policy change in animal research in Australia, will consider how the current system of animal research regulation emerged within the cultural and political climate of the last four decades. This inquiry will use the development of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (the Code) across its eight editions as a framework for understanding changes in standards for animal research practices. Expanding on the evaluation of the Code provided by Rose and Grant (2013), this paper will consider the aims outlined in each edition to determine how these reflected policy changes and the broader social and political climate of the time in relation to the value placed on the experimental animal. The development of the 3Rs: Reduction, Replacement and Refinement (Russell & Burch, 1959) will be considered concurrently with the development of the Australian system of self-regulated Animal Ethics Committees (AECs), as these principles have continued to inform the regulation process from its inception, to the present day. The history of the Code is relevant in evaluating the current system of animal research regulation, because its various editions were in part driven by changes in attitudes in this country both within the public sphere and the scientific community.

Rose, M., & Grant, E. (2013). Australia's ethical framework for when animals are used for scientific purposes. *Animal Welfare, 22*(3), 315-322.
Russell, W. M. S., & Burch, R. L. (1959). *The principles of humane experimental technique*.

Exploring attitudes to research animals through 'the Code' 1969-2013

The current project is still evolving and the findings presented here are preliminary. There are two areas of inquiry within this project. The first uses the development of the Australian Code for the Care and Use of Animals for Scientific Purposes (the Code) as a framework for understanding changes in the social and political sphere, and how these changes subsequently impacted upon animal research practices. This approach assumes that the attitudes towards animals outlined in the Code reflected the attitudes being voiced by the wider public. Moreover, it also assumes that the social and political climate of the time can be seen as a catalyst for changes in the Code. This approach also sees the Code as a proxy for legislation subsequently enacted because State and Territory governments use the standards outlined in the Code as a framework for building legislation to regulate animal research. Changes in attitudes towards research animals are considered by analysing the aims of each edition of the Code.

The second area of inquiry addresses claims about the review process undertaken by animal ethics committees (AECs) in Australia. This inquiry has evolved very recently and is in the early stages of data collection and analysis. There has been a suggestion that no projects are rejected outright

by these committees (D. Russell, 2012); however, due to confidentiality, these claims remain neither confirmed nor refuted. The primary aim of this inquiry is to obtain quantitative data on application outcomes from AECs within Australia categorised as: 'number of applications received', 'number of applications approved without modification', 'number of applications approved with modification' and 'number of applications rejected'. The process of revision is both extensive and effective, and it is anticipated that this will be reflected in a high proportion of the total applications being modified prior to approval. These data will not be used to misrepresent the scientific community, or the functioning of AECs more specifically. However, a study of this nature will be important in addressing sustained issues of transparency between the scientific community and the broader public. The availability of this kind of data within the public domain is an important step to moving into a productive scientific future with greater openness to the public.

The Principles of Humane Experimental Technique by Russell and Burch was published in 1959, outlining the principles of Reduction, Refinement and Replacement (the 3Rs) in the context of animal research. The book was not an immediate success. In a reflective paper 'The Three Rs: past, present and future', W. M. S. Russell (2005, p. 280) states that "after two decades of little or no notice, the Three Rs had suddenly taken off in about 1980". Interestingly, it is around this time that the 3Rs first appeared in the Code. These principles have come to be regarded as the cornerstone of animal research regulation across the international arena over the last forty years, and their role within regulatory systems has evolved to become a prominent structural feature of many of these systems. However, the value and effectiveness of the 3Rs has been challenged. In her paper 'Why animal ethics committees don't work', Denise Russel argued that the way these principles have been applied within animal ethics committees in Australia, has perpetuated the indispensable role of animals within scientific research (D. Russell, 2012). In this paper Russell builds an argument around answering the question of 'why animal ethics committees don't work'. In particular, she problematizes the principle of replacement and its applicability within AECs. Primarily, replacement is not appropriately considered within the review process because there is no member of the committee who is equipped with an up-to-date knowledge of the alternative models that are developing in fields outside of traditional science, such as computer modelling and epidemiology. Consequently, this principle entrenches the existing paradigm of animal models in scientific research. According to Russell, because AECs review applications at the end of the approval process and, importantly, after the research has received funding, "the institutional pressures are such that there is no possibility that the research will be rejected outright" (D. Russell, 2012, pp. 135-136). It should be noted that this paper was published in 2012 and there is likely to have been some change within the review system since then. Moreover, while adhering to the same review process, every AEC functions differently because the committees are made up of people; it is likely therefore, that while some of the issues that Russell outlined may have been present in some AECs, it is unlikely that her criticisms were correct of all committees. Nonetheless, this paper will address the claim that no applications for animal research are rejected outright because this statement can be objectively tested using quantitative data.

The issue of transparency is relevant to the review of applications for animal research. In this context, transparency refers to the flow of information from the scientific community to the broader public. This information could be the practices used within research, the species and number of animals used, the husbandry and housing practices, or the aims and anticipated value of the research. Russell (2012) touches on the issue of transparency in her paper in the context of AECs, where confidentiality safeguards the flow of information beyond the committee members. This lack of researcher transparency in Australia has been conceptually linked to the alleged 'terrorist' activities of animal rights activists especially during the 1980's, and largely within the US and the

UK. Fear has been said to perpetuate this reluctance to be open with the public about animal research practices, in spite of the agreement from all parties that greater transparency is needed.
This paper traces the conceptual changes in the aims of the Code over its eight editions as a way of representing how attitudes towards animals evolved over the period of 1969 to 2013. This is clarified with a media discourse analysis over the period from 1967 to 2013 using the database Informit: Australian Public Affairs-Full Text (APA-FT), which is a collection of journals, newspapers, magazines, books and conference proceedings on Australia's political, economic and social affairs. This database will provide a comprehensive picture of the kinds of issues that were being discussed in a broad range of media outlets, including within the animal rights community. The search terms used in APA-FT can be found in Appendix 1 of that publication. The online database is available from 1978 onwards, and a search using this database obtained 108 records, twelve were eliminated from this list for not fitting the criteria of the search. The hardcopy indexes of APA-FT for the period 1967 to 1977 produced an additional 5 records, bringing the total to 101 records.

For the quantitative analysis on AEC outcomes, data will be collected on the total number of submissions received within a calendar year, categorised as 'number of applications received', 'number of applications approved without modification', 'number of applications approved with modification' and 'number of applications rejected'. It should be noted here that there is not a consistent framework used by all AECs; different AECs use different terminologies, and often entirely different category labels. Some AECs will have more categories, which break down some of the categories identified here into sub-categories. To give an example, the rejected category label used here will cover outright rejections as well as rejections with the option to resubmit after making major modifications. These data will be used to visually represent broad patterns in the review process undertaken by animal ethics committees. The analysis will involve standardising the data from each institution so that the data can be combined into a single data set.
The eight editions of the Code are used as a framework for understanding changes in standards for animal research practices as a response to the broader social and political climate surrounding this issue. These changes are subtle but can be observed in the wording of the overarching aims of each edition.

In the first edition of the Code, published in 1969, it outlined that "the aim is to define a code of conduct which will promote humane behaviour towards experimental animals, the use of which is vital to the growth of biological knowledge as well as to biologists". (National Health and Medical Research Council, 1969, p. 1). This aim is problematic in hindsight because it frames the humane treatment of animals in the context of the growth of scientific knowledge for human gain.

In the second edition, published in 1979, one of the aims was "to emphasise the responsibilities associated with experiments involving the use of animals" (National Health and Medical Research Council, 1979, p. 1). It can be seen that this introduces a greater degree of accountability for those involved in animal research. Another aim was "to promote an attitude that will encourage the efficient and considerate treatment of animals so that the degree of stress or discomfort produced is no greater than would be accepted as reasonable and tolerable by community standards" (National Health and Medical Research Council, 1979, p. 1). As in the first edition, the sentiment here is relatively detached from the experience of the animal, conceptualising the animal as a tool of research.

In the third edition, published in 1983, one of the aims was "to encourage the considerate treatment of animals used for research purposes" (National Health and Medical Research Council, 1983, p. 1). This aim is adjusted in the fourth edition published in 1985, to "ensure the considerate treatment of these animals" (National Health and Medical Research Council, 1985, p. 1). The change in

terminology from “encourage” to “ensure” demonstrates the beginnings of change in attitudes towards research animals, the welfare of animals was being made a priority rather than just a suggestion. However, it should be noted that it was also considered good research practice to make sure animals were suffering as little as possible, because this change in physiology could invalidate the results. The fourth edition also includes the aim: “to promote the development and use of techniques which replace or complement animal experiments and so reduce the numbers of animals used” (National Health and Medical Research Council, 1985, p. 1). This is the first time the concepts of ‘Reduction’ and ‘Replacement’, as proposed by Russell and Burch are introduced into the aims of the Code. In 1983, a Senate Select Committee was established for the purpose of conducting an enquiry into animal welfare within Australia, the culmination being a report which was released in 1989. According to Rose and Grant (2013, p. 316) “[t]his Report identified the need to ensure that the use of animals is justified through ethical review and the application of the principles of the 3Rs”.

The fifth edition of the Code, published in 1990, perhaps in response to the Senate Select Committee report, puts greater emphasis on the principles of the 3Rs in its aims to “minimize the number of animals used in projects and limit or avoid pain or distress” and to “promote the development and use of techniques which replace animal experiments” (National Health and Medical Research Council, 1990, p. 1). This edition shows the transition to a review system that actively seeks to minimize the numbers of animals in research and minimize pain and distress. According to Villanueva (2015, p. 556) “the frequency of revisions [of the Code over this period] reflected a rapid pace change in both scientific practice and knowledge, and in shifting community attitudes to animal experimentation”.

In the sixth edition, published in 1997, one of the aims was to “ensure the use of animals is justified” (National Health and Medical Research Council, 1997, p. 1) and this represents the beginnings of the cost-benefit thinking that asks the question: do the potential benefits of the experiment outweigh the potential harm inflicted upon the animals. This paradigm is now established within the review process undertaken by AECs (e.g. see National Health and Medical Research Council, 1990, p. 6) with the aim of minimising research that does not contribute significantly to improving the wellbeing of humans, animals, or the environment. Another aim of the sixth edition was to “avoid pain or distress for each animal used in scientific and teaching activities” (National Health and Medical Research Council, 1997, p. 1). The use of the phrase ‘each animal’ seems to reflect a change in attitude from seeing the animal as a tool of research, to being an individual life that should be considered.

In the seventh edition, published in 2004, the cost-benefit analysis was fleshed out in the aim to “ensure that the use of animals is justified, taking into consideration the scientific or educational benefits and the potential effects on the welfare of the animals” (National Health and Medical Research Council, 2004, p. 1). The refinement principle of the 3Rs is also stated more plainly here in the aim to “refine methods and procedures to avoid pain or distress in animals used in scientific and teaching activities” (National Health and Medical Research Council, 2004, p. 1).

The eighth, and most recent edition, published in 2013, states that “An obligation to respect animals underpins the Code. This obligation brings with it a responsibility to ensure that the care and use of animals for scientific purposes is ethically acceptable, balancing whether the potential effects on the wellbeing of the animals involved is justified by the potential benefits to humans, animals or the environment” (National Health and Medical Research Council, 2013, p. 1). This is the first time the term ‘respect’ appears within the aims of the code in relation to the treatment of research animals. This timeline of aims of the Code spanning from 1969 to 2013, shows a progression of

the consideration of the capacity for animals to suffer, with the changing terminology demonstrating this progression.

A visual representation of the publication trends (see Figure 1) for the search terms shown in Note 1 (below) within the database APA-FT. The relationship between publication of a new edition of the Code and the number of articles published in APA-FT within a given year has not yet been analysed; however, it is interesting in itself to see the way the trend for these search terms peaked and troughed over the period from 1967 to 2013. The 101 records came from 73 different sources from diverse publishing locations such as the Age, the Australian, Women's Day, the Sydney Bulletin, Animal Liberation, Animals Today, Australian Veterinary Journal and Medical Journal of Australia. This diversity is important because it represents a wide area of interests, in support

(animal* AND (experiment* OR vivisection OR research) AND (rights OR ethic*)) OR (animal AND research AND experiment*)

of and opposed to animal research, as well as the breadth in between.

Note 1: Search terms used in the database APA-FT

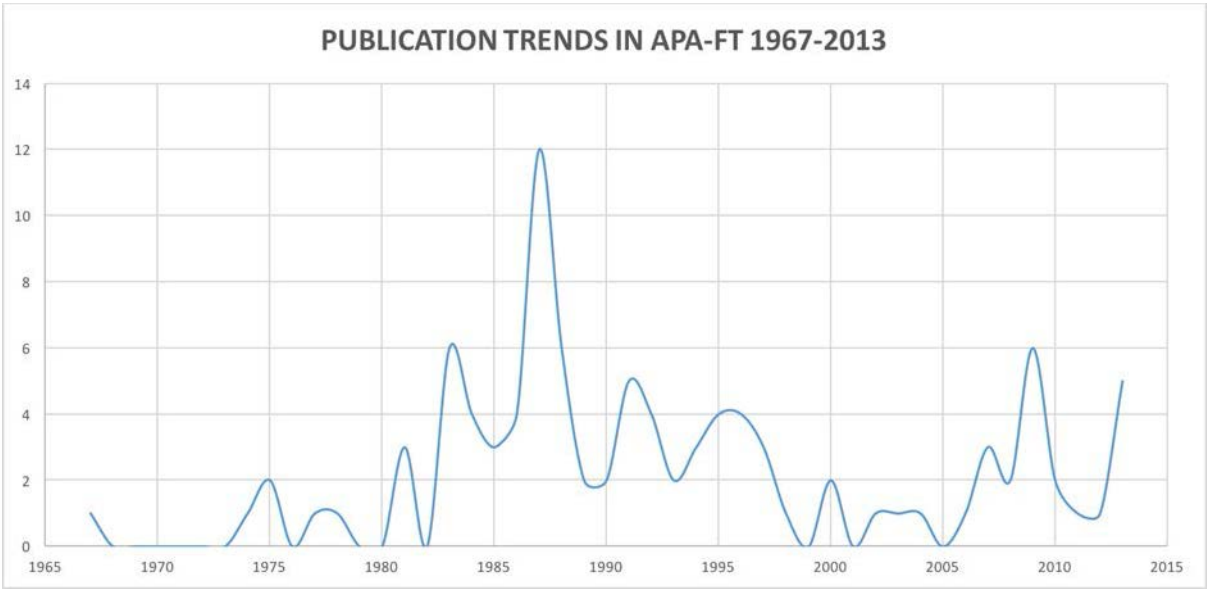

Figure 1: Graphic presentation of changing publication trends between 1967 and 2013.

A representation of the data that has been received for the inquiry into the patterns in application outcomes for AECs is provided below, with a breakdown of data received from five institutions for the calendar year of 2016 and 2017 shown in Figure 2. While Figure 3 is a representation of the data received for all five institutions over both 2016 and 2017. Both graphs show outcome categories as a percentage out of 100 to protect the anonymity of the institutions. This project is still in progress so the results presented here are only a sample of the potential total. For this reason, these results should only be taken as an indication of expected findings. The first thing to note is that, the claim that no projects are rejected cannot be fully supported; however, the trend largely demonstrates that few projects are rejected. Another notable trend is that it is uncommon for projects to be approved without modification. This demonstrates the rigorous process of review that animal ethics committees undertake in adhering to the regulatory system they exist within.

This paper has presented a way of observing the evolution of attitudes towards research animals in an Australian context, by analysing the aims of each edition of the Code. The changing terminology in this document could be seen to represent both the attitudes of the scientific community and the wider public through the influence that public opinion may have had upon the regulation of animal research. This inquiry will develop further when the results of the media search are analysed further, the themes emerging from these articles will paint a picture of the kind of discussions happening within the public sphere regarding animal research. Additionally, quantitative research using the data set obtained from the media search will be used to consider patterns in publication frequency and location. The second area of inquiry was able to tentatively suggest that outright rejection, while rare, is not unheard of within the animal ethics committee review process. This finding will become clearer when a larger data set has been obtained. It is important for AECs to be able to be more reflective in their practices to ensure that a steady progression of change continues into the future.

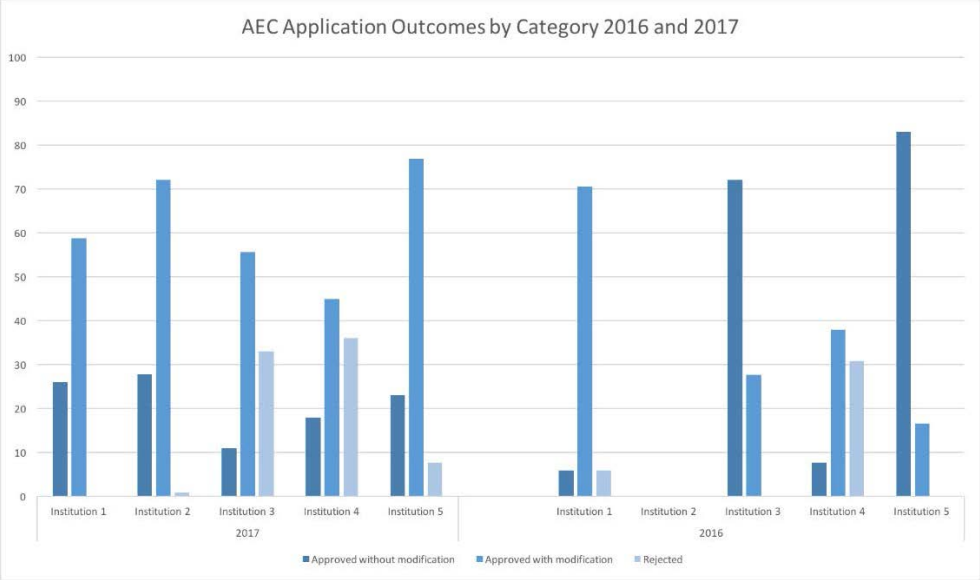

Figure 2: AEC application outcomes for five institutions in 2016 & 2017

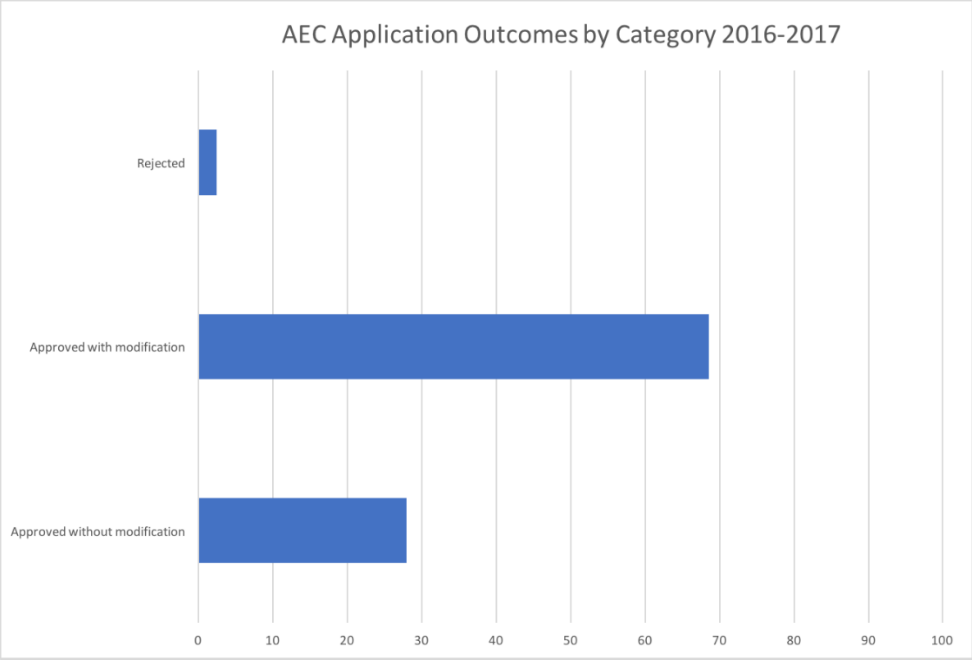

Figure 3: Combined AEC application outcomes (2016 – 2017)

References:

National Health and Medical Research Council. (1969). *Code of practice for the control of experiments in animals*: National Health and Medical Research Council.

National Health and Medical Research Council. (1979). *Code of practice for the care and use of animals in research in Australia*: Australian Government Publishing Service.

National Health and Medical Research Council. (1983). *Code of practice for the care and use of animals in research in Australia*: Australian Government Publishing Service.

National Health and Medical Research Council. (1985). *Code of practice for the care and use of animals for experimental purposes*: Australian Government Publishing Service.

National Health and Medical Research Council. (1990). *Australian code of practice for the care and use of animals for scientific purposes*: Australian Government Publishing Service.

National Health and Medical Research Council. (1997). *Australian code of practice for the care and use of animals for scientific purposes*: Australian Government Publishing Service.

National Health and Medical Research Council. (2004). *Australian code of practice for the care and use of animals for scientific purposes*: Australian Government Publishing Service.

National Health and Medical Research Council. (2013). Australian Code for the Care and use of Animals for Scientific Purposes: Canberra: National health and medical research council.

Rose, M., & Grant, E. (2013). Australia's ethical framework for when animals are used for scientific purposes. *Animal Welfare, 22*(3), 315-322.

Russell, D. (2012). Why animal ethics committees don't work. *Between the Species: an online journal for the study of philosophy and animals*, 15 (1), 127-142.

Russell, W. M. S. (2005). The three Rs: Past, present and future. *Animal Welfare, 14*, 279-286.

Villanueva, G. (2015). “In the Corridors of Power”: How the Animal Movement Changed Australian Politics, 1979–1991. *Australian Journal of Politics & History, 61*(4), 546-561.

Aim high for high containment: Welfare in a high containment laboratory

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The Australian Animal Health Laboratory is a vital part of Australia's biosecurity infrastructure and helps protect Australia's humans and animals from emerging and exotic disease threats. It is one of only six laboratories in the world that can work with animals at the highest biosecurity level 4. Biosecurity level 4 conditions are applicable for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy. Approximately 70% of emerging infectious diseases in humans originate from animals and therefore much of the animal work at AAHL is work with zoonoses, which are diseases that can be transmitted from animals to humans. This talk will discuss the restrictions of a high containment laboratory, how it has the potential to create a high impact adverse environment for both animals and human staff, and what strategies we can use to prevent this.

Aim high for high containment: Welfare in a high containment laboratory.

The CSIRO Australian Animal Health Laboratory (AAHL)

The CSIRO Australian Animal Health Laboratory is a vital part of Australia's biosecurity infrastructure. The facility helps protect Australia's humans and animals from emerging and exotic disease threats. The initiative for the Australian Animal Health Laboratory came about in discussions in the late 1950s amongst Australian Veterinary Association. In 1967 there was a Foot and Mouth Disease outbreak in the UK which resulted in 442000 animals being culled and in the 1960s and 1970s there was increasing concern about the spread of Bluetongue virus across Australia. The construction of AAHL started in 1978 and it was officially opened in 1985 at a cost of over $160 million. AAHL would cost approximately $1.2 billion to replace today and includes more than 600 concrete piles, 42000 cubic metres of reinforced concrete, 2300 pre-cast concrete wall panels, 520 air-lock doors, 62 air-handling systems and 1000 high-efficiency particulate air (HEPA) filters. The establishment of AAHL meant exotic diseases could be diagnosed within Australia, rather than being sent overseas.

AAHL's animal facilities

AAHL has two animal facilities and an additional satellite facility in Werribee. The Werribee animal facility houses agricultural species and the Small Animal Facility (SAF) houses specific pathogen free (SPF) chickens and laboratory rabbits and rodents. These two facilities are certified for up to biosecurity level 2 containment, so for work involving agents of no known, mild or moderate potential hazard to personnel and the environment (i.e. - don't cause disease in humans, indigenous diseases, no aerosol transmission).

My presentation focused on the Large Animal Facility (LAF) which has 26 biosecurity level 3 capable animal rooms and 2 biosecurity level 4 animal rooms.

Biosecurity Levels (BSL)

Biosecurity Level 3

Biosecurity level 3 containment is applicable to work done with exotic or indigenous agents that may cause serious or potentially lethal disease through the inhalation route of exposure. The manipulation of infectious material is conducted within biological safety cabinets or other physical containment devices, or by personnel wearing appropriate personal protective clothing and equipment. There are many thousands of BSL 3 capable facilities worldwide and they include specialised design features such as controlled access, directional air flow which draws air from “clean” towards “contaminated” areas and HEPA filtered exhaust air. Examples of BSL 3 agents are Influenza, African Swine Fever, Japanese Encephalitis, Murray Valley Encephalitis, Rift Valley Fever, West Nile Virus and Yellow Fever.

Biosecurity Level 4

Biosecurity level 4 containment is applicable to work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments, or a related agent with unknown risk of transmission. All manipulations of potentially infected materials and isolates pose a high risk of exposure and infection to personnel, the community and the environment and work with this material must be carried out in a Class III biological safety cabinet and/or a full body, air-supplied positive pressure personnel suit. Examples of BSL 4 agents are haemorrhagic fever viruses, such as Ebola, Marburg and Lassa virus and particularly important for Australia, the Henipah viruses (Hendra and Nipah virus).

Most BSL 4 capable facilities are a separate building or a completely isolated zone, with specialized ventilation and waste management systems.

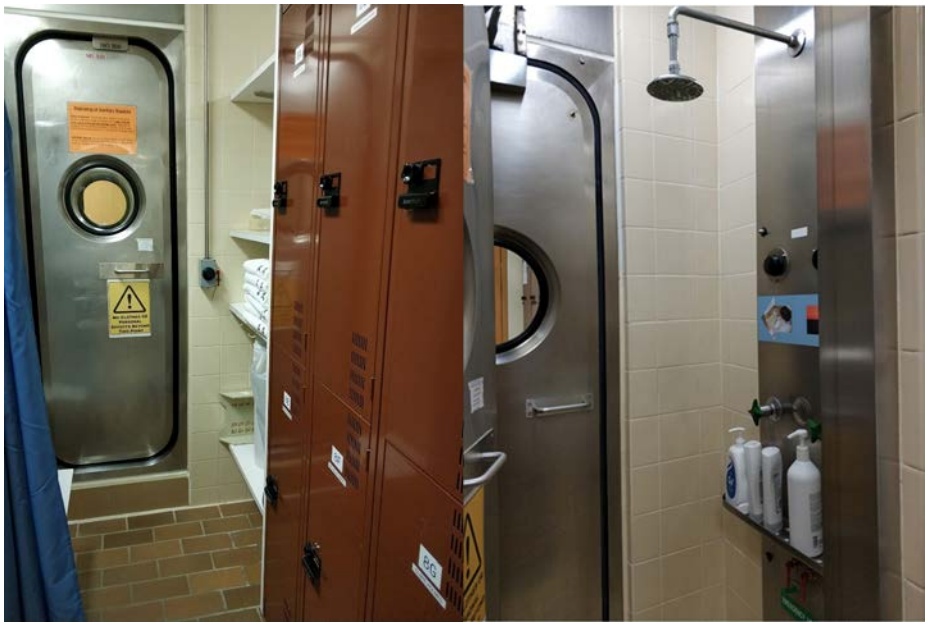
AAHL is unique

There are approximately 38 BSL 4 facilities worldwide, but only about 10 of these have the capability to do animal studies and generally this is work with small laboratory mammals. Three to five of these facilities can do work with “large” animals at BSL 4, depending what species is being referred to. AAHL is the only facility with the capability to work with horses in BSL 4 conditions.

How to enter and exit the LAF animal rooms

- AAHL has controlled access to the site using Cardax security cards for all employees.
- There is a Cardax access point for entry to the site and also to the building.
- Then Cardax access points at the Central Monitoring System door, turnstile and change room door.

- Once in the non-secure change room, all personal items must be removed and placed into a locker before passing through a double airlock with a shower. Showers are unnecessary on entry as the principle is decontamination when leaving the secure area.



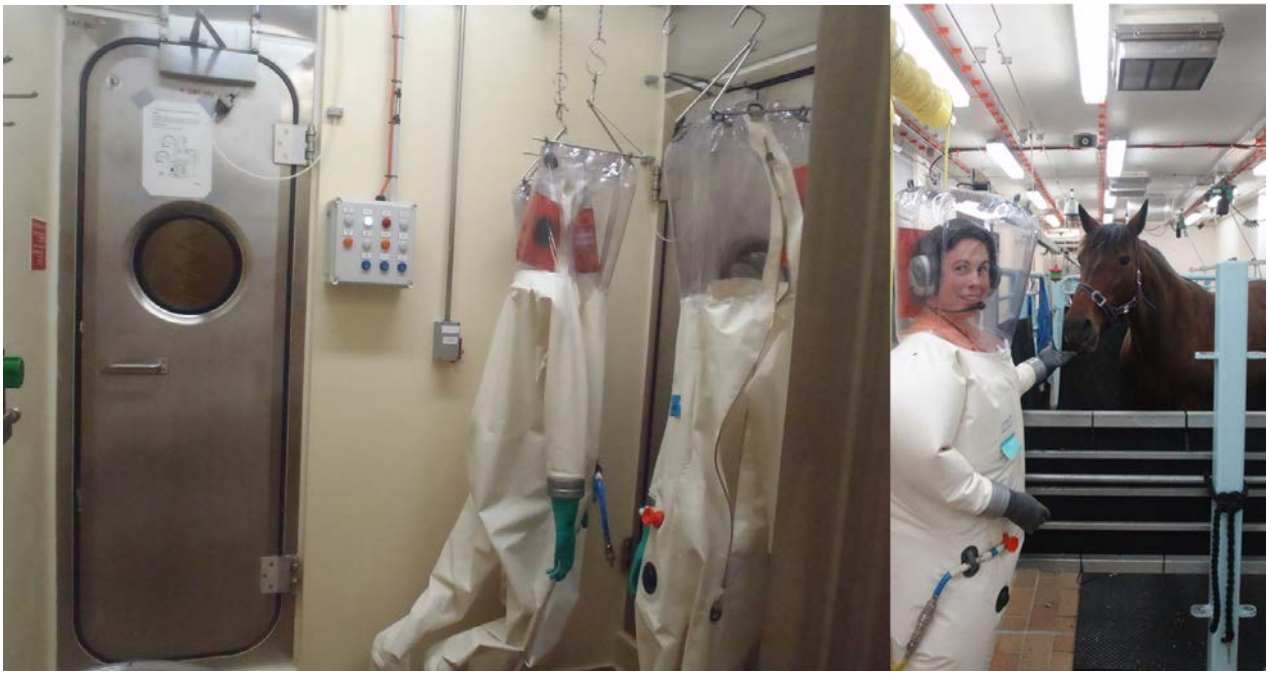
- Once in the secure area there is personalised clothing and shoes to wear.

- To gain access to the LAF from the secure area, enter the Cardax access change room and remove all clothing.
- Proceed through a double airlock with a shower and change into orange overalls to access the LAF corridor.

- Enter into the LAF animal room:

- BSL 3 – Don Tyvek overalls, enter the security door using a PIN on a keypad, pass through a double airlock with a shower into the workroom and put on a powered air purifying respirator (PAPR).

- BSL 4 – Enter the security door using a PIN on a keypad, pass through a double airlock with a shower into the workroom, put on full body, air-supplied positive pressure personnel suit.



- Exiting the LAF animal room:
  - BSL 3 – Move from animal room to work room, spray Tyveks with Virkon disinfectant and undergo a three minute wait for air change in work room. Undress and take three minute personal shower in animal room airlock shower.
  - BSL 4 – Enter the chemical shower and take a 10 minute glutaraldehyde shower in the suit. Remove the suit in the work room, undress and take three minute personal shower in animal room airlock shower.
- Exit security door with PIN into corridor.

- Enter LAF entry change room, remove overalls, pass through LAF airlock and put secure area clothing back on.
- Enter secure area change room, remove secure area clothing, take a three minute personal shower in secure area airlock shower and put regular civilian clothing back on.

Why do we use animals at AAHL?

We need to pursue research on diseases in this highly contained environment for the benefit of both animals and humans.

- More than 60% of known infectious diseases in people are spread from animals. These diseases that transmit from animals to humans are known as a zoonoses.
- Infectious diseases previously unknown in humans have been increasing steadily over the last three decades. For example Severe Acute Respiratory Syndrome (SARS), Middle Eastern Respiratory Syndrome (MERS), Nipah virus and Hendra virus.
- Seventy-five percent of these emerging diseases are zoonotic.
- Increasing spill over events in humans (e.g. – Ebola).

The animal studies undertaken directly contribute to exotic disease preparedness for Australia and AAHL is Australia's facility for detection and characterisation testing in the case of newly discovered agents. The facility also develops models for infectious disease with the ultimate goal of developing treatments and/or vaccines for animals and/or humans. The laboratory produces polyclonal antiserum for many diseases, such as influenza and pestivirus, which is used for diagnostic tests and development. AAHL also supports Australian state veterinary laboratories by testing thousands of samples and contributes to international disease preparedness and eradication.

The challenges of AAHL

Biosecure rooms and decontamination

AAHL's secure animal rooms are constructed from thick reinforced concrete walls and steel air lock doors. It is designed to withstand emergencies such as fires (no combustible materials) and explosions. Once empty, rooms are pressure washed with glutaraldehyde and then gassed with formaldehyde gas. While experiments are underway, items and disposables cannot be freely accessed or moved in or out of the rooms.

Prior planning is required to help overcome this issue. The majority of the items required for the experiment are organised within the room, this includes animal food, syringes, needles, anaesthetic machine/drugs, postmortem instruments, etc. However, this strategy must also be minimalist to prevent waste and clutter and extra effort at decontamination. Technology in the room must be productive, as electronics must be protected during the washing of the room and able to withstand the formaldehyde gas. Animal enrichment items be either disposable, for example polar fleece cloth bedding, wooden chew blocks and cardboard or paper nesting materials. Toys for animals are usually plastic or rubber with no small holes or pockets so that they can be easily cleaned and decontaminated.

Sewerage system

AAHL has a highly technical sewerage treatment system in place to effectively treat waste from the high containment area, potentially containing BSL 3 and 4 pathogens. The code stipulates that “the care and management of animals must be based upon current best practice that takes into consideration the relevant aspects of species specific biology, physiology and behaviour”. The beneficial effects of providing hay to ruminants and horses as environmental enrichment is well documented. The AAHL Animal Ethics Committee and animal care staff considers the provision of hay to these animals as best practice and a species-specific need which should be provided whenever possible.

Primary catchment and movement of fluids from drains, floors, sinks, etc. is collected into pipes 100mm diameter and up to 150 metres in length. Hay swells and floats when exposed to liquid potentially blocking the pipes and if a blockage occurs it can disable multiple animal rooms and expose engineering staff to untreated, potentially infectious effluent. Sewerage is collected in 6000L capacity tanks that discharge into larger tanks (30000 – 36000L) via a macerator. The macerator is effective at chopping up most solids, but inefficient at chopping up feathers and hay. The sewerage is treated by heating to 125^{o}C and then discharged through a cooling system into public sewers that results in slow drainage. This slow drainage

can result in residual solids remaining in the tank and hay solids can then bake into a hard crust rendering the tank unusable for extended periods. Cooling system pipes are also very susceptible to blockages caused by excess hay.

Animals held in the LAF at AAHL are provided hay to improve their welfare during experiments. By employing the following methods, there have been no major blockages of the sewerage treatment system at AAHL during the many years hay has been used for enrichment purposes. Sharing these strategies has also convinced other high containment facilities overseas to institute the provision of hay to ruminants.

Quantities

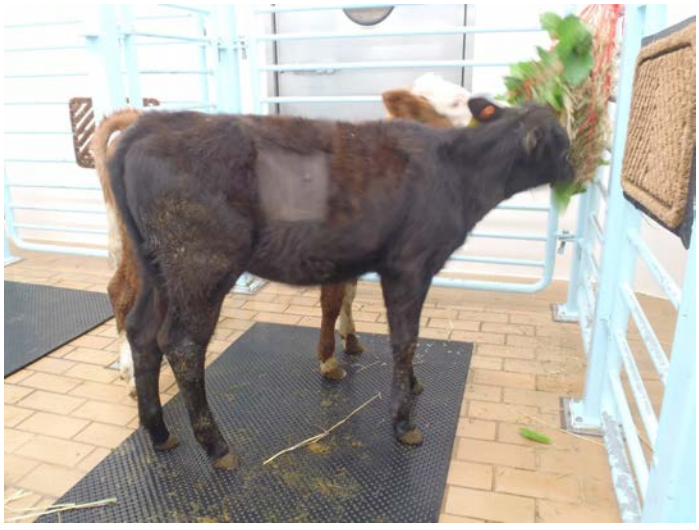
Hay is fed in conjunction with other foodstuffs, including chaff and commercial extruded pellet diets. The provision of hay is primarily for environmental enrichment and therefore large amounts are not required. Using relatively small amounts of hay with feeding apparatus to slow intake is more effective in this regard, as it encourages foraging by the animals and occupies time.

Provision

Hay is provided to the animals in dispensing apparatus, rather than in troughs. This requires animals to work to get the hay, reducing idle time which can encourage the development of negative behaviours. It also minimises the amount falling on the floor, which could contribute to drain blockages.

Cleaning

Animals in the LAF are housed with as much free range room as possible and the rooms are cleaned by hosing the floors on a daily basis. When providing hay, the floors are thoroughly swept and as much dry matter as possible is collected and placed into bins prior to hosing. The room has large drains that are covered with the slotted cover and contain a perforated metal bucket which catches and collects most large particles to prevent them from entering the sewerage system. These buckets are emptied into bins daily in rooms where hay is being utilised.



High training requirements

The competency for working with animals in a BSL 3 and 4 environment takes a long time to develop. This is because staff in the LAF work with a multitude of species with many different disease and must have familiarity with normal versus clinical signs. They are also dealing with sharps handling in a very hazardous environment with reduced dexterity and sensory ability due to personal protective equipment, the development of personal safety awareness in this situation only comes with experience.

Therefore AAHL's LAF has its own animal services team. The team is a group of highly trained animal staff that carry out the majority of animal procedures. This gives consistency in monitoring, procedures and sample collection. It also ensures that animal welfare is the top priority if humane intervention is required and enables better science through the ability to more easily use blinding in experiments.

Animal and human welfare impacts

Infectious disease worked with in the LAF are potentially terrible diseases that can have severe clinical signs, leading to the potential for animal suffering. The animal services team have a high empathy for animals, leading to the potential for personal emotional impacts.

To ensure the wellbeing of animals we can follow the Australian Code for the care and use of animals for scientific purposes Section 3 - Strategies to support and safeguard animal wellbeing. When investigating the pathogenicity of emerging/new diseases or new host species we aim to use:

- Conservative evidence-based end points.
- Small animal number pilot studies.
- Identify appropriate host species for models.

An example of this would be AAHL's investigation of Cedar virus, a novel Henipah virus isolated from pooled bat urine collected in Cedar Grove, Queensland. A pathogenicity experiment was undertaken using small numbers of animals (2 ferrets, 4 guinea pigs, 5 mice) using the same planned humane endpoints determined from previous Hendra and Nipah virus experiments. Fortunately these intervention were not required as the virus was non-pathogenic, although the ferrets and guinea pigs did produce antibodies which would be expected as these species are naturally susceptible to the other two Henipah viruses.

In ongoing disease research models, the *continual refinement of humane and scientific endpoints to ensure early intervention*. Examples of this at AAHL would be the in the humane endpoints for H5N1 antibody production in chickens; birds are initially given inactivated virus and only boosted with live virus if all birds in the room have titres are >32 which gives more chance of being protected against disease. Therefore since antibody production should be a low animal welfare impact and we know that with H5N1 birds that show clinical signs will succumb to the virus, even mild clinical signs will trigger euthanasia.

Another example of endpoint refinement, would be the AAHL Ebola virus ferret model. A drop in body temperature (based on subcutaneous microchip temperature scanners) of ≥1°C within 24 hours of a recording of fever (≥40.1°C) has been a strong indicator nonsurvivable disease in previous ferret filovirus studies and is be used as a trigger for euthanasia

Observation of normal individual behaviour prior to experiments commencing enables the development of a baseline of temperament. This can also enable early intervention by allowing the identification of changes in individual animals. This allows us to distinguish between timidness and lethargy in quieter individuals. In ferrets, AAHL has developed a play score to gauge individual animal's general demeanour and activity.

Frequent monitoring is also essential and this means rostering of staff in high impact studies. For Ebola studies at AAHL, eight hourly monitoring at 8am, 4pm and 12am is instituted from day 4-8 post infection during the expected time of peak clinical disease. Monitoring reverts back to two daily once all animals in the experiment have had normal clinical signs for 24hrs.

To ensure the wellbeing of our animal services team, the most important factors are empowerment and support. This includes an objective animal ethics application with clear and conservative endpoints to avoid

any unanticipated situations. There should be effective communication and collaboration with scientific staff, so that animal staff understand the science justifications behind the experiment; why it is important to do the work with a particular disease, why the chosen endpoints are required. They also need assurance that any decisions which need to be made in an unanticipated animal welfare situation will be supported by superiors. This also contributes to ensuring animal wellbeing is safeguarded, as well as human wellbeing, and indeed they are interlinked. The animal services team are also perform all the husbandry for animals in the LAF and are all involved in designing, trialling and refining the environmental enrichment so that they can contribute to the wellbeing of animals on a day to day basis.

Occupational health and safety restrictions

Due to the extreme hazards of animal work in a high containment environment, animals must be anaesthetised before handling in BSL 4 conditions wherever possible particularly those animals which may bite or scratch.

Hazards must obviously be minimised where appropriate, such as the use of indwelling intravenous catheters. However, this issue is managed predominantly by effective handling and restraint. Examples of this would be:

- Gaseous anaesthesia in home cage or padded forceps and Kevlar gloves for mice.

- Squeeze mechanisms – care must be taken to avoid the development of aversion (short duration, positive reinforcement rewards, etc.).

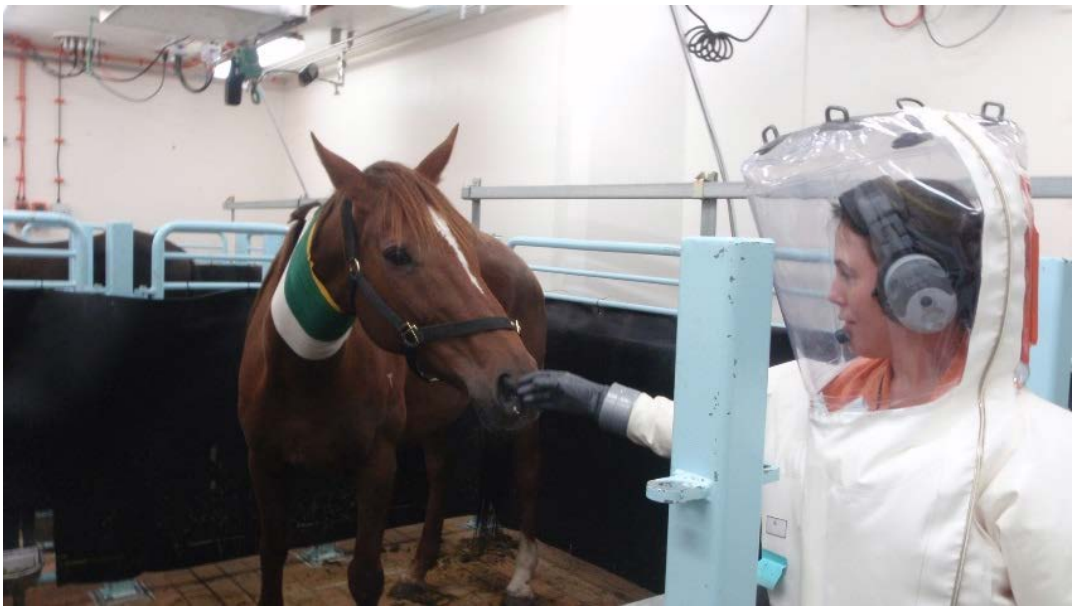
- Training of animals for handling in appropriate cases.

This allows positive human-animal interaction and an ideal species for this approach is, of course, dogs. AAHL had dogs in the LAF in 2011 for a specific study which needed dogs as the species. This was subsequent to a dog on a quarantined property in Queensland testing positive for antibodies during a Hendra virus outbreak and it needed to be determined whether dogs could transmit Hendra virus from horses to humans. This was virtually a new species in the LAF, as there have only been these 18 dogs in used at the facility in over 20 years. Dogs were trained with positive reinforcement to enter crates, walk into noose leads and accept muzzles so that anaesthesia could be administered.

- Temperament selection prior to the experiment in the LAF.

This is a very important factor for certain species. In the case of dogs housed in the LAF, adult dogs from an existing laboratory colony were not as interested in human interaction or positive reinforcement training and there was concern over fear aggression and bites when dealing with Hendra virus. Therefore, puppies 10-12 weeks old were sourced and staff did extensive positive reinforcement training as detailed above.

Horses are another species where temperament selection is very important as particular individuals can have a high flight drive. More relaxed horses need to be selected initially as suits worn at BSL 4 can be very frightening. Although horses were exposed to the suits in a clean environment prior to coming in to the LAF, conditioning was not very successful. This could be due to the change in environment (from a paddock to the secure facility) and small changes such as attachment of the air hose to the suit.



Small pool of expertise

Worldwide there is a very small pool of people who are capable of doing animal experiments in high containment. This applies particularly to BSL 4 animal work as there are only around 10 facilities worldwide with this capability, even fewer for species larger than laboratory rodents. This means there is a limited ability to share refinements or collaborate physically. Facilities are also vastly different in design and age, so that shared information may not always be applicable.

This hurdle can only be overcome by the investment of money and time by institutions. There needs to be a willingness to send employees to other facilities or remote collaborations negotiating different time zones. AAHL is a member of the recently established Biosafety Level 4 Zoonotic Laboratory Network (BSL4ZNet), this group brings together eleven high consequence animal health and public health laboratories from Australia, Canada, Germany, the UK and the USA. Its focus is an integrated global capacity to respond to current and emerging high consequence bio-threats through strengthened partnerships and aims to share information and identify potential collaboration opportunities.

AAHL is 33 years old

AAHL's LAF was originally designed for Foot and Mouth Disease research and so the animal rooms have gates and large pens – i.e. for sheep, cattle. The main species that are housed now are poultry, ferrets and mice, however depending on requirement the LAF must be able to house many different species.

While AAHL is old, it is adaptable. This means the space and enrichment opportunities can be adapted to the species required. It also means more thought around housing and purpose built equipment is required which can lead to opportunities for improved animal welfare. Opportunely, it allows the free range housing of animals in many cases, except where transmission is a factor. There is also an effort made to ensure the animals spend the shortest amount of time in more restrictive conditions, for example housing ferrets free range or livestock species in a paddock after a vaccination and only moving to cages or LAF pens during the infectious phase of a project.

Examples of animal housing at high biosecurity containment

Mice

- Group housing
- Shelters
- Nesting materials – tissues placed on top of the cage daily are pulled in by the mice. If this doesn't occur it can be an indicator of mild clinical signs
- Chewable items
- Food treats (e.g. – muesli) scattered for foraging
- +1 mouse – so that mice are not left alone in the case of other cage mates reaching endpoints

Chickens

- Free range group housing
- Perching opportunities

- Deep litter retreat – foraging and dust bathing
- Cardboard boxes – shelter and pecking
- Artificial turf
- Food treats – corn cobs, mealworms, sprouts, etc.
- Toys – plastic bird toys, bells, mirrors, xylophones, etc.



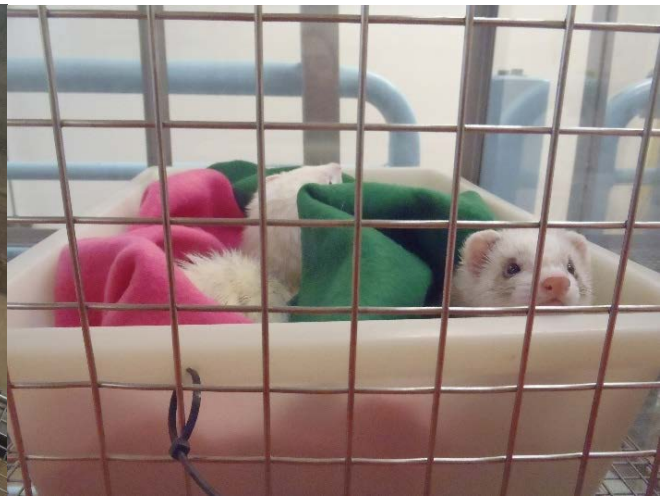

Ducks

- Similar to chickens
- Wading pool

Ferrets

Cages

- Paired housing
- Polar fleece bedding
- Carpet square
- Tunnels
- Plastic or rubber toys
- Food treats



Free range and perspex floor pens

- As above
- Group housing
- Digging pit
- Ferret balls

Guinea Pigs

- Floor pens at BSL 3 or opaque storage tubs at BSL 4
- Group housing
- Soft litter substrate
- Hay
- Toys
- Food treats

Pigs

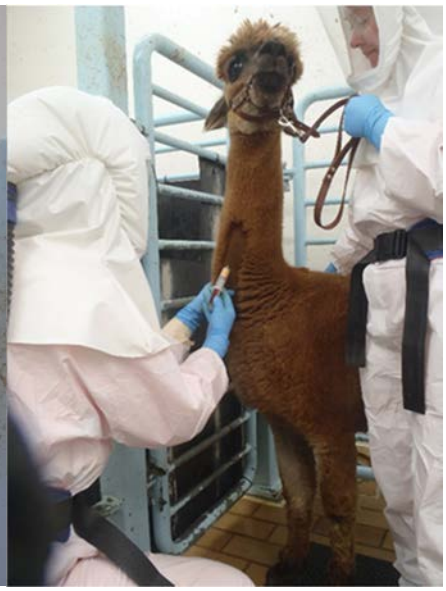
- Group housing
- Straw bed area
- Rubber matting
- Food treats
- Treat balls
- Toys
- Training/human interaction (e.g. – training pigs to play with rope allowed the remote collection of saliva samples and fewer interventions for swabbing)




Alpacas

- Group housing
- Straw bed area
- Rubber matting
- Food treats
- Browse and hay

- Human interactions

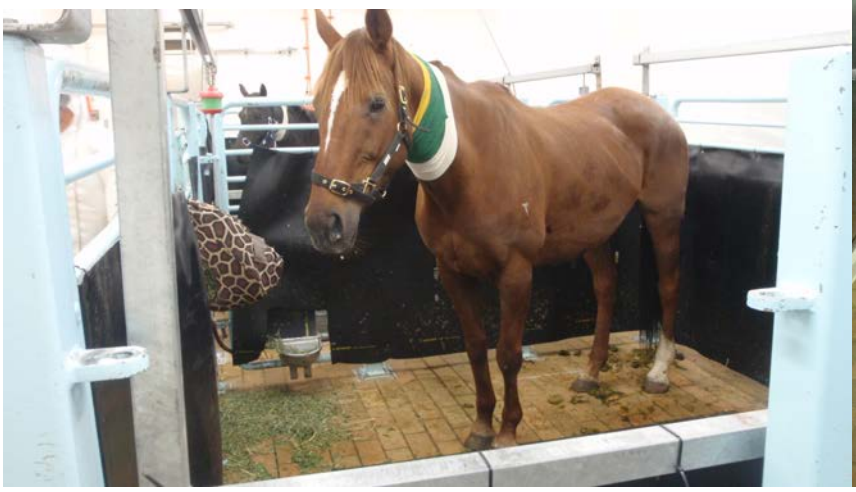


Ruminants

- Group housing
- Rubber matting
- Food treats
- Browse and hay
- Salt licks
- Human interactions
- Grooming boards
- No solitary animals
- For sheep – “safe-zone” retreat made of rubber sheets or shade cloth

Horses

- Stable type housing with the capacity for three horses at one time
- Rubber matting on floor and sides of pens to prevent leg and hoof injuries
- Food treats (e.g. – carrots, apples, molasses, horse muesli)
- Browse and hay
- Jolly stall snack salt lick toys
- Human interactions and grooming



What Can a D Do?

Leigh Taylor
Category D Member, Multiple South Australian AECs

The Category D Member occupies a unique position on the AEC, sharing none of the background experience and knowledge of the Category A, B and C members. In fact, the Category D Member must not fit the requirements of any other category. The other categories must have, to a greater or lesser degree, considerable experience with animals and scientific knowledge in relation thereto. In such circumstances, the Category D member may well feel isolated, particularly given that the deliberations of the AEC revolve very much around the use of animals in a scientific setting.

What then is the role of the Category D? What can a D do? In what areas can a Category D member feel confident that their contribution can add value to the AEC's deliberations beyond simply asking questions and seeking clarification about various aspects of the application.

Subject to the responsibility to consider the application as a whole and partake as much as possible in the discussion regarding all aspects of the application; it is suggested that there are particular aspects that the Category D should be encouraged to focus on and feel confident to contribute to the discussion on an equal footing with Categories A, B and C members. This paper will highlight some of these aspects.

The list is not intended to be exhaustive but might simply provide some guidance for novice Category D members and serve to some extent, to prevent such members becoming disenchanted with their role because of what thee perceive are limitations of experience.

Lay Description

Primarily and most importantly: Category D members will need to determine the value and clarity of the Lay description. This is essential as this may be required to help describe the work being undertaken to the general public or media and so clarity is essential. Clearly the Category D cannot participate appropriately unless the nature of the project is clearly stated in plain English terms. Often other categories comment on the clarity of the lay description, sometimes involving he Category D members by asking "what do you think?" The category D should be confident to take a view on whether or not the description of the project provides an understanding of what is proposed in terms of the use of animals and what will happen to them. One qualification in this might be that in seeking to justify the work, a simple statement like "we are seeking to cure breast cancer" is not sufficient. However, a more detailed statement setting out a specific aspect of a problem might be beyond the Category D's ability to evaluate the significance and / or the novelty of the work. The category D is not in a position to know whether the work is novel or relevant to a particular issue. In this regard, he may look to the scientific experience of the other categories.

Numbers

The new category D may be alarmed at the large number of animals used. Reduction is important and a natural tendency, although it should always be kept in mind that too few animals may invalidate the findings and therefore result in unnecessarily wastage.
Statistics is a specialised area. Most committees rely on a particular member for consideration of the numbers, or in special cases, seek outside experts. The Category D should look to these sources when considering the numbers.

Procedures

In considering the procedures to be involved in applications, the Category D can look to life experience to form attitudes to aspects such as pain, trapping of animals, the isolation of companion animals, etc. The Category D members or independent members of the AEC should not be swayed by statements such as "we always do it this way" or "the alternative costs too much". In considering the monitoring of animals, staff availability and inconvenient times should not overrule the wellbeing of animals. The category D members can be pro-active in these deliberations bringing a fresh approval not influenced by well-established practices and long-term involvement with animals.

There is one aspect of the procedures where the Category D member's experience will not help. In the past, researchers have promoted their competency by stating "I have many years' experience with rats or mice or the particular animal". This is no longer acceptable; more specific experience with the particular procedures is required and in its absence, training is necessary. The Category D member can only look to others or the committee as a whole to ensure that competency is appropriately dealt with.

Trifling

There are matters that arise in considering an application which, although not directly involving animal welfare are matters upon which a Category D may well have a particular view.

(a) Spelling – multiple spelling mistakes could well reflect a casual approach by the researcher to the formal task of applying for approval or a language problem. This may sound a warning for the committee on how to approach the application. Further multiple spelling mistakes may be indicative of other mistakes in the document, particularly in numerals. Such mistakes may be fatal in relation to dosage and other areas. The Category D member may lack knowledge in matters such as dosage, but never the less, the warning should be noted.

(b) Likewise, wrong answers in some instances, particularly in multiple applications may reflect a general misunderstanding of the question. A response by others on the committee that "we know what they meant" should not satisfy the Category D member. If it is a constant problem, the Category D member should push for a revision of the question.

In the above circumstances where matters are not addressed perhaps because they do not directly relate to animal wellbeing or the science involved, it should be borne in mind that should the document be considered outside the committee by an external review panel for example, or worse in the public arena, such matters will reflect badly and embarrass all parties. The category D members can play a part in avoiding that situation.

Animal House Inspections

The Category D member can be proactive in relation to the inspection of animal facilities. A scientific background is not necessary to observe the condition of premises, painting, lighting, ventilation, noise, etc. Comment can be made in relation to the smell of the facility, the tidiness, the disposal of waste. Do the staff appear at ease with the work; coping with the care of animals. None of this is to ignore essential matters such as the proper identification of animals and projects and compliance with monitoring and records thereto.

Annual Reports

These are a requirement of the Code. The Category D member can play an active role in assisting the committee in this regard, checking use of animals, reporting adverse events, complying with approval conditions, modifications, etc. Scientific issues that arise can be referred to other categories of member.

External Review

External review is required by the Code. Usually the review team attend a meeting to observe proceedings. The team also provides an opportunity for members of the committee to meet privately to assist the review. The Category D members should not hesitate to take advantage of this and any other opportunity offered, to discuss how the role of the category D members is functioning and any other general concerns.

Summary

In summary, although the Category D member is to be encouraged to be involved in all aspects of the committee's work, it is hoped that the above suggestions will be a useful guide in the initial phases of involvement and that with experience, new Category D members will gain confidence and develop to participate as a fully independent member of the committee.

Presentations given

on

Wednesday 25th July

Education and training programs: trans-Tasman convergence or divergence?

Deirdre Bourke and Melissa Lindeman

University of Western Australia

In 2017, we conducted an online survey of education and training programs for researchers, teachers, animal care staff and Animal Ethics Committee members in both Australia and New Zealand[1]. At the 2017 ANZCCART Conference combined data from the survey was presented, which clearly showed that there was strong support for standardisation of education and training requirements for people working with animals in science. It was concluded that if the trans-Tasman nations were able to work toward a common framework and shared educational resources, not only would this be cost effective and avoid the need to 'reinvent the wheel', but it would also facilitate mutual recognition of standards.

However, the relevant legislative and regulatory frameworks are different in Australia and New Zealand, and there is no direct guidance on required standards of education and training for people involved in the care and use of animals in research and teaching in either country.

The potential success of a future regional education and training initiative would be dependent on the partner countries being largely 'on the same page', and we considered that it was pertinent to analyse the data to determine if there was trans-Tasman convergence or divergence.

The aims were to determine if there were any significance differences between the countries in relation to:

- Current education and training programs or opportunities.
- Identified issue and problems.
- Suggested improvements and future aspirations.
- Interests in future standardisation and accreditation of education and training in the region.

The results of these analyses will be presented and discussed.

1. Bourke, D.A, Lindeman, M.J. (2017) Survey of education and training programmes in Australia and New Zealand: current status and future prospects. In M. Rands & G Sutherland (Eds.) Proceedings of 2017 ANZCCART Conference: Maintaining Social License in a Changing World, (pp 71-75) Australian & New Zealand Council for the Care of Animals in Research & Teaching, 2017.

At the 2017 ANZCCART Conference combined data from an online survey of education and training programs for researchers, teachers, animal care staff and Animal Ethics Committee members in both Australia and New Zealand was presented[1]. The results clearly showed that there was strong support for standardisation of education and training requirements for people working with animals in science. It was concluded that if the trans-Tasman nations were able to work toward a common framework and shared educational resources, this would not only be cost effective and avoid the need to 'reinvent the wheel', but it would also facilitate mutual recognition of standards. However, the relevant legislative and regulatory frameworks are different in Australia and New Zealand, and there is no direct guidance on required standards of education and training for people involved in the care and use of animals in research and teaching in either country.
The potential success of a future regional education and training initiative would be dependent on the partner countries being largely 'on the same page', and we considered that it was pertinent to analyse the data to determine if there was trans-Tasman convergence or divergence.

The aims were to determine if there were any significance differences between the countries in relation to:

- Current education and training programs or opportunities.
- Identified issue and problems.
- Interests in future standardisation and accreditation of education and training in the region.

Survey design & distribution

A survey was created using the Qualtrics survey tool and was designed to be voluntary and anonymous. Approval to conduct this research was provided by The University of Western Australia, in accordance with their human ethics review and approval procedures. The survey was distributed via email, by ANZCCART to their membership list. The survey requested responses on current programs and potential future direction of education and training for people involved in the care and use of animals in science within Australia and New Zealand.

RESULTS

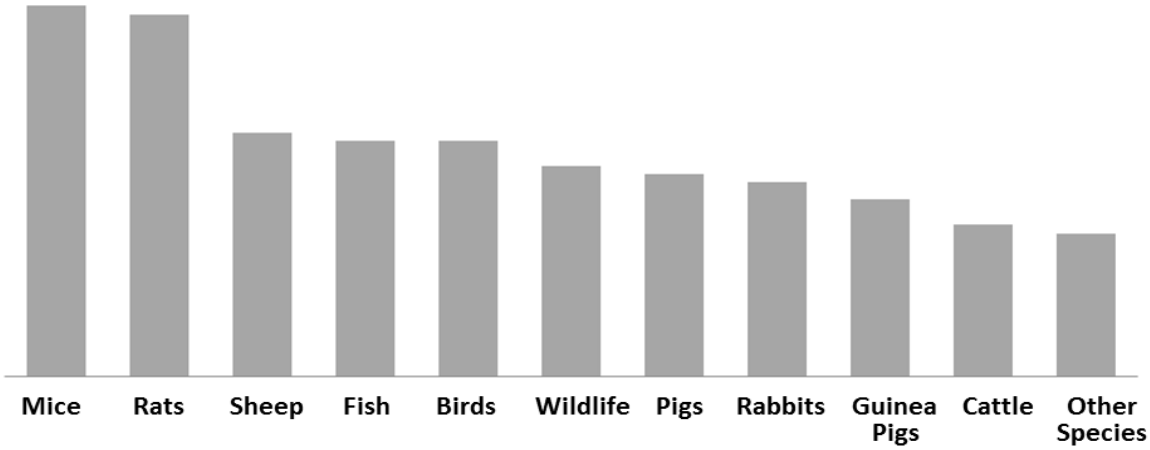
Response rate and characteristics of respondents

In summary, 133 responses were received during the specified survey access period. Of these, 58% were resident in Australia, and the remaining 42% lived in New Zealand.
Responses were received from a broad range cross section of people involved with the care and use of animals in science, in both Australia and New Zealand. These included Animal Care staff, Animal Ethics Committee (AEC) members, Animal Ethics administrators and Animal Welfare Officers. It was noted that researcher and teaching staff, and postgraduate students (PhD & Master) were well represented, and overall accounted for >35% of the respondents.

Animal Species used in Research and Teaching

In both countries, mice and rats are still the most common animal species used in research and teaching (Fig 1). In New Zealand, responses indicated that rats were slightly more frequently used than mice, and the next most frequently used species were sheep and birds. In contrast, in Australia, the top four species were (in descending order): mice, rats, fish and wildlife.

Figure 1. Animal Species used in Research and Teaching Organisations in Australia and New Zealand (combined data for both countries)



Views on current education and training programs

Only 61% of organisations had a comprehensive education and training program. New Zealand responses indicated a lower rate (55%) of full programs offered than in Australia (65%) (Table 1).

Table 1. Percentage of organisations with current training and education and training programs for people working with animals in science

	New Zealand Respondents (%)	**Australian** Respondents (%)	**Combined** Respondents (%)
Full program	55	65	61
Partial program	20	15	17

The majority of respondents had attended all (73%) or part (15%) of their organisation's education and training program. Attendance rates at full programs were higher in Australia (79%) than in New Zealand (65%) (Table 2)

Table 2. Respondent attendance at organisational education and training programs for people working with animals in science

	New Zealand Respondents (%)	**Australian** Respondents (%)	**Combined** Respondents (%)
Attended full program	65	79	73
Attended part of program	22	10	15

Overall, a wide range of topics were delivered but there was no stand out mode of delivery in either Australia or New Zealand. In general, the mode of delivery of commonly offered topics (e.g. regulations, legislation, animal handling, pain & distress, monitoring etc.) was highly variable and across all formats, with a relatively balanced split across delivery modes, that is, online modules; face to face theory sessions and practical skills training (hands on with animals) all used. In addition, some other delivery formats, e.g. ad hoc seminars, were also used. Australia had slightly higher rates for online delivery (32% (AUS) vs 26% (NZ)), and in contrast New Zealand had slightly higher use of the practical skills mode (34% (NZ) vs 27% (AUS)). In both of the countries less than half of the programs were formally assessed.

Views on education and training programs

Overall, most respondents (75%) who had attended education or training programs were either 'extremely' or 'somewhat' satisfied. In Australia, 45% were 'extremely satisfied', and another 30% were 'somewhat satisfied' with the education and training they received. In New Zealand, the satisfaction rates appeared to be the opposite with only 23% 'extremely satisfied' and 54% 'somewhat satisfied'. Overall, from the combined data, less than 10% were 'somewhat' or 'extremely dissatisfied.

For staff transferring between organisations, more than 40% of respondents did not feel confident accepting or recognising prior education and training related to use of animals in science which had been acquired at other organisations in Australia and New Zealand. This included organisations within their respective countries. Respondents were even more reluctant to recognise prior education and training from other overseas countries.

Overall, most respondents (75%) thought it would be useful if Australian and New Zealand Institutions introduced standardised education and training requirements for people involved in the care and use of animals in science. This response was slightly stronger in Australia (78%) in comparison to New Zealand (70%).

Furthermore, the majority of respondents (67%) in both countries thought that education and training programs should be accredited by a responsible authority.

Conclusion

Overall, in relation to the stated aims, there were no significance differences between Australia & New Zealand in relation views on current and future education and training programs; opinions were generally well aligned; and both countries were supportive of standardisation and accreditation.

There was an identified lack of confidence in recognising prior education and training obtained in Australia and New Zealand, as well as other countries. Consequently, this means that staff transferring between organisations will generally be required to go through another animal user education and training program at the receiving organisations.

The data supports that Australia and New Zealand are well aligned and there were no 'Trans-Tasman' barrier to forward progress for development of a standardised approach to education and training in this region.

If Australia and New Zealand created a common accredited and shared educational resources, this would this be beneficial and cost effective, and would also facilitate mutual recognition of standards within the region.

In view of recent trends towards international collaborative research, it would also seem logical for Australia and New Zealand to develop a joint approach to structured accredited education and training for people working with animals in science, which aligns with contemporary standards and expectations and authenticates reciprocal recognition of prior education & training in our region.

References

1. Bourke, D.A, Lindeman, M.J. (2017) Survey of education and training programmes in Australia and New Zealand: current status and future prospects. In M. Rands & G Sutherland (Eds.) Proceedings of 2017 ANZCCART Conference: Maintaining Social License in a Changing World, (pp 71-75) Australian & New Zealand Council for the Care of Animals in Research & Teaching, 2017.

Recent NHMRC initiatives to promote the 3Rs and research quality

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High quality research that is rigorous, transparent, reproducible and conducted with integrity contributes to scientific progress, fosters translation of outcomes into practical and clinical applications and ensures value for research investment. These values also support public trust in scientific findings and their use in evidence-based policy. In recent years, however, concerns about the reproducibility of research — including studies involving animals — have been reported extensively in the international literature and in forums such as annual conferences of the Australian and New Zealand Council for the Care of Animals in Research and Teaching (*ANZCCART*). The attention given to reproducibility speaks to its importance to research quality but it is only one element of a strong research sector. For research involving animals, the application of the 3Rs (Replacement, Reduction and Refinement) is also critical in ensuring quality research and the ethical and humane use of animals for scientific purpose. However, despite the importance of the 3Rs, there appears to be limited documented evidence about their use in Australia and factors that enable or hinder their development and adoption.

This presentation will provide an overview of recent and current activities undertaken by the National Health and Medical Research Council (NHMRC) to promote and ensure research quality and reproducibility in animal and human studies. It will also outline the progress of work by NHMRC and its Animal Welfare Committee aimed at addressing the information gap about the development and adoption of the 3Rs in Australia, in order to promote informed discussion and guide recommendations for improvement if required. This session will include a presentation of preliminary results from the 3Rs survey conducted by ORIMA Research on behalf of NHMRC in February–May 2018 and will be of interest to investigators, animal ethics committee members, institutions and regulators.

Introduction

This paper provides an overview of initiatives undertaken by the National Health and Medical Research Council to promote and ensure high quality research and the replacement, reduction and refinement of the care and use of animals for scientific purposes.

National Health and Medical Research Council (NHMRC)

NHMRC is Australia's leading funding agency for health and medical research. Its purposes are to fund high-quality health and medical research and build research capability, support the translation of health and medical research into better health outcomes and promote the highest

standards of ethics and integrity in health and medical research. These purposes support NHMRC's mission of 'building a healthy Australia' and reflect the role for NHMRC set out in its enabling legislation, the *National Health and Medical Research Act 1992.* The purposes are implemented through the three underpinning strategic themes in NHMRC's 2017–2018 Corporate Plan[i] of investment, translation and integrity.

Research integrity framework in Australia

Australia's research integrity framework is underpinned by three national standards developed by the NHMRC and its co-authors, the Australian Research Council (ARC) and Universities Australia (UA):
Australian Code for the Responsible Conduct of Research, 2018
National Statement for ethical conduct in human research, 2007 (updated July 2018), and
Australian code for the care and use of animals for scientific purposes, 2013.

The *Australian code for the care and use of animals for scientific purposes* is also co-authored by the Commonwealth Scientific and Industrial Research Organisation (CSIRO). Together these three standards provide guidance on the responsible and ethical research conduct across all research disciplines. The overarching document is the *Australian Code for the Responsible Conduct of Research, 2018* (the Code of Conduct), which establishes a framework for responsible research conduct and a foundation for high-quality research across all disciplines.

The 2018 Code of Conduct was released in June 2018 and is a revision of the 2007 edition. It outlines the principles that are the hallmarks of responsible research conduct. These principles also support the conduct of high quality research.

P1. Honesty in the development, undertaking and reporting of research.
P2 Rigour in the development, undertaking and reporting of research.
P3. Transparency in declaring interests and reporting research methodology, data and findings.
P5. Respect for research participants, the wider community, animals and the environment.
P6. Recognition of the right of Aboriginal and Torres Strait Islander peoples to be engaged in research that affects or is of particular significance to them.
P7. Accountability for the development, undertaking and reporting of research.
P8. Promotion of responsible research practices.

The Code of Conduct also outlines responsibilities of institutions and researchers in upholding the principles of responsible conduct of research. Sections that specifically relate to the care and use of animals for scientific purposes include the following responsibilities of researchers:

R17. Comply with the relevant laws, regulations, disciplinary standards, ethics guidelines and institutional policies related to responsible research conduct. Ensure that appropriate approvals are obtained prior to the commencement of research, and that conditions of any approvals are adhered to during the course of research.

R20. Ensure that the 3Rs are considered at all stages of research involving animals and minimise the impacts on animals used in research and in so doing support the welfare and wellbeing of these animals

The *Guide to Managing and Investigating Potential Breaches of the Australian Code for the Responsible Conduct of Research, 2018* was developed to as supplementary guidance for the Code

of Conduct. The development of additional supplementary guidance is underway including guidance about authorship, and guidance about data management.

Research quality

High quality research that is rigorous, transparent and reproducible contributes to scientific progress, is essential for the translation of research outcomes to practical and clinical applications and evidence-based policy that benefit the community, delivers the highest possible value for research investment and promotes community trust in scientific findings. Concerns about research quality have been reported extensively in the international literature and the general and scientific media, with issues highlighted in several areas including:

establishment of research questions
research design, methods and analysis
research regulation and management
accessibility of research information, and
reporting of research.

Ensuring research quality is an international issue and requires action by multiple participants in the sector including institutions, funding agencies, researchers and ethics committees (human and animal). Journals and publishers also have a role to play. Concerns about research quality have been reported across all scientific disciplines including physics, chemistry, environmental science, biology and medicine.

NHMRC has commenced the Research Quality Project, which aims to ensure the highest quality of NHMRC-funded research through rigour, transparency and reproducibility. This project is a priority for the agency. An expert advisory committee – the Research Quality Steering Committee – has been established to provide advice to NHMRC on short and long-term strategies for ensuring research quality and mechanisms for measuring and reporting on the effectiveness of any strategies implemented. Finalisation of NHMRC's Research Quality Strategy in anticipated in early 2019 with implementation of the associated Action Plan throughout 2019–2021. NHMRC's Research Quality Strategy and Action Pan will also be reviewed annually.

Framework for high quality animal-based studies

The ethical use of animals for scientific purposes and the value of the outcomes of their use is dependent on the studies being rigorous, transparent and reproducible. The ethical costs of poor quality animal-based studies include the unnecessary use of animals where such use is based on previous studies that are poorly performed or reported, unnecessary negative impacts on the wellbeing of individual animals, and the wastage of animals that are used.

The *Australian code for the care and use of animals for scientific purposes, 2013* (the Code) has been adopted under legislation in each Australian state and territory. The governing principles outlined in Section 1 of the Code are based on the requirement that 'Respect for animals must underpin all decisions and actions involving the care and use of animals for scientific purposes' which is demonstrated by:

using animals only when it is justified
supporting the wellbeing of the animals involved
avoiding or minimising harm, including pain and distress, to those animals
applying high standards of scientific integrity

applying the 3Rs (replacement, reduction and refinement) at all stages of animal care and use knowing and accepting one's responsibilities.

The principles in Section 1 of the Code support the conduct of high quality animal-based studies. For example, those about scientific integrity are very clear. Regardless of the potential benefits of a project, the methods used must be scientifically valid, feasible, well designed and carefully conducted so that there is a reasonable expectation that the aims of the project will be achieved (Clause 1.15). Investigators must use methods that accord with current best practice that (i) take into consideration relevant aspects of species-specific biology, physiology and behaviour (ii) are based on the best available scientific evidence, which includes the potential adverse impact of conditions and procedures on the wellbeing of the animals (ii) include strategies to minimise adverse impacts (Clause 1.16). Animals used must be suited to the purpose of the project or activity, taking into account their biological characteristics (Clause 1.17).

The application of the 3Rs as outlined in Clause 1.18–1.32 of the Code is also critical in ensuring research quality and the ethical and humane use of animals for scientific purpose.

Best practice methodology in the use of animals for scientific purposes, 2017

In recognition of the need for additional guidance about ensuring the quality of animal-based studies, NHMRC developed the *Best practice methodology in the use of animals for scientific purposes, 2017* (the Guidance) which is intended to support implementation of the principles in the Code. The Guidance is supported by those organisations that also endorse the Code – ARC, CSIRO and UA.

The Guidance was developed with advice from NHMRC's Animal Welfare Committee. The process for its development included targeted consultation conducted in 2016. The aim of the targeted consultation was to seek feedback regarding the accuracy of the issues raised, and the practicality of the proposed strategies to address the issues.

The key elements of the framework for best practice in animal-based studies are:

- The application of good scientific method, which requires the formulation of a relevant and legitimate research question and a clear and valid hypothesis and the use of robust and unbiased practices.
- The application of the 3Rs at all stages of animal care and use. The Guidance discusses how the 3Rs provide a structure and a tool for research quality. It also highlights factors such as the necessity for assessment of validity and relevance of a proposed animal model; the conduct of systematic review, which can minimise the unnecessary use of animals and can inform the validity of the proposed animal model; and the importance of statistical design to ensure that the appropriate number of animals are used.
- The effective and transparent reporting of key information about how studies are designed, conducted and analysed. This element also encompasses pre-registration of protocols and plans for analysis, reporting about the negative impacts on animals, ensuring full reports and data are accessible including negative or neutral results, and reporting to the animal ethics committee (AEC) of the outcomes of previous or related work.

To increase awareness of poor research practices and hence encourage changes and improvements in practices, the Guidance highlights the consequences of poor methodology, and outlines the common flaws in methodologies and issues that impact on research quality in the following areas:

the design of the studies
the statistical design and analysis
techniques and procedures used, and reporting of the studies.

The Guidance provides practical strategies that institutions, investigators and AECs can consider to ensure high quality animal-based studies. The scope of the Guidance is the same as that for the Code – that is, it applies to the use of all live vertebrate animals and cephalopods, and to the use of animals for '*scientific purposes'* as defined in the Code and is not limited to biomedical research. The practical strategies in the Guidance are therefore provided in broad rather than prescriptive or detailed terms to facilitate their application to any specific circumstance. Not all strategies will be applicable to some types of animal use.

References to key governing principles in the Code and the Code of Conduct are included throughout the Guidance to highlight where the principles and requirements in these two documents apply. Information about international guidance is included as examples of current best practice for high quality animal-based studies. There is also an extensive reference list for more detailed guidance and resources.

The 3Rs Project

The application of the 3Rs is critical in ensuring research quality and the ethical and humane use of animals for scientific purpose. The 3Rs are included in the governing principles of the Code and underpin the requirements in the entire Code including the responsibilities of institutions, animal ethics committees, investigators and animal carers. Despite the importance of the 3Rs, there appears to be limited documented evidence about their use in Australia and factors that enable or hinder their development and adoption. To address this information gap, NHMRC is conducting the 3Rs Project, which is being overseen by NHMRC's Animal Welfare Committee. The major components of the project are a literature review and a survey of users of animals for scientific purposes – investigators, animal ethics committee members and institutional representatives. The outcomes from the literature review and the survey will inform the development of an Information Paper providing evidence about the implementation of the 3Rs in Australia. The intent of the Information Paper is to promote informed discussion of the subject and guide recommendations for improvement if required. The reports from the literature review and the 3Rs survey will be published concurrently with the Information Paper.

[1] NHMRC's 2018–2019 Corporate Plan https://nhmrc.gov.au/about-us/publications/nhmrc-corporate-plan-2018-2019

Use of Simulators in Technical Skills Training to Reduce Animal Use in Teaching

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Overview In keeping with the '3Rs', medical and veterinary trainers have turned increasingly to the use of simulators to achieve the initial competencies in technical skills training. Both medical and veterinary curricula are driven by the need to achieve Day One competencies as mandated by the Royal Colleges in the UK and the Australasian Veterinary Boards Council in Australia and New Zealand and the Australian Medical Council. Increasingly, there is greater reluctance by the public to condone the training of undergraduate and postgraduate students on patients or animals. These factors have driven the development of simulated skills trainers that vary from low-fidelity models to the extremely sophisticated haptic devices that give biofeedback to the trainee. This talk will outline the range of skills training models as well as give some background on their costs and efficacy as educational tools.

Types of models Low-fidelity models are constructs that do not mimic the feel and texture of the scenario being modelled unlike high-fidelity models that use complex anatomic structures and sophisticated materials to closely mimic the form and texture of the system being modelled. Cost is the big difference as low-fidelity models can often be made in-house from readily available materials, where high-fidelity models often run into the thousands of dollars per model (www.vetsimulators.com).

Venepuncture Models are used for training for blood collection and catheter placement in humans, canine, bovine and equine models. Models are available also for cystocentesis training, i.e. sterile urine sample collection through the body wall.

Suture models are used for teaching knot tying and suture patterns for all species and the development of manual dexterity prior to dealing with an anesthetised animal or patient.

Surgery models are especially well developed for canine and feline ovariohysterectomy or neutering females. Both low and high-fidelity models will be discussed.

Theriogenology models for obstetrics training, pregnancy diagnosis and rectal examination skills development in cattle and horses have been well developed. These models can be used with cadaveric material from abattoirs or synthetic calves for dystocia management training.

Models for **minimally-invasive** modalities such as arthroscopy, endoscopy and laparoscopy will also be highlighted.

Models as Educational Tools Students using models feel more confident in that they can repeatedly perform the technical task without fear of failure and harm to the animal or patient. They repeat the skill until they feel competent to move on to the real-life scenario with its added complexity of patient reaction/discomfort, or anaesthesia and haemorrhage. This pre-training allows the trainee to move more quickly in competency development than if this was left to random case-based experience for complex procedures. It also ensures that all students achieve a similar level of competency during their training, as they will all have had exposure to the same training opportunities on models allowing a more consistent level of skills acquisition between trainees.

Measures of student salivary cortisol levels and heart rate suggest that pre-training using models prior to live horse exposure decreased student anxiety[1]. Numerous other studies suggest that fidelity of the model does not impact on the model's efficacy as a teaching tool.

Reference Nagel C, Ille N, Aurich J, Aurich C. Teaching of diagnostic skills in equine gynecology: simulator-based training versus schooling on live horses. Theriogenology: 2015:84 (7)1088. DOI 10.1016/j.theriogenology.2015.06.007

I would love to share some of my experiences as a surgical teacher in veterinary settings, using inanimate models for skills training, to decrease animal use and to build confidence in students prior to live animal contact.

Many of the simple models illustrated come from my time in the West Indies at Ross School of Veterinary Medicine in St Christopher and Nevis, a very small country near Jamaica in a chain of islands called the Less Antilles. These were made in house as the school had a very limited number of teaching animals so got creative about teaching methods. I would like to acknowledge Dr Robin FioRito/Farrell and the technical staff there who are largely responsible for these trainers used at Ross. They have hosted a meeting called INVEST that attracted many international vet teachers to see and learn how these are made.

Three Rs exort us to reduce animal use for procedures that do not benefit the animal.

Welfare considerations and public opinion make the use of animals for terminal procedures very difficult e.g. invasive surgical training.

Surgical residents in the UK cannot "train" on patients so how do trainees acquire the requisite technical skills?

Medical and veterinary curricula, and the Code itself, require competencies to be gained prior to initiation of procedures or graduation.

Skills training using inanimate models allows non-threatening safe reproducible environment for students to gain confidence and not be hurt.

Why use models?

Public expectations about the use of animals in teaching means that invasive and terminal procedures are much less acceptable in both human medical and veterinary training and for research training.

Accreditation boards in both veterinary and human medicine require that basic competencies are met by graduates entering into professional practice. This tension between competency attainment, and increasing acceptance of animal use, has driven the development of models for technical skills training.

Using models allows the student to practice a skill in a very low stress way. There is no anesthesia of the animal to worry about, there is no bleeding, no pain and no threat of discomfort to that animal or injury to the student from bites kicks or scratches.

The donkeys above are the "Ross horses" in St Kitts – beautiful creatures and very tolerant of students. The legs are horse limb models used for bandage practice in Adelaide at the vet school. They almost never kick the students….

Complex task skills training

- For complex tasks - need to break down the skill into small components
- Experts move seamlessly through complex tasks without conscious recognition of the multiple steps involved in a procedure
- Novice has to consciously focus on each step and only gradually do the skills become part of the neural network and unconsciously enabled
- Neurobiologists now recognize the increased connectivity of the brain when new tasks are learned
- 10,000 hrs to become proficient

Anyone that has developed expertise in a complex skill moves from one component of that skill to another seamlessly and largely without conscious recognition of the multiple steps involved. It is actually quite challenging for the expert to remember how to dissect the steps to reproduce and explain them accurately to a novice in the area.
Many models are designed to perform only one skill of a multistep process so that the task seems more straight forward and less daunting for the novice.
Technical skills training is an iterative and time consuming process that takes approximately 10,000 hours to fully mature, be it a surgical skill, or a musical instrument's mastery.

Implications for training with animals?

- Small steps for the novice
- Repetition required to gain competence
- Subtle variation in the model(s) will be useful
- Stressed/anxious students learn less
- Traditional "military" surgical training model did not provide an environment conducive to relaxed learning
- Using inanimate models for early skills training decreases stress in students relative to direct early animal encounters e.g. equine rectal training, Nagel et at 2015
- Relevant to mouse handling and research procedure training as well

The use of models has the other advantage that the student is not stressed.
It is known that learning is not best done in stressful environments and students gain a much better understanding when learning in a relaxed setting.
Nagel's paper shows that students learning to do rectal exams in horses that start using a model before progressing to the live animal, are less stressed and more comfortable that they have gained some competence prior to animal contact.
Research students handling rodents for the first time do not want to hurt or injure, what they perceive as tiny animals. Rather than starting by picking them up by the tail, using a Perspex tube gives the mouse and the student a much calmer experience to gain confidence. If we can use tubing more for routine cage transfers the mice are less aversive to being handled and perform better in behavioural tests.

Low- and High-Fidelity Models

- Low-fidelity model – simplification of the scenario that does not attempt to mimic the complex system either in look or materials e.g. suture / knot tying board

- High-fidelity model – uses sophisticated materials and systems to closely mimic the system being modelled e.g. flight simulator for pilots

The top illustration is a surgical knot tying board that can be used for practicing instrument ties or hand ties in various configurations.
Sutures can be tied around the various objects making square knots that will not slip or come undone.
Repeated attempts can be done until the knot is symmetrical and will not slip and tensioning can be practiced until the knots are 100% secure.
High fidelity models include almost all the aspects of the method being modelled – are much more complex and thus also more expensive. There are simulated CPR patients with pulses and respiration and ECGs but of course all that technology makes the cost suited only to university type settings for medical training.

Venepuncture models – often low fidelity

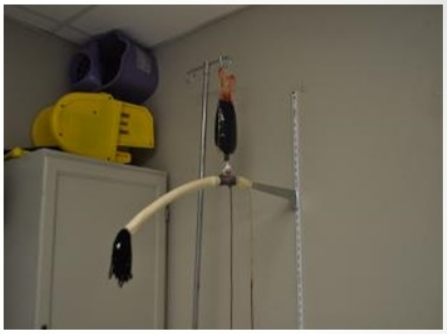
- Catheter placement intravenous – dog forelimb
- Simple dowel rod wrapped with Vetwrap bandage over a iv tubing line primed with fake blood
- Cost $<$50, re-useable, repairable, higher fidelity $100s

Before being let loose on some unsuspecting patient, the student can practice getting the needle and syringe together, the angle of vessel entry correct and learn to draw back on the syringe plunger to withdraw fluid.
The venipuncture model is filled with red fluid to mimic blood and the entire system can be made from readily available materials found in most clinical settings.

The “dog leg” on the right is made from silicone over a dense polymer skeleton but is essentially the same model with an IV tube under a skin to allow palpation and identification of the vein prior to venipuncture.

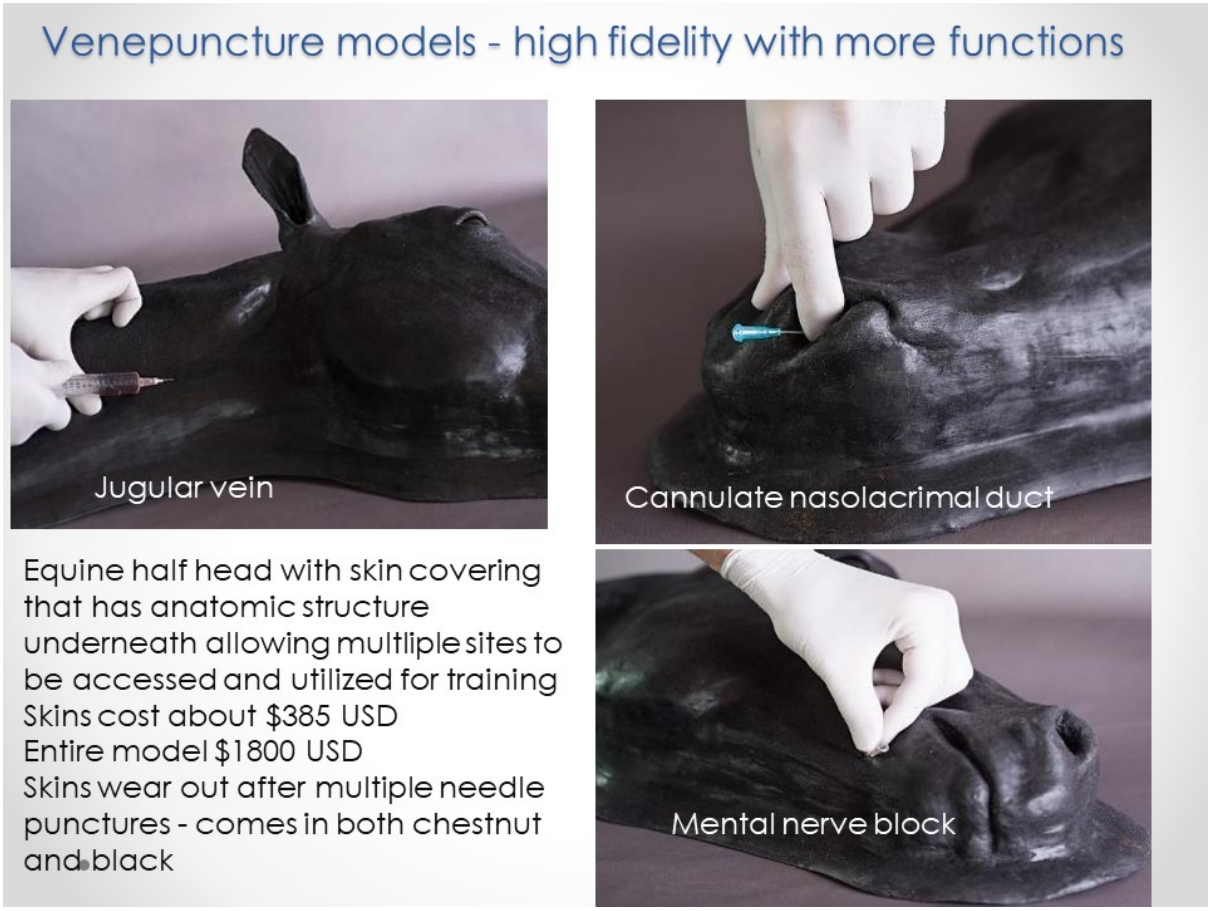
Venepuncture models continued

Cattle tail vein blood collection trainer
Wall bracket, silicone tuning covered with vetwrap bandage material with a iv tubing wrapped into the ventral aspect and an IV bag of fake blood.
Total cost < $50



Ovine / porcine jugular venipuncture trainer -
Foam bolster wrapped in thin neoprene with a fine walled rubber tube used as the vein under the neoprene cover, fake blood primed into the vein.
Cost < $50

Depending on the species that you might want to model, the vein for venipuncture will vary.
The right slide shows a cattle tail vein model used against a wall so that the student needs to feel the ventral aspect of the tail as they would in the live animal.
Essentially the same as the dog legs in that the vein has fake blood to allow sampling.
The foam rolls on the bench on the left are for sheep jugular models or pig necks where the vein is less obvious. Again the set-up is the same but vary the materials and depths for the vein placement in the model.



Horses head model that has a skeleton under it that is polymer and a silicone skin that is punctured to do procedures such as venipuncture of the jugular vein or nerve blocks.
The mental nerve block has the correct foramen in the skull to palpate to place the local anesthetic injection accurately after palpation of the structures.
The nasolacrimal duct can be palpated and cannulated on the inside of the nostril.
Obviously the silicone skin is reasonably durable but will need to be replaced after many uses as the integrity of the silicone eventually is disrupted by multiple needle punctures.

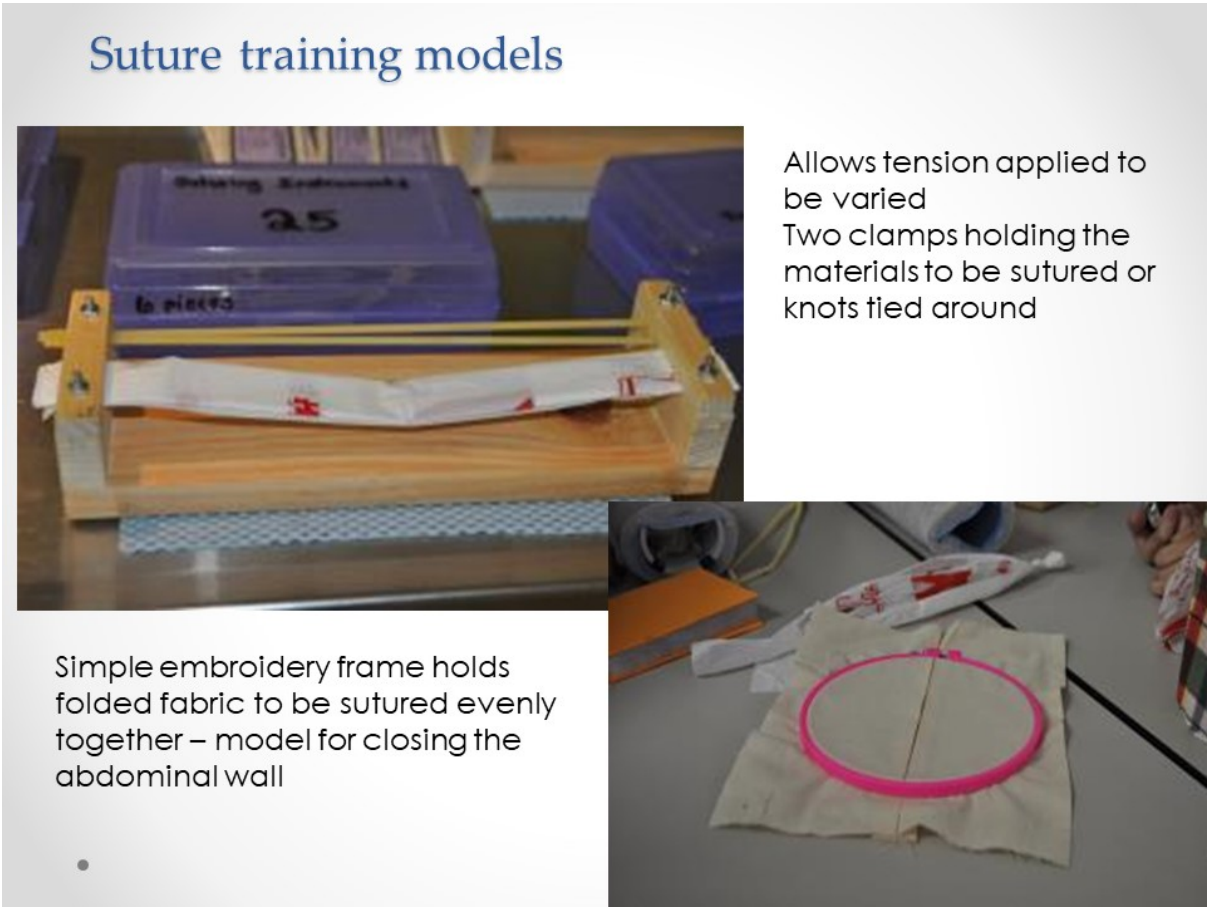
Equine Head model continued..

Using the jugular vein for vacutainer blood collection

The skin replacement part..$385USD

The horses head can be positioned more anatomically for training.
The skin without the underlying skeletal support shows it’s a separate piece to the head as a whole and can be purchased separately.

The knot tying boards given to students in Nth America by Ethicon were not available to us at Adelaide when we started the new school - so we made them ourselves.
The brass hook, the Penrose tubing and the hook inside the Perspex tubing allow knots to be tied at various depths and with various tensions.
The lower illustration is the “factory” in the spare room at home.
Upper right: the first cohort of Adelaide vet students learning their introductory surgery skills in a relaxed non-threatening environment with no blood and no anesthetic gases and no pain.

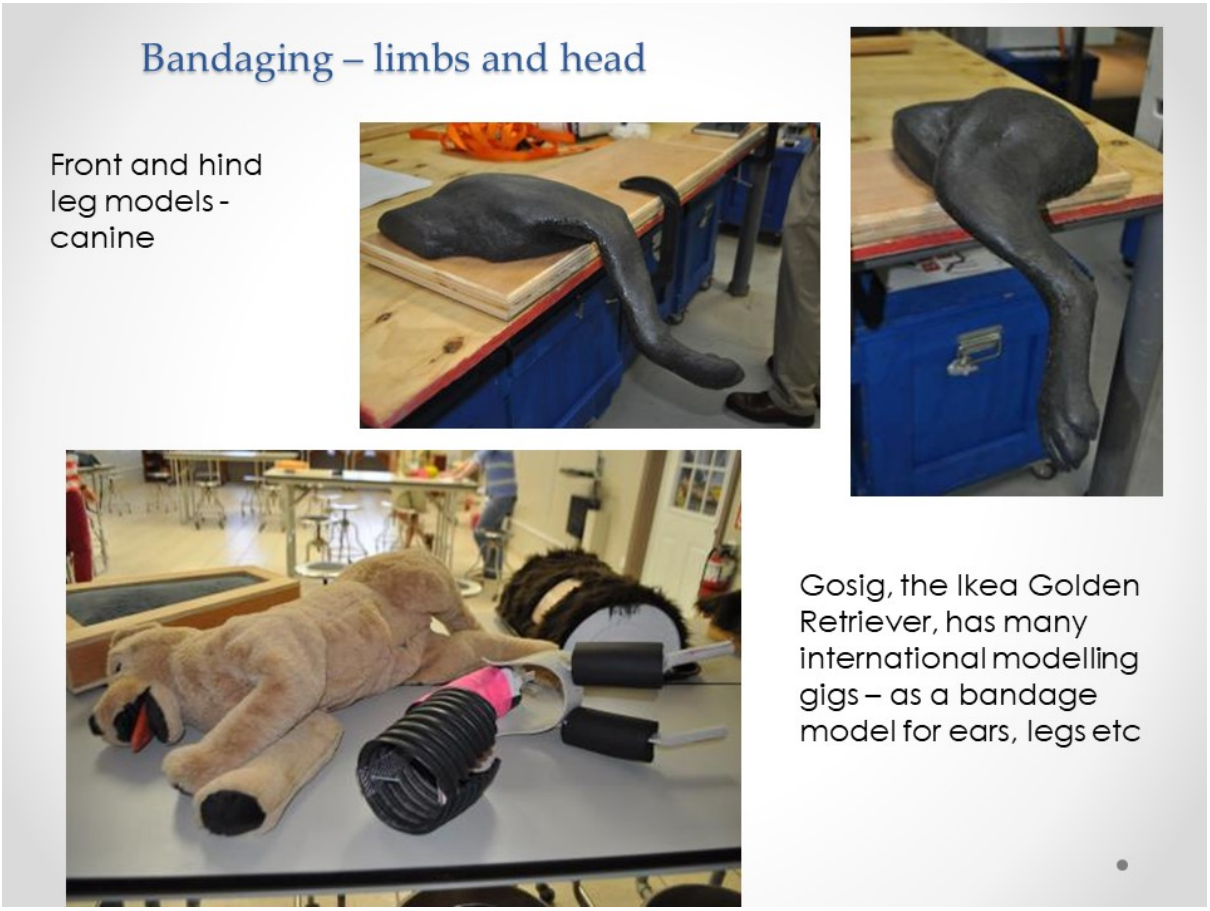


The lower right image shows a very simple embroidery frame being used with folded fabric to allow modeling of the closure of the abdomen with various suture patterns possible. The students can perform inverting, apposing or interrupted sutures in this setting.

You can get less pink embroidery rings also to make this more gender neutral – cane ones are good in this respect.

The Vet Simulators models have been developed in conjunction with the University of Calgary Vet school and are high fidelity models that have replaceable components.

This one mimics the anatomic layers of the body wall and skin for a more realistic experience - but a more expensive outlay by the teacher than the low fidelity in-house type of suture models. The silicone components do wear out and require replacement due to needle puncture fatigue of the materials.



Gosig, the IKEA Golden Retriever has multiple uses. The ears can be bandaged for head bandaging to illustrate the need for indicating where the ear is inside the bandage when it is being cut off so as not to take the ear flap as well.

The limbs can be used floppy for bandaging or can have a foam and metal skeletal structure placed inside to allow them to be sued for IIM injection training.

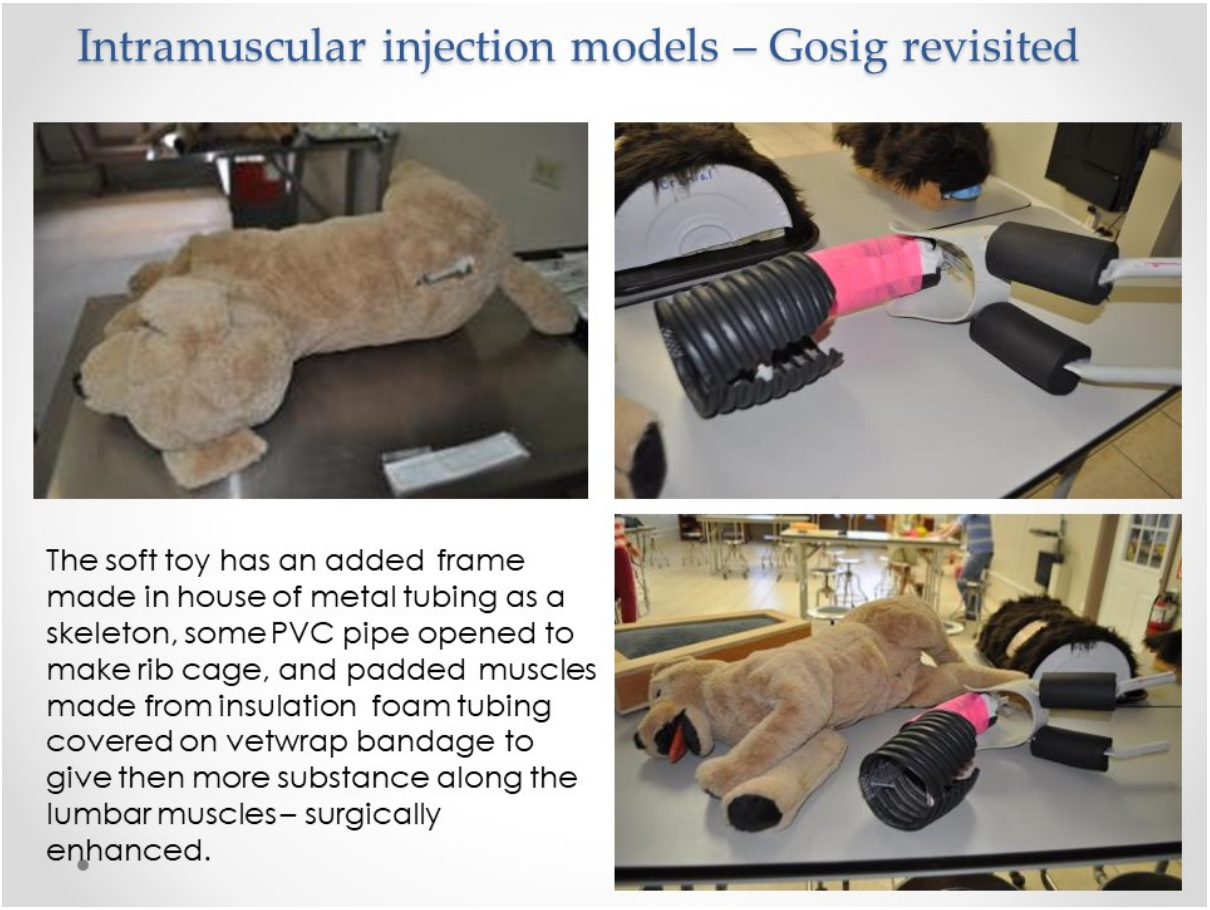
Illustrated beside Gosig is the skeleton with a rib cage and lumbar muscles, and hind limbs as well for injection training.

The top models are silicone dog limbs for bandaging practice.

Another higher fidelity model from Vet Simulators with a skeletal inner and a skin over it for radiography positioning training.

It has joints for performing joint taps with local anesthetic, as well as palpation of joints.

The entire limb can be used for bandage or cast application practice as well.



A closer view of Gosig's skeleton and ribs. Some minor back surgery is required to insert his frame and injection muscles.

Chest drainage or tube placement – low and high

- Thoracocentesis - draining air/ fluid from the chest after trauma
- Chest tube placement
- Plastic bucket cut in half and a window made
- Silicone tubing in the window of the shaved area as skin
- Foam as the lung
- Lid to seal the chest
- Can practice placing a chest drain or using a needle or catheter to drain air.

Critical Care Gerry - $2782USD

The placement of chest drains is always a bit daunting for the novice as it means penetrating ribs in a almost completely conscious animal in an emergency situation.

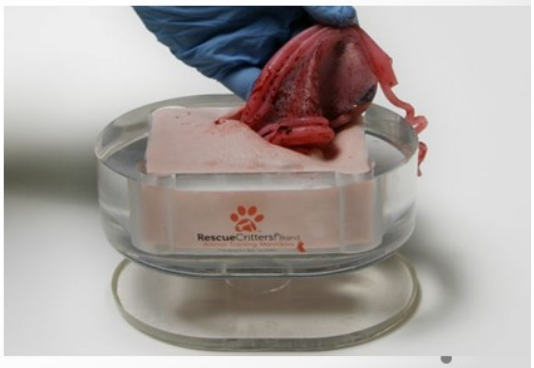
The model at the top is a plastic bucket halved and filled with sponge foam with a balloon pocket that can have fluid or air in it. The bare area is silicone sheeting attached to the rib cage, to replicate skin that can be punctured. The hair some serious faux fur that must have been a polyester Newfoundland dog.

Critical Care Gerry is the higher fidelity option that allows entubation of the airway and practice for ventilation and chest compressions.

Yet another great use for Golden Retriever models..

Training for neutering or ovariectomy

- Fidelity varies enormously
- Multistep process involves
- Finding the correct structures
- Suture placement
- Ligation skills – secure ties
- Closing the body wall
- Closing the skin
- Restricted access



Cost of the Rossie = $30 USD
Cost of the Rescue Critters = $??

Training for ovariohysterectomy – or female neutering –

The Rossie model, developed at Ross in the West Indies, is a low cost in-house manufactured spay model that is low fidelity but has the anatomic layers of the body wall and then the ovaries and uterus inside that the student must locate and ligate. Cost per model is cheap so can be used per student and replace parts are only a few dollars.

The Rescue Critters version is much higher fidelity but also significantly more expensive as each component must be replaced after the reproductive tract is removed surgically by the student.

Vet Simulations model with disposable uterus & body wall

The ultimate in high fidelity spay models with anatomically correct pasts that can be replaced after each student use.

The body wall has layers and an umbilicus.

Bovine Dystocia Model

For teaching bovine reproduction palpation and obstetrics

The reproductive tracts can be replaced and substituted for various stages of the cycle.

Choice of breed as well..

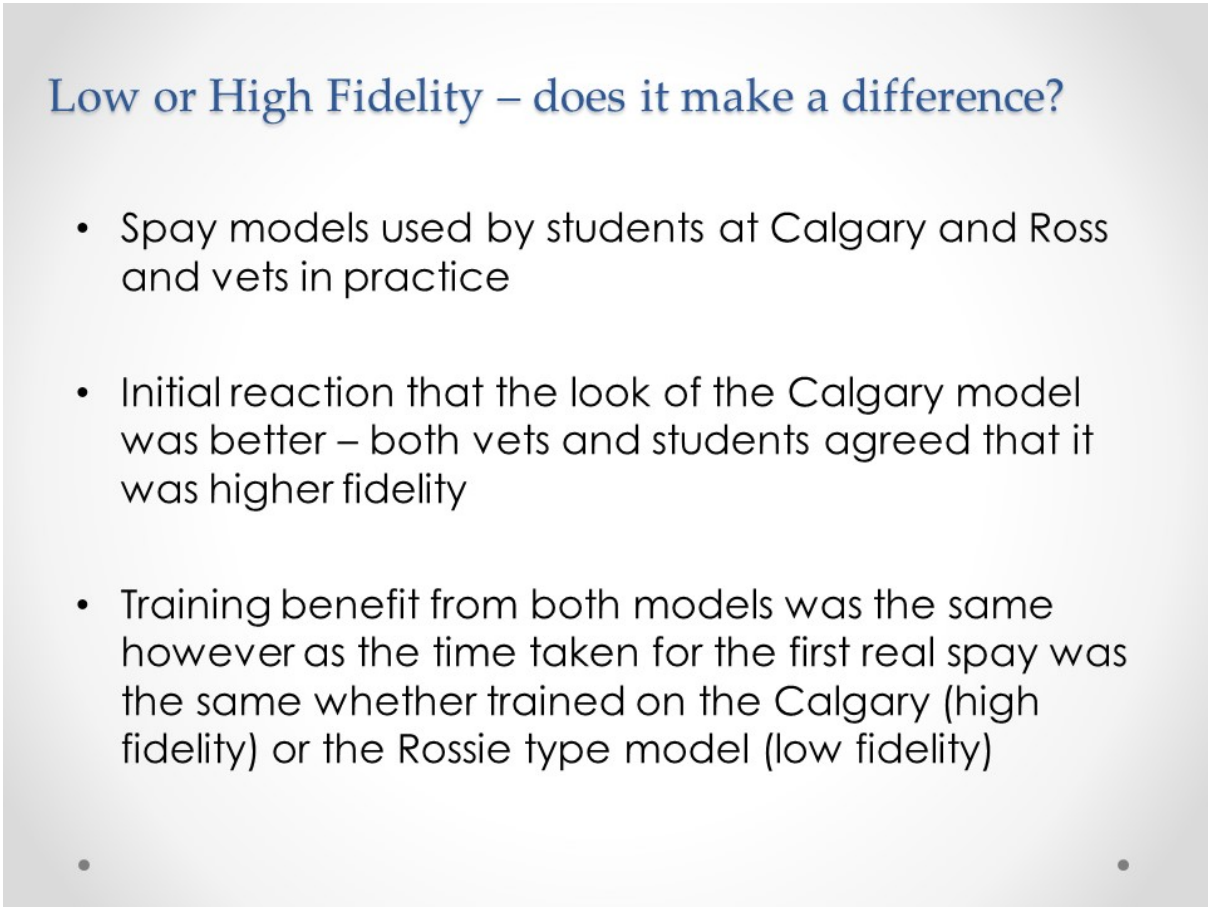
Full reproductive model for cattle complete with calf that is life sized for palpation training, repositioning in utero as needed, and delivery.

Windows can be removed to see inside to better understand mal-presentation postures of the calf and how they might be corrected.

The less glamourous, but very functional, version for reproductive training.

This one can use silicone reproductive tracts of various stages or these can be harvested from the abattoir as fresh specimens as can the calves.

These can be used for artificial insemination trainers as well for technical staff/ student training.



Basically the models were equally effective for training despite the very great difference in look and cost of these.

Evaluation of Veterinary Student Surgical Skills Preparation for Ovariohysterectomy Using Simulators: A Pilot Study

April 2016

Journal of Veterinary Medical Education 43(2):1-24

DOI:

10.3138/jvme.0815-138R1

Emma K Read

Andrea Vallevand

Robin M Farrell

Benefits of models

- Student training with models can take as long as they need to gain the confidence to do a procedure without feeling as though they are hurting an animal
- Relieves student stress and anxiety
- Learners do better when they are not stressed or time pressured
- Diminished fear of failure by students
- No conscientious objections
- Gets all students to similar skill levels over time
- Consistency of experience
- Models do not have to be expensive to be effective
- Reduces animal use for basic skills teaching

The utility for models is great and really only limited by your resourcefulness and imagination.

Think how you can break down the requisite skill into its step wise components and then reconstruct these.

The possibilities for developing trainers for research teaching is great despite to smaller size of our animals. The use of live animals for surgery training should be minimised by the creative use of alternatives to allow students to gain initial technical skills in a stress-reduced setting where they can focus on the basic steps first.

Have fun modelling!

Thank you – questions?

Reliability of animal research data – something old, something new?

Margaret Rose
Prince of Wales Clinical School, University of New South Wales.

It has long been acknowledged that a basic tenet for the acceptability of animal research is that proposed research objectives are achievable [1]. In Australia, national policies governing both human [2] and animal research [3] are unequivocal that it is ethically unacceptable to perform these activities unless there is clear evidence of scientific validity. Thus recent publications focused on the reliability of animal research data raise issues that are integral to both the integrity and the ethical acceptability of this research.

Replication of data is recognized as an issue in science and various strategies are used to identify and manage these risks [4]. Although for many years those opposed to animal research have challenged evidence as to the reliability and relevance of animal research achieving benefits to human health and wellbeing, the recent raft of publications in scientific and medical journals have prompted further in-depth analyses that have identified *inter alia* significant issues with experimental design and selection of animal models when reporting animal studies. In response, recent meetings in the UK and USA have discussed ways to address these issues and resulted in the dissemination of guidelines as to those matters that need to be taken into consideration in the planning, conduct and reporting of animal studies; matters that have also been addressed in a recent NHMRC publication [5].

How these issues impact on our deliberations as to the ethical acceptability of animal research and consideration of strategies to achieve the goals of the 3Rs will be discussed.

1. Hall M (1847) Reprinted in *Lancet*, 135: 58-60.
2. National Health and Medical Research Council (2005) *National statement on ethical conduct in human research* (updated 2012). Canberra: National Health and Medical Research Council.
3. National Health and Medical Research Council (2013) *Australian code for the care and use of animals for scientific purposes, 8th edition*. Canberra: National Health and Medical Research Council.
4. Hewitt JA et al (2017) *ILAR Journal* 58(1): 115-128.
5. National Health and Medical Research Council (2017) *Best practice methodology in the use of animals for scientific purposes*. Canberra: National Health and Medical Research Council.

No Manuscript was received for this presentation

Can ethics be considered a science?

Verrinder, J.M.
Animal Welfare League Qld, Gold Coast, Australia

Despite ethics being legislated into researchers' and Animal Ethics Committees' decision-making through the Australian Code for the Care and Use of Animals for Scientific Purposes, when people are asked to define "ethics" they often have different interpretations and struggle to come to agreement. The definition of "ethics" in the Code is open to interpretation and ethics terms used in the governing principles in the Code are not defined.

Currently no standard education or training program on ethics exists for scientists or animal ethics committees in Australia. Animal ethics education internationally often offers a variety of perspectives and leaves it up to individuals to decide and justify their preference. This approach provides little guidance for deciding collectively what should be done.
Is there something more substantial to ethics? Can ethics be considered a science, and how does animal ethics differ, if at all, from animal welfare, animal science, animal legislation, animal rights, and the various personal and cultural values about animals that differ between people and countries.

This presentation provides answers to these questions to clarify these important concepts and create a clearer basis for ethical decision-making.

Speaker Biography

Joy Verrinder has a doctorate in animal ethics education and moral development and a Masters degree in Professional Ethics and Governance. She has served on the Boards of numerous animal organisations, as well as Queensland Government and Griffith University Animal Ethics Committees, and the Australian Government Animal Welfare Strategy Education Working Group. She is currently the Strategic Director of the Animal Welfare League of Queensland (AWLQ), Australia, working with state and local governments, veterinarians, the pet industry and other animal welfare organisations to reduce the abandonment and killing of domestic cats and dogs in communities across Australia.

Can Ethics be considered a Science?

Dr Joy Verrinder

Strategic Director, Animal Welfare League, Qld.

Ethics

Animal Welfare

Professional Ethics

Laws

Animal Ethics Committees

Australian Code for the Scientific Use of Animals

Social licence

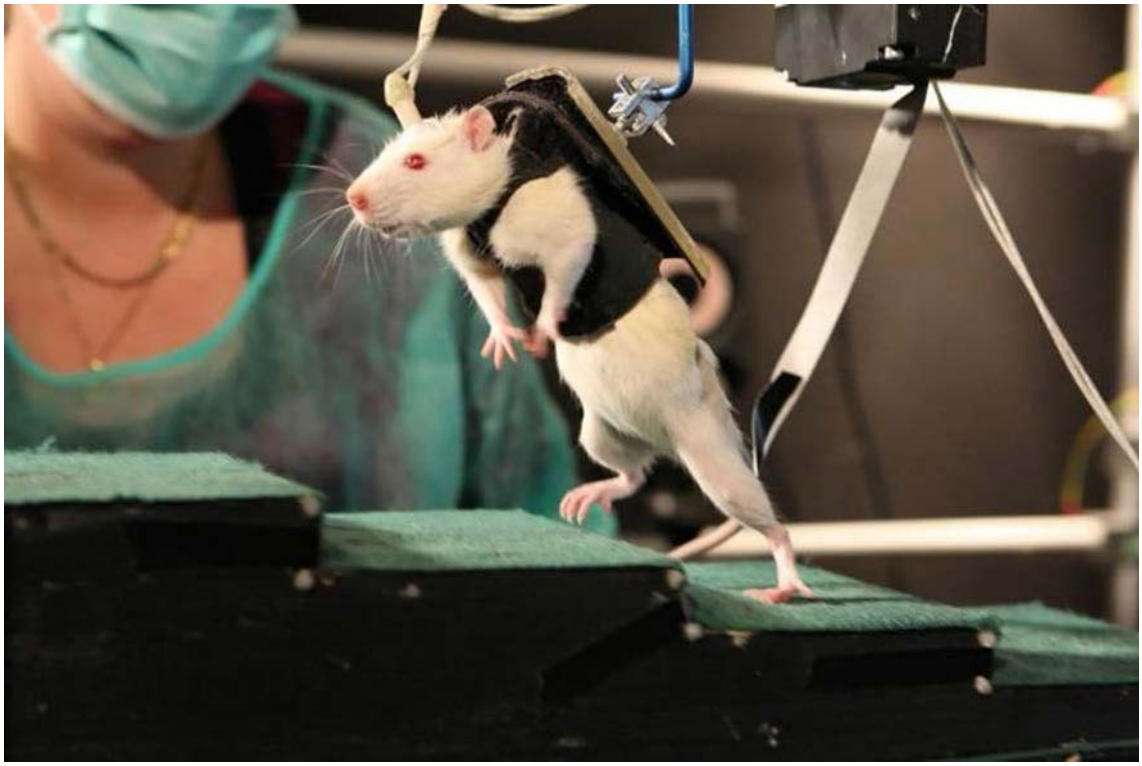
Animal Ethics

Personal Values

Cultural Values

Science

What **should** I/we do?

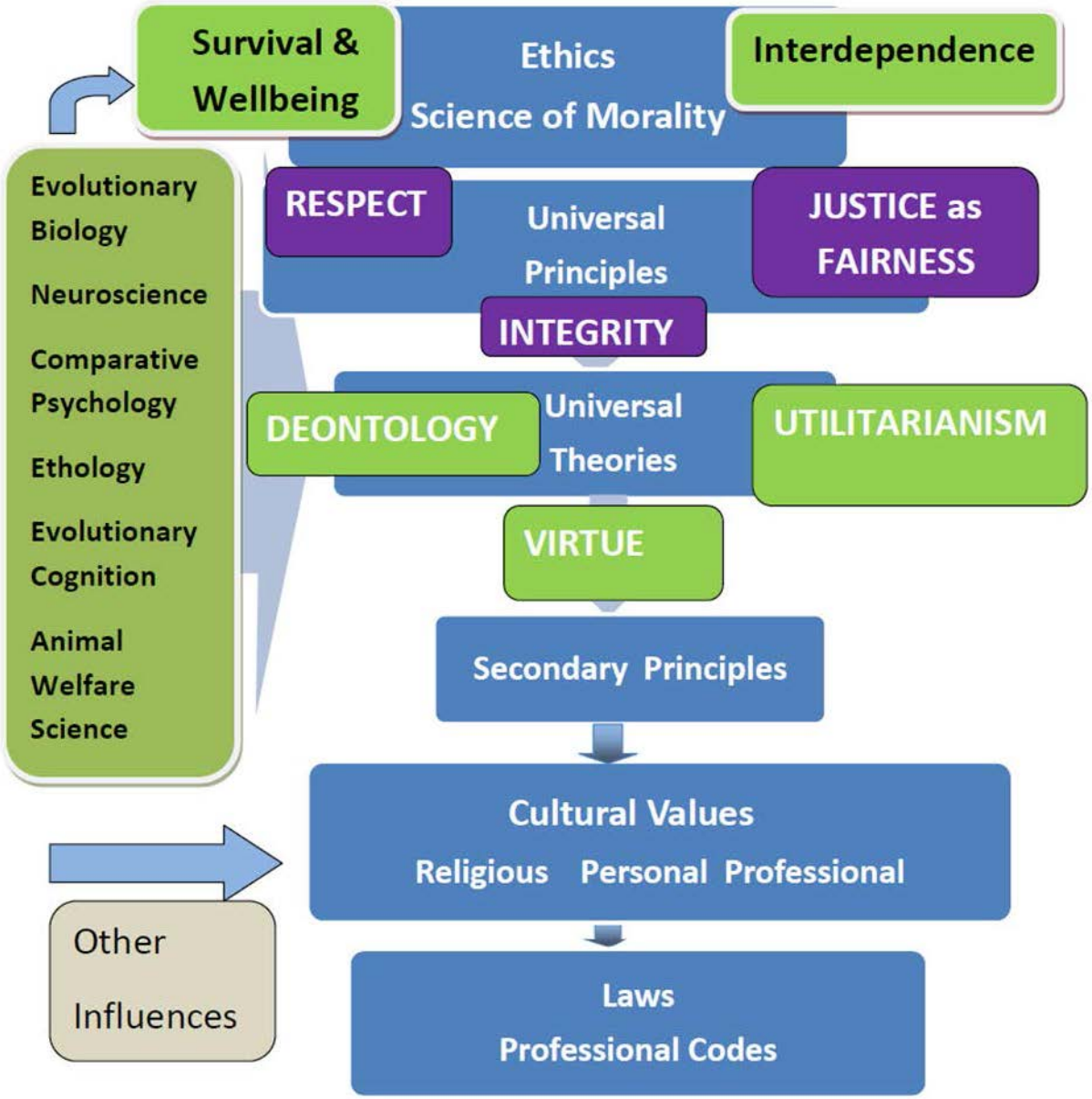


Differs from Animal Welfare

- **Animal Welfare**: An animal's physical and psychological state with respect to the quality and quantity of its experiences in its environment.
- Welfare informs ethics

However **ethical decision-making and ethical behaviour** require **different knowledge and skills** to help us to decide what we should do.

Understanding where ethics fits



Understanding where ethics fits:

The relationship between ethics, morality, values, culture, law & science

Survival & Wellbeing

Interdependence

Ethics is based on 2 Facts

FACT 1

Sentient beings have an inbuilt desire to survive and thrive, to experience pleasure (for growth and flourishing) and to avoid pain (physical and emotional)

Jeremy Bentham

Deontology or the Science of Morality 1834

- “Nature has placed mankind under the governance of 2 sovereign masters, pain and pleasure. It is for them alone to point out what we ought to do, as well as to determine what we shall do.”
- “…when the greatest good, the universal happiness is made the central point round which all action revolves, the golden era of moral science will commence.”

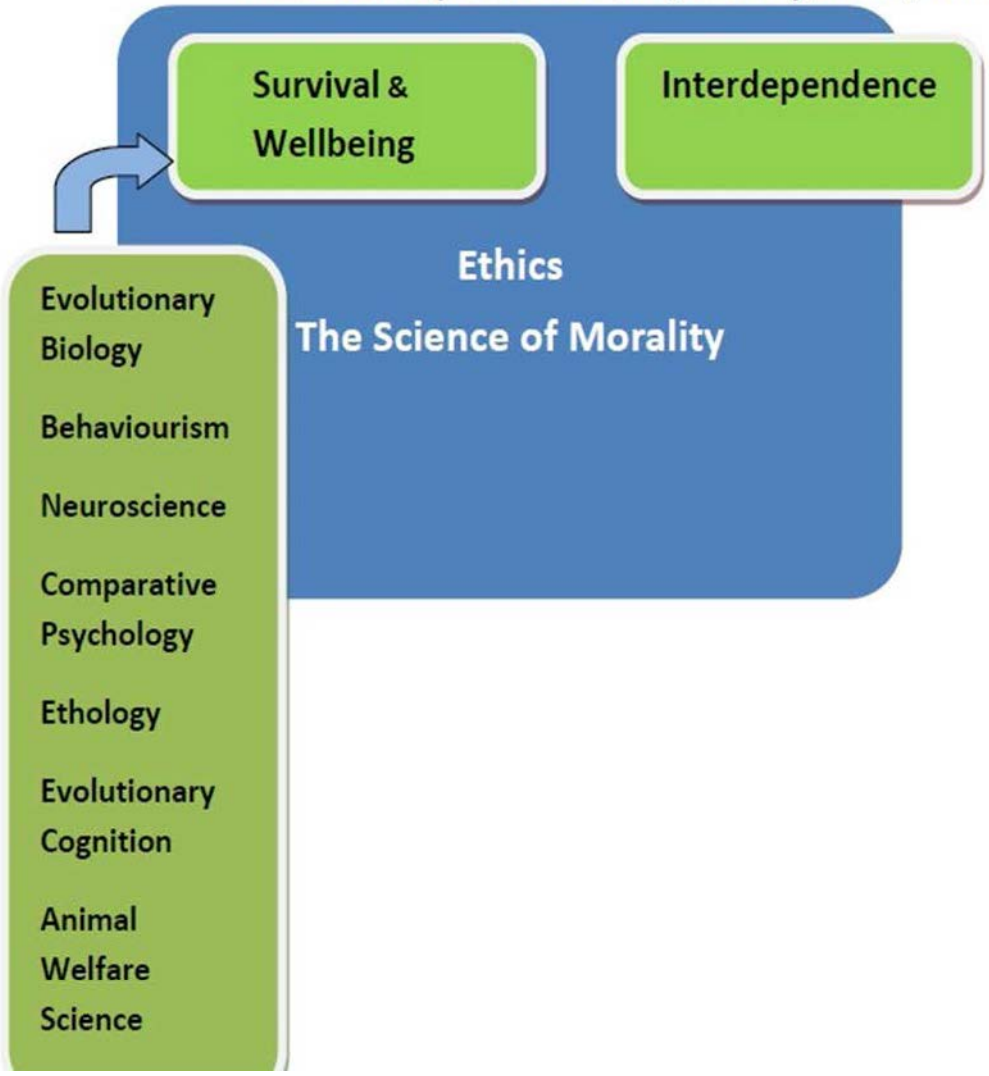
Sam Harris: The Moral Landscape 2010

"Just as there are ways to identify better and worse health, there are ways to identify better and worse well-being. Human and animal well- being are natural phenomena.

As such they can be studied in principle with the tools of science and spoken about with greater or lesser precision."

Understanding where ethics fits:

The relationship between ethics, morality, values, culture, law & science



Nervous systems designed for self-preservation
- Fear of harm and death

The Cambridge Declaration on Consciousness in non-human animals

7 July, 2012

"Artificial arousal of the same brain regions generates corresponding behavior and feeling states in both humans and non-human animals."

2nd Fact on which ethics is based

All life is interconnected

- every behaviour (action we take or don't take) has some impact on other sentient beings, and it is inbuilt within sentient beings to care about others' survival (being) and wellness (well-being).

Oxytocin and Arginine Vasopressin

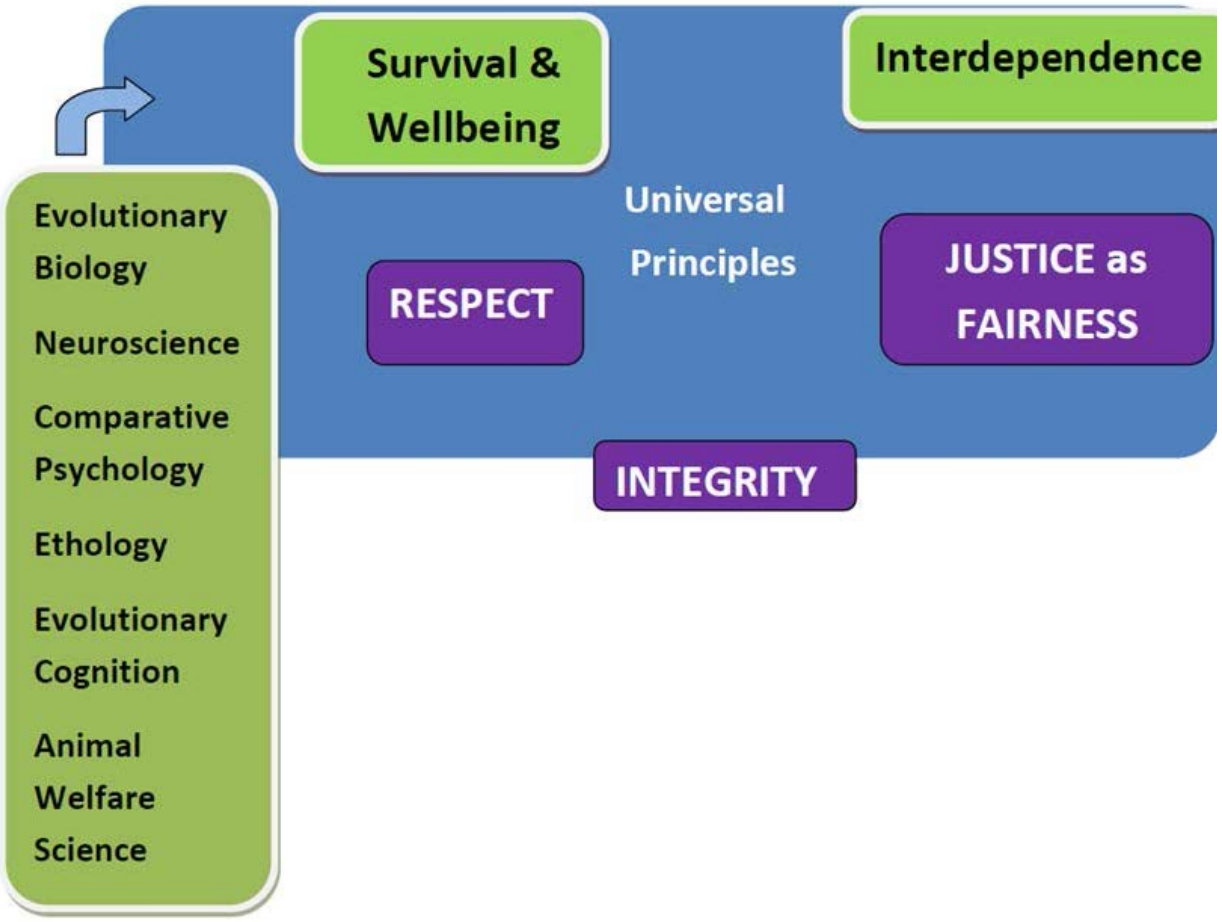
Both the insula, and the anterior cingulate cortex, respond to physical pain, but they also respond to social pain, triggered by separation, exclusion, or disapproval, and to pain resulting from errors and poor prediction

Our conscience

"Our conscience - the pain from being shunned and the pleasure of belonging, along with imitation of those we admire, gives rise to powerful intuitions about the absolute rightness and wrongness of behaviour."

Churchland PS. Braintrust: What Neuroscience Tells Us About Morality. Princeton, New Jersey: Princeton University Press; 2011.

Understanding where ethics fits



1. Respect for all life

“The reverence felt by my will-to-live for every other will-to-live”

(Schweitzer 1949)

- https://www.youtube.com/watch?v=FvkI8c9eALQ

2. Justice as fairness

i.e. Greatest priority to the least advantaged

The veil of ignorance

- **Imagine you don’t know whether you are one of society’s weakest or least fortunate or one of the most fortunate**
- What decision would you make?

John Rawls

Integrity – consistency in beliefs and actions to respect life and well-being and be fair

Sapontzis (2004) disagrees that animals' lives are less important to them than ours are to us.

“the more likely conclusion is that those traditions are based on **a priority principle** (that humans have priority over other animals) which **violates the impartiality which is supposed to be characteristic of morality”**

Sapontzis 2004

3 Main Ethical Frameworks

1. **DEON**TOLOGICAL ETHICS

DUTY TO PRINCIPLES

- The authority for a moral decision is within the person, not in some external authority like God or even the law.
- Duties must be obeyed or rights acknowledged regardless of the consequences

Ethical theories are now being related to biological facts about our brains

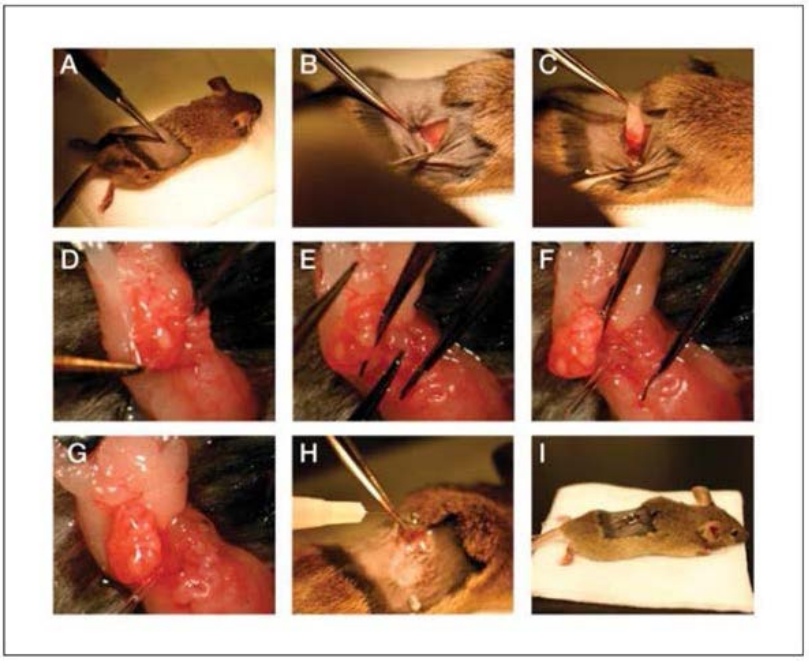
Deontological judgements seem to be driven by intuitive emotional responses (Greene 2008)

2. UTILITARIAN ETHICS

- WEIGHING UP THE BENEFITS AND HARMS TO EACH STAKEHOLDER
- LOOK AT WIDER AND FUTURE CONSEQUENCES
- MAKING A DECISION BASED ON THE GREATER GOOD – THE WELL-BEING OF ALL CONCERNED

Ethical theories are related to biological facts

Utilitarian judgements seem to be more controlled cognitive processes (associated with reduced empathy)



(Gleichgerrcht & Young 2013)

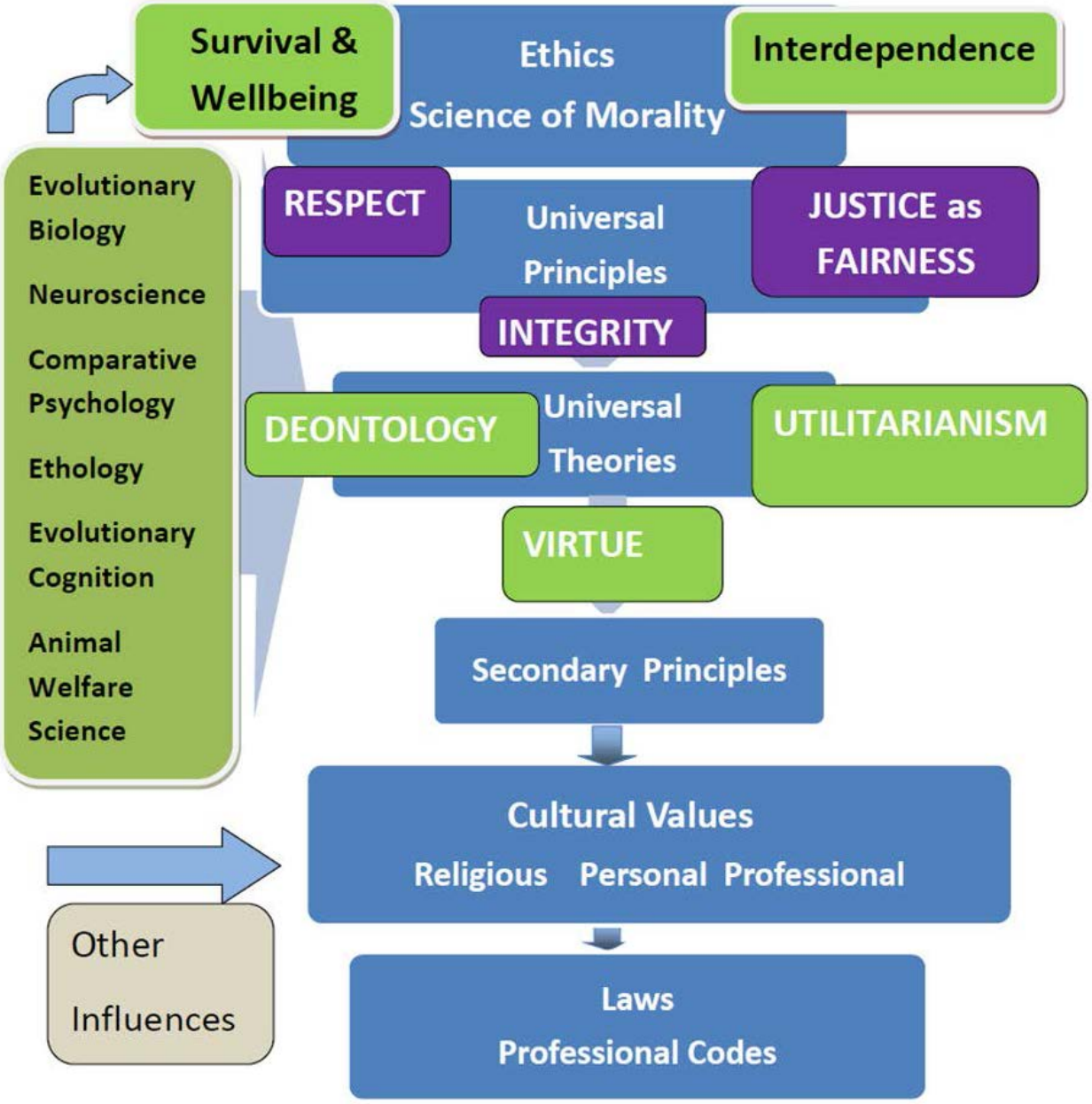
3. VIRTUE ETHICS

How does the decision relate to the person or the professional I want to be?

Aristotle: Happiness - a state of contentment, a life integrated happily with a sense of purpose, lived in a community with others.

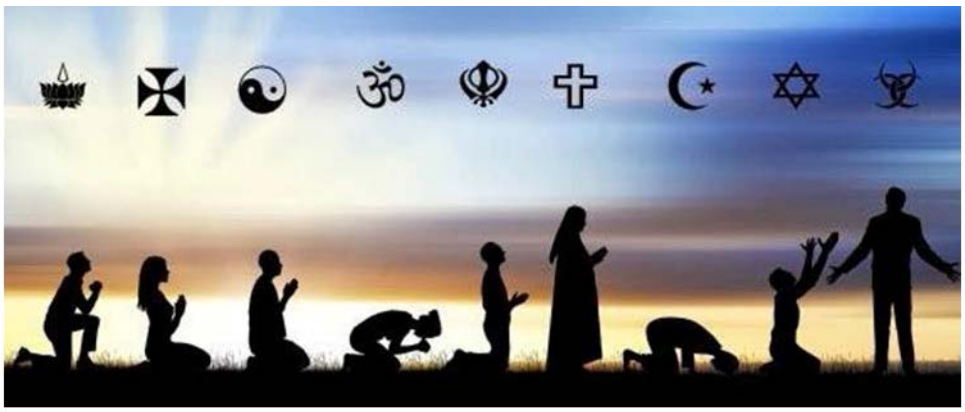
To achieve this requires:
Internal qualities (courage, good temper, truthfulness, compassion) rather than external (fame, power, profit).

Understanding where ethics fits

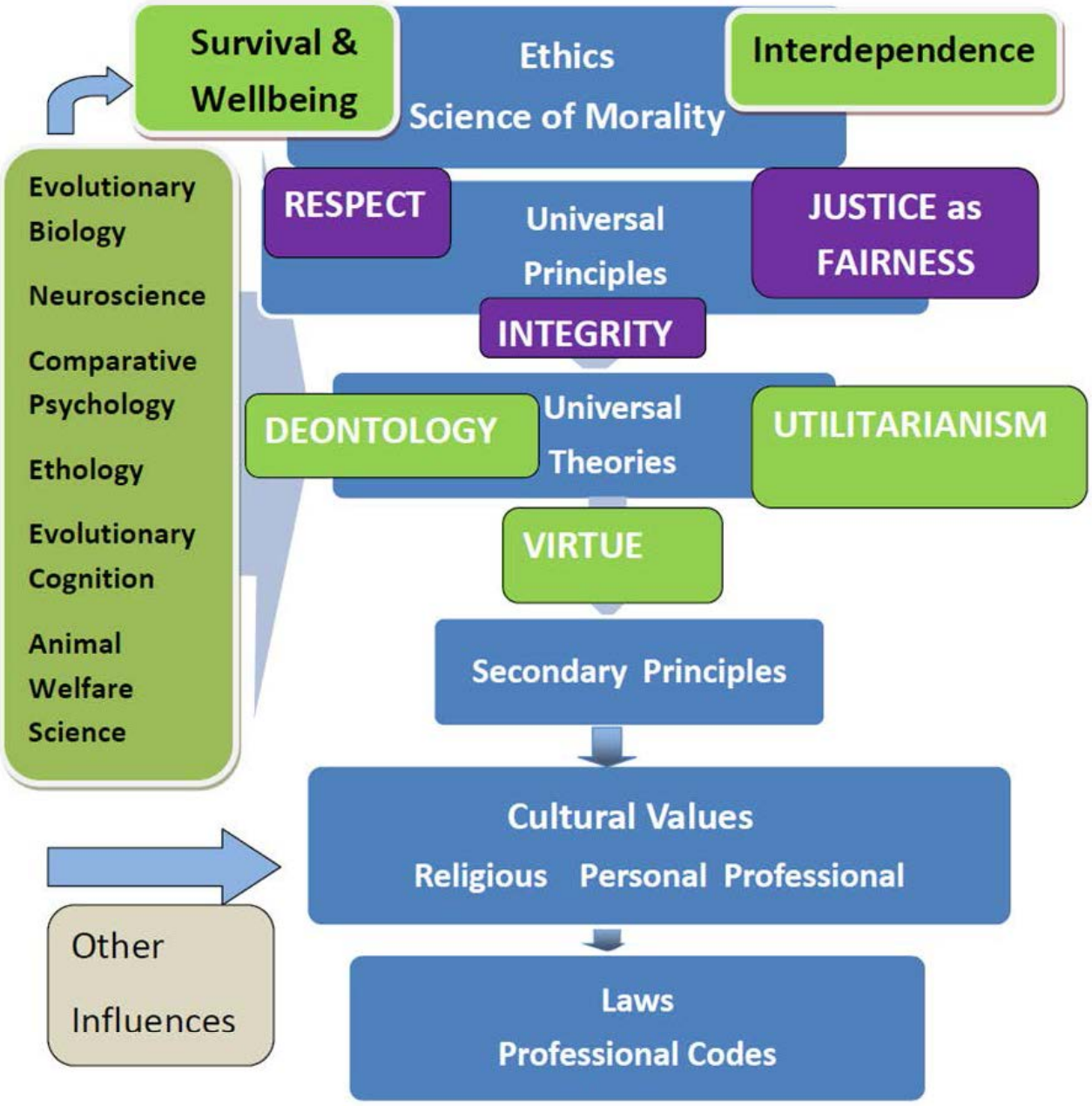


Cultural Values

- Cultural values may, or may not, be informed by ethics and can be distorted by:
  - Religious values
  - Professional values
  - Personal values



Understanding where ethics fits

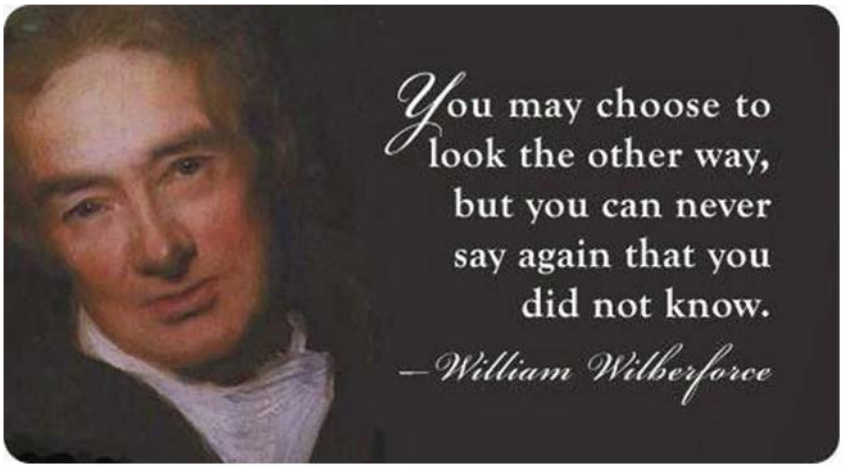


Ethics and law

- Just as welfare informs ethics, ethics should inform laws and codes.
- “Over time laws and codes may undergo modification for many reasons, some that serve the interests of a powerful subgroup (humans), some that serve the well-being of the group as a whole and some that reflect the psychiatric delusions of a manipulative despot.”

(Churchland 2011)


VOTES


You may choose to look the other way, but you can never say again that you did not know.
—William Wilberforce


SAVE
our
EARTH

Animal ethics and animal law

- The recent rapid expansion of animal law reflects the social struggle to ensure that laws reflect the 3 principles – respect, justice as fairness and integrity - in relation to animals.

"The arc of the moral universe is long, but it bends toward justice."

(Martin Luther King)

So if ethics is now found to be based

on the physical structure of our brains,

can ethics be developed scientifically?

YES!

Animal Ethics as a Science based on Oxford definition of science

- Animal ethics can be defined as the intellectual and practical activity encompassing the systematic study of what humans should do in relation to animals' needs and wants, and how to develop this moral behaviour amidst conflicting interests, through observation and experiment.

3. Components of moral behaviour

- Moral sensitivity
- Moral judgement
- Moral motivation
- Moral character

So what should we do in relation to the use of animals for scientific purposes?

A wider ethical approach

1. We can improve ethical knowledge and competency in moral sensitivity, moral decision making, moral motivation and moral behaviour of scientists and animal ethics committee members.

What else should we do?

Align the Code with universal ethical principles

- “Ethics” and “Respect” in the Code can be defined better as acknowledging all sentient beings desire for “survival and well-being”.
- Harm includes loss of life which sentient beings are biologically programmed to fear and avoid.

How do we decide what suffering is too much suffering in research?

- Any loss of life and suffering which is deliberately caused in another sentient being is not ethical, unless it is to benefit that animal or prevent it causing greater harm to other animals.
- Breeding sick animals to endure the same fate as very sick human animals does not increase the protection of life and well-being in the world.

So what else should we do?

Scientists can focus more on addressing the causes of disease (poor or polluting lifestyles and lack of motivation to address these, poor living conditions) to prevent rather than react to disease.

So what else should we do?

- Government policy and resourcing should give preference to prevention.
- Each institution should encourage and resource replacement research to fast track alternatives to the use of animals

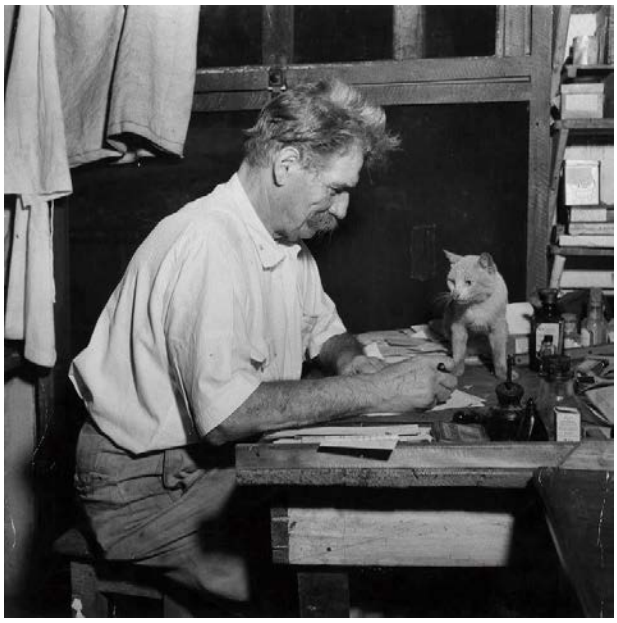
Creating a more ethical world

- With greater trust and ethical understanding, we can pursue more rigorously the **prevention of disease** and **replacement of animals used in research**.
- Institution- wide. Country-wide. World-wide.

We should plan a process for doing this.

Reverence for life

To make progress in ethics “one must become ruled more and more by the longing to preserve and promote life, and more and more obstinate in resistance to the necessity for destroying or injuring life.”



(Schweitzer 1949)

Developing Ethical Knowledge Skills & Behaviour

- Interested in a workshop or

course to develop:

- Moral sensitivity
- Moral judgment and use of a universal framework for ethical decision making
- Moral motivation
- Moral character?

Email: jverrinder@awlqld.com.au

Keeping it relevant in primary and secondary schools.

Linda Hearder
Principal Consultant, Department of Education Western Australia

A critical discussion of the relevance of the Code to the primary and secondary school context in Australia is overdue. The majority of animal use activities being conducted in schools are low impact and of short duration. As such, the mechanism associated with AEC oversight is cumbersome, inefficient and ill-suited to manage animal welfare in schools.

The use of live animals for scientific purposes in primary and secondary schools (excluding farm schools) is undertaken intermittently and is overseen by teachers and school principals, across Australia. Examples of common primary school activities include aquaponics, breeding mice or guinea pigs and hatching chicken eggs.

Total compliance with the Code is impossible for school systems due to the large number of sites across wide geographical areas. As a result, each State's AEC has implemented strategies to manage this task by mitigating the risks of inexperienced teachers undertaking low risk activities, while approving only applications for activities with perceived higher risks. One of the most commonly used strategies across all jurisdictions includes categorising certain school activities as pre-approved. However, the Code requires that *all* activities must be subject to ethical review, approval and monitoring by an AEC and approval must occur at a quorate meeting of the AEC.

Activities perceived to have the most risk are undertaken by farm schools and, technically, do not require approval by an AEC under the Code. In addition, some jurisdictions still require the AEC to consider applications for dissections even though schools obtain specimens for dissection pre-euthanized.

School AECs across Australia are approving applications for activities that do not require approval and are not approving applications for activities requiring approval. Achieving strict compliance with the Code in all circumstances remains an ongoing challenge for schools. This is primarily associated with the application of a complex regulatory framework designed to protect the wellbeing of animals potentially being used in high level scientific research, to activities which are low impact, of short duration and are conducted only occasionally by personnel not routinely familiar with that regulatory framework.

The relevance of the Code to schools across Australia must be questioned and ultimately reviewed.

No Manuscript was received for this presentation

Feedback to the conference from the Wednesday discussion groups

Discussion groups for this session were pre-arranged by ANZCCART staff and aimed to ensure a broad, but representative mix of delegates in each group. Key aspects of mixing up the members of each group included representation from each of the standard membership categories, different regions of Australia and New Zealand as well as different institutions as much as possible. This process is essentially designed to help ensure a broader range of experiences and processes as possible can be introduced to the group and influence their discussions and possible conclusions.

For this session, the following list of topics was offered for discussion:

- Would you approve an application to use Gene Drive technology to exterminate feral species? (See background publication provided if you are unsure about the technology)
- Pain Research: - How much pain is too much pain? Where do you set the boundaries?
- Public Access to science and maintaining the social licence. How do you balance security of research against the public 'right to know' – after all, most is publicly funded.
- Consistency of decisions made by your AEC – how good are we and how do we improve? This would include consistency between researchers, between applications and possible issues with evolution of techniques and reagents over time.
- Facilities inspections – who should do them and what are you looking for? Do you report on your findings and if so, to whom and why? How do you cope with facilities that are outside the main animal facility and / or off – campus?
- Problem applicants – what can we do to help them, help us?
- Mutual recognition of other AEC approvals. Do you have a system in place that works and if so, how is it set up?
- Are your applicants really addressing the 3Rs completely? In particular, how do they address the issue of replacement or how should this be addressed?
- Should Pentobarbitone Sodium (Lethabarb) be reclassified as an S8 to help prevent Veterinarian suicides? If it is, what effects will this have on researchers in the lab and in the field and how can these be addressed?

As with previous discussion group sessions, groups were free to discuss another topic or topics if there was something they particularly wished to consider. At the end of the designated period for discussion, one member of each group was invited to briefly outline their discussions and any conclusions they were able to draw. These reports are listed below:

Group 1

Whether everyone is addressing the 3Rs – Replacement

- Felt there was some confusion on the 3Rs – may need training on all 3 parts not just reduction
- There were a number of people who were addressing the 3Rs by using videos to view animals.
- In reducing numbers - demand there is review literature done so it's not repeated and for the terms to be included so that they know what research was being done and what might be looked at for finding the information relevant to that project
- Most people said there were questions about the 3Rs on the application form which means they were thinking about it

- In training 3Rs is often overlooked
- Onus is on researchers not on AECs
- Evidence to be required that the researchers are actually looking at the 3Rs
- Material can be shared between researchers - not sure how this can be done
- Training on cadavers – issues as it's not easy to replace some animals - use tissue culture
- Screening process in some AECs – the applications have been well screened and the researchers have applied the 3Rs
- Promote pilot studies
- Need better ways for networking and thank ANZCCART for this opportunity and maybe collectively find a way to share samples in research

Group 2

Problem applicants

- remind people they can reject applications
- the importance of understanding what is going on for first time applicant
- repeat offenders need more work
- some training programs have been run as a mock AEC meeting which was very successful
- Face to face mentoring from a scientific perspective
- Using the AWO is important
- Some applications have many amendments – discussed ways to limit multiple amendments as project changed
- Some people do not wish to comply. In one instance, the head of school agreed with him and it went through the complete dispute resolution at the Institution. Research can be stopped.

- Transfer of ethics approval across jurisdiction
- Monitoring issues in large states and across great distances ie WA
- Issues of public access
- Death threats over an AEC application which was rejected.

Group 3

Consistency of decision making across AECs

- The Code is a good guideline and we obviously adhere to that – assists with decision making
- Need a good set of SOPs
- AEC Guidelines are important
- Realise the importance of education re investigators – it needs to include everyone, irrespective of seniority - it's mostly easier online and it should be assessed at the end. It should occur every 3-4 years.
- Importance of lay language – especially for Ds and Cs makes it easier for them to assess application
- Contribution of As and Bs and they can assist with Cs and Ds with this process
- Relevance and usefulness of pilot study information and it can help make a quick decision on how you are going with the project

- Resources provided by the institution are important and amongst this is the attitude and support of your EO, Deputy DVC and they can make it a lot easier
- The importance and value of animal staff on the committee
- Importance and value of attending conferences ie ANZCCART
- Committee turn-over and could affect consistency for a while.
- Re Euthanasia drug? - need to be able to euthanise animals as quickly and painlessly as possible.

Group 4

Gene drive technology

- Associated risks and unknowns with unintended consequences
- Great potential for this technology eg killing mosquitoes that cause malaria
- Decided the risks were higher than the benefits

Pain Research

- Draw boundaries
- Pain is one small element in assessing quality of life.
- How complex it is and humans are not very good at expressing or understanding pain
- Different measures to be used which can assess the animal before pain sets in
- Using analgesia and anaesthesia are appropriate to use in pain studies as that most closely mimics how humans manage pain
- Pilot studies to see if using analgesia and anaesthesia does have an impact on results
- It needs to be assessed on a case by case basis and you would need very clear end points in conducting pain research studies

Public access to information

- Honesty and transparency is very important
- Discussed an initiative overseas where it is a requirement to publish the lay summaries or proved projects which has been a positive and successful initiative
- In conclusion, transparency is very important and needs to be put into context for it to be really valuable to the public.

Group 5

Mutual recognition

There are a lot of systems at place in some states and we focussed on SA where several institutions have collaborated to recognise the other institutions approvals. There was a lot of thought as it is about how animals are being used across different sites. Generally, the site where the animal is held is the site where the research is applied for AEC approval. This site would take responsibility. This is not always the case as in another case the primary institution was not taking responsibility

How much pain is too much pain?

- When AECs look at applications where pain is the result there may be a tendency to view pain as a consequence differently to pain that is being deliberately inflicted to study that pain. This can sometimes lead to difficulty determining where to put your limits. Everyone has their personal subjective opinion as to how much is too much which affects each AEC.

- Need to agree where the limit is. Look to scientific information - Grimace scales.
- Researchers could explain their research to the AEC and reassure members.
- Pilot studies could be used to determine pain
- Fear amongst investigators to score their pain level high

Problem investigators

- Co-operation and working with these investigators in the first instance is valuable.
- Those that constantly push boundaries are worse.

Felt that the drug wasn't necessary

Group 6

Problem applicants

- Reprimand and punishment was not necessarily the way to go.
- Manage them positively
- More pilot studies particularly for high risk or new research
- Ask for researcher's feedback on the AEC to understand them more
- Invite researchers to meetings for discussion
- Have a research ethic advisor on the AEC – can be used for liaison
- Have statisticians available to review applications prior to submission which gives AECs confidence stats are right
- Training for researchers and investigators
- Offer somebody to observe procedures for the first few times and report back to AEC
- Provide a resource library, SOPs, listing of other research in that field
- Have large or complicated projects divided into phases so that they can report on each phase
- Valuable for AECs to have valuable relationships with supervisors

Group 7
Pain research

How do you qualify pain in fish and other difficult groups - not as much research?

- At times researchers argue against using analgesics for pain as they feel it affects their research. Argument that the previous research didn't use analgesics. Animals feeling pain could affect the results.
- When does pain get too much? Researchers fearing, they can't euthanize animals when appropriate as there may be repercussions from the AEC.
- Chronic pain can result from acute pain not being managed
- Ways of assessing pain eg weight loss/gain and in some instances that may not be the reason eg a tumour growing
- Distress – single animal housing may affect this
- Heat treatments which mimic pain
- Differences between Australia and NZ. In NZ drugs don't need to be included in applications. The AWO deals with that.
- Pilot studies

Group 8
Pain research

- AECs need a clear understanding of the project.
- Opportunity to relate the pain back to a human analogy, could be relevant but could also be confusing
- Monitoring sheets
- Needing clear intervention points
- Customised to suit the application
- Managing pain vs scientific validity – where do you draw the point? Its an ethical dilemma. To mitigate that we leverage the experience of our AEC and value and respect their opinions and with their combined knowledge we move forward with a clear direction.

Public access

- do the public really want to know everything?
- Risks to AEC members
- Intellectual property issues, confidentiality issues, financial obligations
- Organisations have real risks which need to be managed and addressed
- If we focus on the positive there are many opportunities of the good work we are doing, and we should be advertising this
- Educating the next generation is the way to bridge this public opinion and pressure.

Replacing In-Vivo and Animal Testing with Supercomputers to Gain Insight into Effectiveness and Specificity of Medications for Chronic Pain and Epilepsy

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Your nervous system is responsible for the coordination of your actions and detection of environmental sensations. It does this is by sending electrical signals throughout your body as a means of communication and coordination with the body's other systems. Voltage-gated sodium channels are one of the integral transmembrane proteins responsible for generating these electrical signals, more specifically in nerve and muscle cells. As such, mutations of sodium channels are responsible for a variety of disorders, including cardiac arrhythmias, epilepsy and chronic pain. Thus, they are key targets for a variety of compounds, including local anaesthetics and anti-epilepsy medications.

Existing medications, such as local anaesthetics and anti-epileptic medications, are effective but often have unintended and potentially harmful side effects. These side effects arise from medications interacting both with intended and unintended targets. Investigating how these drugs interact with their targets on the molecular level is imperative in not only understanding their underlying mechanism, but crucial for designing more specific and efficacious medications.

The normal pathway for drug development involves extensive cell and animal testing, and the suffering of animals is increased when the main target of the research is new pain medications. This is because animal testing usually involves the infliction of pain upon test animals to assess pain tolerance. For epilepsy models, epileptic mice strains are specifically bred, leading to a poor quality of life for these animals. It is therefore critical that we reduce and ultimately replace such studies by utilising careful predictions from computational models.

As an alternative approach to the design of new compounds, we have used Molecular Dynamics simulations to better understand how a wide range of drugs interact with sodium channels, including two local anaesthetics, two anti-epileptic medications, and two sodium channel inhibitors developed by Pfizer inside a sodium channel pore. With this data, we were able to determine an important trend in the compounds; namely that the charge state matters for the efficacy and specificity of compounds. This study, which is supported by funding from the Medical Advances Without Animals Trust (MAWA), highlights the utility of molecular simulations, and the utility of supercomputers as an alternative method to animal-based research.

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Protonation state of inhibitors determines interaction sites within voltage-gated sodium channels

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Voltage-gated sodium channels are essential for carrying electrical signals throughout the body, and mutations in these proteins are responsible for a variety of disorders, including epilepsy and pain syndromes. As such, they are the target of a number of drugs used for reducing pain or combatting arrhythmias and seizures. However, these drugs affect all sodium channel subtypes found in the body. Designing compounds to target select sodium channel subtypes will provide a new therapeutic pathway and would maximize treatment efficacy while minimizing side effects. Here, we examine the binding preferences of nine compounds known to be sodium channel pore blockers in molecular dynamics simulations. We use the approach of replica exchange solute tempering (REST) to gain a more complete understanding of the inhibitors' behavior inside the pore of NavMs, a bacterial sodium channel, and NavPas, a eukaryotic sodium channel. Using these simulations, we are able to show that both charged and neutral compounds partition into the bilayer, but neutral forms more readily cross it. We show that there are two possible binding sites for the compounds: (*i*) a site on helix 6, which has been previously determined by many experimental and computational studies, and (*ii*) an additional site, occupied by protonated compounds in which the positively charged part of the drug is attracted into the selectivity filter. Distinguishing distinct binding poses for neutral and charged compounds is essential for understanding the nature of pore block and will aid the design of subtype-selective sodium channel inhibitors.

Voltage-gated sodium channels (Navs) are transmembrane proteins responsible for generating action potentials in nerve and muscle cells. By opening in response to a small stimulus, they facilitate the passage of Na^+ ions into the cell, leading to rapid depolarization of the membrane potential. Mutations of sodium channels are responsible for a variety of disorders, including cardiac arrhythmias, epilepsy, and pain syndromes (1– 5). Thus, they are key targets for a variety of therapeutic com- pounds (6, 7), including the broad family of "local anesthetics." While current local anesthetics inhibit all nine sodium channel subtypes found in the human body, there is a great interest in developing subtype-selective compounds to treat conditions like chronic pain (4, 5) or to reduce the side effects of epilepsy and arrhythmic medications. While there has been some progress in finding subtype-selective channel inhibitors (8–12), this has not yet been translated into clinical success. Thus, there is a great desire to better characterize the mode of action of existing channel inhibitors to determine what makes them inhibit all human subtypes, to define the common binding sites and pharmacophores, to elucidate the potential for making subtype- selective variants, and to gain a broader understanding of basic sodium channel function.

The publication of a number of bacterial Nav structures (13– 22), as well as the most recent published structure of a eukaryotic channel (23), have opened the door to gaining detailed insight into the mechanism of action of sodium channel inhibitors. While bacterial channels have many differences from eukaryotic sodium channels, there are many similarities that make these a reasonable starting point for biophysical studies. Bacterial Navs consist of four identical subunits, while eukaryotic channels are one long sequence consisting of four homologous domains. However, the overall architecture of the channels is similar, with each subunit or domain containing six transmembrane helices (labelled S1–S6). The first four of these form an independent voltage-sensing domain, while the last two helices from each come

together to form a central ion-conducting pore. The pore region itself contains: (*i*) a narrow selectivity filter at the extracellular end of the channel (Fig. 1, yellow box); (*ii*) a water-filled cavity in the center of the pore; (*iii*) a cytoplasmic activation gate that controls whether the channel is open or closed; and (iv) hydrophobic lateral fenestrations that extend from the center of the pore to the center of the bilayer (13–16, 23, 24). Mutagenesis studies have indicated that local anesthetics bind in the central cavity with key interactions with a number of polar and/or aromatic residues on helix S6 (25–32) (Fig. 1, orange boxes) and supported by a recent X-ray structure in which an inhibitor was partly resolved (16).

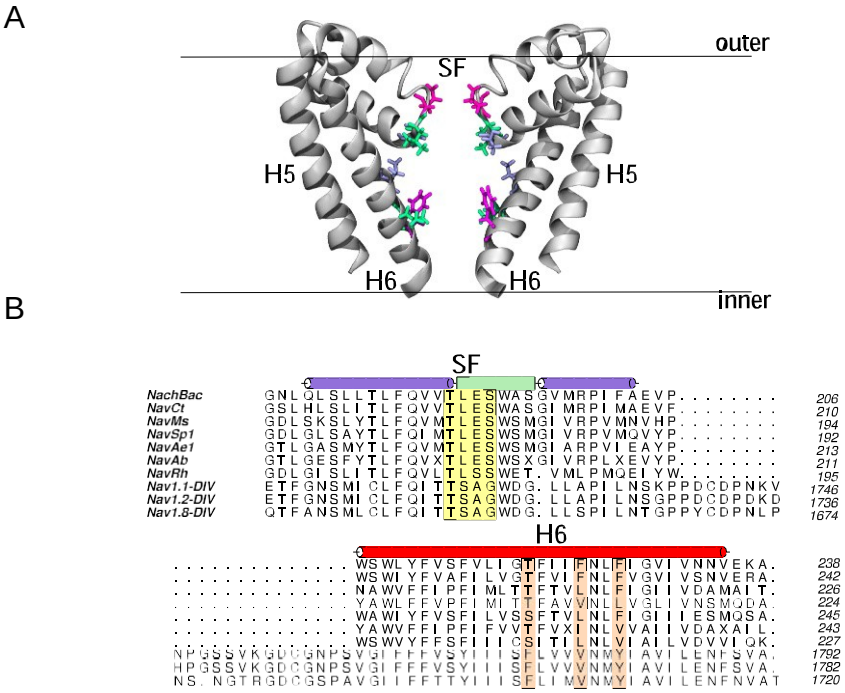

Fig. 1. (*A*) Structure of the pore of the bacterial sodium channel NavMs. The backbone of the protein is shown in gray cartoon, and residues of inter- est that are highlighted in *B* are shown in licorice. The bilayer is represented by a black line. (*B*) Alignments of sequences of bacterial sodium channels and select eukaryotic channels, consisting of residues in the selectivity filter (SF) to the S6 helix (only domain IV of the eukaryotic channels is shown). The helices on each side of the selectivity filter are shown in a purple cylinder, with the selectivity filter shown as a light green box. The S6 helix is shown in a red cylinder. Residues of interest in the selectivity filter are highlighted in a yellow box, whereas residues on helix S6 are in orange boxes.

Local anesthetics are known to be able to inhibit sodium channels in at least two distinct ways. The first is “tonic block,” where the drug blocks resting channels. This is believed to occur when the compound enters the pore directly from the membrane via hydrophobic lateral fenestrations in the side of the pore (13, 19, 24, 33–36). Alternatively, anesthetic compounds can display “use-dependent” block (24, 37), in which the compounds only inhibit the channel after it has opened, presumably by first crossing the bilayer into the cytoplasm of the cell and entering through the open activation gate. Although close together, different residues contribute to the binding site of tonic and use-dependent block (30, 31), suggesting possible conformational changes occurring upon binding. Both pathways require the drug to first either enter or cross the bilayer. Interestingly, many local anaesthetics (especially use-dependent blockers) have a pKa near 7, meaning that there are both neutral and charged forms at physiological pH. It is most likely the neutral state that crosses the bilayer, before regaining its charge in the cytoplasm and binding to the Nav protein; however, this has yet to be clearly shown. Simulations provide one way to examine which protonation state is likely to be present in each situation, but a comparative study of charged and neutral variants of local anaesthetics has not yet been published.

The bilayer partitioning and energy barriers faced in crossing the membrane have been determined from simulations for a variety of both perpetually neutral and protonatable compounds. These include benzocaine (35, 38–40), lidocaine (41, 42), articaine (43), and phenytoin (35, 40). All show that, irrespective of charge, compounds prefer partitioning into the bilayer from the aqueous phase to sit just under the lipid head groups, and they all face a barrier to pass through the hydrophobic bilayer core. In addition, a number of molecular dynamics (MD) studies have been conducted to examine the interaction of perpetually neutral local anaesthetic compounds with Nav channels. Direct simulations of drug–channel interactions have been conducted for benzocaine, phenytoin, sevoflurane, and isoflurane (33–36, 44). These studies indicated that the aforementioned compounds can enter the pore via the lateral fenestrations or through the activation gate, and they highlight potential binding sites inside the activation gate and at the internal mouth of the fenestrations. However, most of these studies have used unbiased simulations, which may oversample binding poses of the drug in metastable states. In addition, these same studies have only examined the behavior of at most two neutral compounds in the pore, making it difficult to determine the common mechanisms of action of this broad class of compounds and to examine the differences between the charged and neutral forms.

Here, we have used MD simulations to better understand the binding of a range of known pore-blocking compounds to sodium channels. The compounds studied include three perpetually neutral compounds (benzocaine, lamotrigine, and carbamazepine) and three protonatable compounds in both neutral and charged states (PF-5215786, PF-6305591, and lidocaine). By studying a range of compounds, we are able to not only appreciate the com- mon aspects of binding, we can tease out differences between protonatable and neutral compounds, as well as tonic and use-dependent blockers. Furthermore, to overcome the limitations in sampling the preferred locations of compounds in the pore, we used the approach of the replica exchange with solute scaling/tempering method (REST2) (45, 46). Using MD and REST2, we were able to (*i*) determine the ability of these compounds to enter and cross the bilayer, (*ii*) greatly increase sampling of the compounds inside the pore compared with unbiased simulations, and (*iii*) uncover distinct modes of binding for neutral and charged compounds. While they shared some interactions with the protein, charged compounds effectively blocked the pore by extending into the base of the selectivity filter, whereas neutral compounds resided lower in the central cavity, both in prokaryotic and eukaryotic channels. These findings support the hypothesis that the protonatable amine group is common to many Nav inhibitors, because it allows the compounds to lose their charge before they traverse the bilayer, only to regain the charge in the cytoplasm to more effectively bind to and block the channel via interactions with the selectivity filter.

Results

Parameterization and Validation of Compounds. Nine compounds were initially modelled and parameterized for use in MD simulations [Fig. 2; structures are shown along with their corresponding potentials of mean force (PMFs)]. The validity of the parameters for each compound was tested by calculating the water/octanol and water/cyclohexane partition coefficients in simulations (logP) and comparing them to experimental values. Although experimental data were not available for all compounds (Table S1), the simulations accurately reproduced the trends seen in existing values for the experimental logPs. The main exception to this was lidocaine, for which we overestimated the water/octanol partition coefficient. However, we only predicted the value for the neutral species of lidocaine, due to difficulties in running free energy profile (FEP) calculations for non-charge-conserving processes, whereas the experimental value would include a contribution from the charged form of the molecule. This trend was expected to extend to PF-5215786 and PF-6305591, as their partitioning coefficients would also have contributions from both charged and neutral species. In addition to partitioning coefficients, we determined the PMF of each compound traversing a

dipalmitoylphosphatidylcholine (DPPC) lipid bilayer to determine how the newly parameterized drugs would compare with previous studies of drug–lipid interactions and to gain new information about trends in sodium channel inhibitors. All compounds in this study (Fig. 2), irrespective of charge, had an energy minima between 1 and 1.5 nm away from the center of the bilayer. This position corresponded to the drug partitioning into the membrane, just below the lipid headgroups. In addition, each compound then faced an energy barrier when crossing the hydrophobic portion of the membrane and was in excellent agreement with simulation data for previously simulated compounds (35, 38–43). These PMFs showed that all of these compounds will partition into the membrane; however, both the proportion of each compound that enters the bilayer rather than remaining in the aqueous phase, and the rate at which they will cross the bilayer differed. While you may expect that neutral compounds will more readily partition into the bilayer, there was no clear trend in the depth of the energy minima presented in Fig. 2. The perpetually neutral compounds benzocaine, lamotrigine, and carbamazepine all had relatively small minima in the range of ~2–4 kcal/mol, similar in depth to most of the charged versions of the protonatable compounds. Lamotrigine had a larger energy barrier to pass through the bilayer center than either carbamazepine or benzocaine, but this was not entirely surprising, given the presence of polar groups on this molecule. The neutral protonatable compounds, surprisingly, had deeper wells (in the range of ~6–8 kcal/mol) than the perpetually neutral compounds, with lower energies as they inserted themselves into the hydrophobic core of the bilayer. In general, our results reflected the expected trend that larger and more polar compounds will have slower bilayer crossing rates. All of the charged protonatable compounds, however, had very large energy barriers to cross the bilayer in the charged form, which was significantly reduced when in the neutral form. This strongly suggests that the protonatable molecules are more likely to permeate the bilayer core in the neutral form.

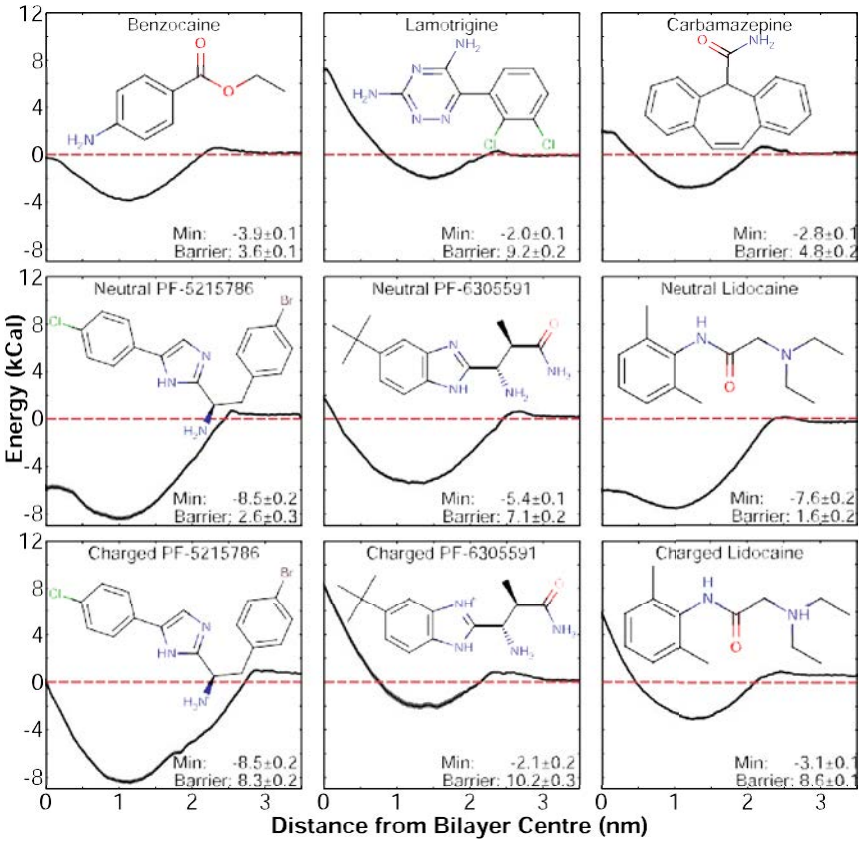

Fig. 2. PMF for lipid partitioning of the compounds used in this study, along with their corresponding molecular structure. The dashed red line is at an energy of 0 kcal/mol. The value of the energy minima (Min) and the barrier to cross the bilayer are indicated.

Understanding Anesthetics Behavior in the Pore.

Increased sampling of benzocaine inside pore. To best study the likely binding sites of local anesthetics in the sodium channel, we used REST2 (45) as implemented in NAMD (46). Replica- exchange methods have been shown to more efficiently improve conformational sampling than alternative methods such as meta-dynamics or accelerated MD (34, 46, 47), and the solute tempering method allows the approach to be used efficiently in large systems. To show that replica exchange solute tempering (REST) simulations indeed increase sampling of a compound in the pore, we chose a test system that has been well-characterized in simulation: the structure of NavAb [Protein Data Bank (PDB) ID code 3rvy] with benzocaine inside the pore (34–36), embedded in a pure 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) bilayer. This system was simulated for 100 ns by using both unbiased and REST simulations. To measure sampling efficiency, and indirectly convergence, we chose to track the conformational sampling of the drug as measured by the physical volume visited by the center of mass of benzocaine. As seen in *SI Appendix*, Fig. S1*A*, the volume visited by the center of mass of benzocaine increased rapidly at the beginning of the REST simulations (purple line), compared with the unbiased simulation (orange line), indicative of more rapid sampling. In addition, the volume asymptoted toward a much lower value in the unbiased simulations than with REST, indicating that it is unlikely that even an extremely long unbiased simulation would achieve the same conformational sampling obtained by using REST. Energy landscapes obtained from both the unbiased and REST simulations (*SI Appendix*, Fig. S1 *B* and *C*, respectively) supported the conclusions from the volume sampling. The energy landscape from the unbiased simulation showed that benzocaine only sampled the known binding site near the fenestrations, and did not explore the cytoplasmic activation gate where benzocaine is also known to bind. However, when using REST, both the known binding sites were sampled. In addition, when cluster analysis was done on both the unbiased and REST simulation (*SI Appendix*, Fig. S1 *D* and *E*, respectively), the four most populous clusters associated with the unbiased simulations were all close together and near the same binding site. In the REST simulations, these clusters represented both the activation gate and fenestration binding sites, agreeing well with previous simulations on NavAb with benzocaine (34, 36). All these results indicated that REST not only increased sampling in the pore, it also identified binding modes that may not be seen in unbiased simulations.

Simulations determine two distinct binding modes of PF-5215786 with NavMs. To gain a direct comparison between the simulation results and experimental data, a system was constructed with NavMs (PDB ID code 4p9o) embedded in POPC, with the brominated compound PF-5215786 inside the pore. The structure of NavMs with this compound bound has previously been solved, and the electron density of the bromine atom is visible near the internal opening of the fenestrations (16). When both the neutral and charged states of PF-5215786 were simulated with NavMs, different energy landscapes were produced (Fig. 3 *A* and *B*; neutral and charged respectively), indicative of distinct binding modes of the neutral and charged versions. While both neutral and charged PF-5215786 bound in the central cavity, the free energy surfaces showed that the neutral version of the molecule bound lower in the cavity ($z \sim 4$ Å) and away from the central axis ($r \sim 3$ Å) (Fig. 3*A*). In contrast, the charged version sat higher in the pore ($z \sim 6$ Å) with its center of mass close to the pore axis ($r \sim 0$ Å) (Fig. 3*B*). This difference in the binding modes is visualized in Fig. 3 *C* and *D*, by looking at the most populous binding pose. Neutral PF-5215786 preferred to bind close to helix S6, where it interacted with residues on both the S6 helix (T207 and L211; Fig. 3*E*) and at the bottom of the selectivity filter (T176 and L177; Fig. 3*E*). In this position, the amine (blue, Fig. 3*E*) pointed into a lateral fenestration. Charged PF-5215786, in contrast, spanned the width of the pore (Fig. 3*D*), with the halide (colored in brown and green for Br and Cl, respectively) on either end of the molecule inserting itself into the entrance of a fenestration. The protonated amine (Fig. 3*D*, blue) pointed toward the negatively charged selectivity filter, preferentially interacting with T176 and L177 (Fig. 3*F*),

while the other parts of charged PF-5215786 were interacting with T207 and L211. As a final indication of the different binding modes of the neutral and charged compounds, we plotted the average interaction energy of each compound with each protein residue in *SI Appendix*, Fig. S2. While the neutral compound had a fairly strong interaction for the pore chamber, the charged compound had stronger interactions, especially with residues in the selectivity filter. In short, while both the neutral and charged compounds interacted with residues on S6 at positions found to be important in eukaryotic channels (30), the charged compounds had additional interactions with the selectivity filter, which was likely to result in stronger binding.

To compare our predicted binding poses with the crystallographic structure containing resolved bromines [PDB ID code 4p9o (16)], we aligned representative structures from the most populous binding poses with this crystal structure. As seen in Fig. 4 *A* and *C*, the bromine atom of neutral PF-5215786 did not overlap with any of the crystallographic bromines. Instead, the amine group was closer in position to the crystallographic bromines than either the bromine or chlorine atom on either end of the molecule. In contrast, the bromine and chlorine atoms on charged PF-5215786's were in close proximity to the crystallographic bromine (Fig 4 *B* and *D*). The same pattern of binding for both protonation states was seen for all of the 10 most populated clusters, as demonstrated in *SI Appendix*, Figs. S3*E* and S4*F*, in which we plotted the positions of the bromine atom for all clusters. For neutral PF-5215786, there was very little over- lap in the positions of either halide with the crystallographic bromine (*SI Appendix*, Fig. S5 *A* and *C*). In the case of charged PF-5215786, the bromine atom often was near or overlapped with one of the bromine positions seen in the crystal structure (*SI Appendix*, Fig. S5 *B* and *D*). In addition, the chlorine atom sat near to another crystallographic bromine position, either in the opposite or adjacent fenestration, suggesting that the chlorine atoms could be contributing to the halide density seen in the crystal structures. This observation was supported by the occupancy of both the bromine and chlorine atoms for each cluster, as shown in *SI Appendix*, Fig. S5. The closer agreement between both the bromine and chloride position in our simulations of the charged compound with those in the crystal structure, as opposed to the neutral compound, strongly suggests that the compound Bagneris et al. (16) cocrystallized with the pore of NavMs was in the charged state.

Our simulations also suggested that the presence of the inhibitor in the pore alters the position and number of Na+ in the selectivity filter. The charged form of the compound partially inserted itself into the selectivity filter via the protonated amine group, occluding the pore and influencing ion permeation. To highlight this point in Fig. 5*E*, we showed that in an unbiased simulation without the compound present, the number of Na+ ions inside the filter fluctuated between one, two, and three. The average Na+ density was spread throughout the filter (Fig. 5*A*), in agreement with previous simulations of the channel (48–50). When the neutral PF-5215786 was present, it lowered the number of sodium ions in the filter to between one and two (Fig. 5*E*), both in the whole simulation and in the most populous binding pose. Despite this, the distribution of the sodium density in the selectivity filter was similar to the apo form (Fig. 5*B*). In contrast, for charged PF-5215786, there was a marked decrease in the number of Na+ ions present in the filter, with the protonated amine effectively replacing a sodium ion. This reduced the most frequently occurring number of ions in the pore to one (Fig. 5 *C* and *D*) and significantly altered the distribution of Na+ in the filter for both the whole simulation and for the most populous cluster (Fig. 5*E*). This observation correlated with the lack of electron density seen in the selectivity filter between apo and PF-5215786 crystals, indicating that something was occluding the selectivity filter (16). Bagneris et al. (16) used molecular docking to predict a binding pose of PF-5215786 in which the bromine atom overlapped the crystallographic bromine and the chlorine atom pointed into the selectivity filter, where it was proposed to alter the ion densities and occlude the pore. We did not see this binding pose in any of our simulations, and think it unlikely that the electronegative Cl− atom would either be attracted to the filter (given

repulsive electrostatic interactions) or displace resident Na+ (given the attractive electrostatic interactions). We suggest that the lack of density seen in the selectivity filter in the crystal structure was not caused by the chlorine-containing end of PF-5215786 blocking the filter, but rather by the protonated amine group in the middle of the molecule interacting with the selectivity filter residues.

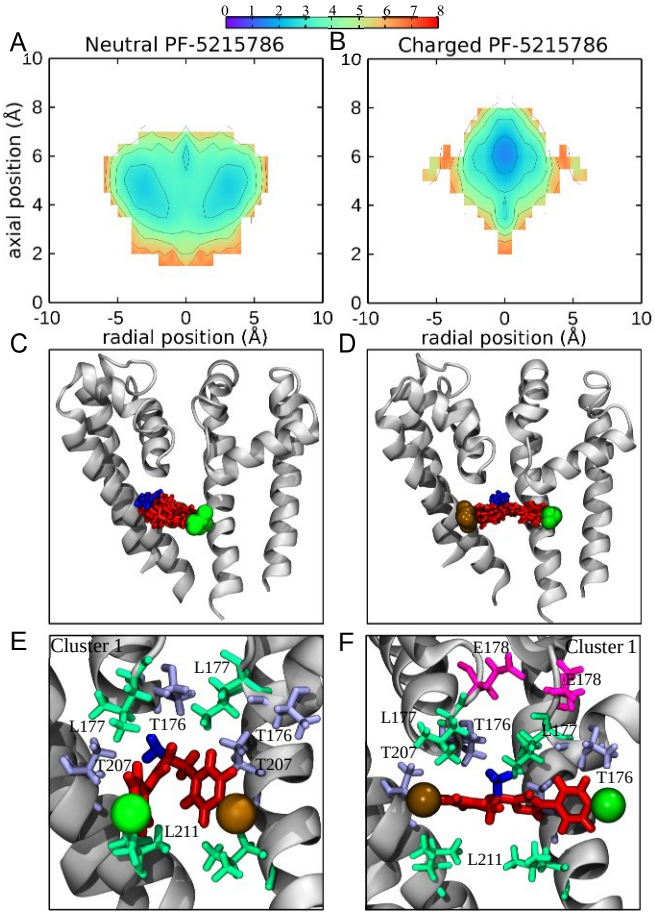

Fig. 3. Binding of PF-5215789 to the NavMs pore. (*A* and *B*) Energy land- scapes (in kcal/mol) of the centers of mass of neutral PF-5215786 (*A*) and charged PF-5215786 (*B*) inside the channel. (*C* and *D*) Snapshots from the most populated clusters for neutral PF-5215786 (*C*) and charged PF-5215786 (*D*) are shown, with the channel in gray cartoon and the compound in red licorice. (*E* and *F*) A representative frame from the cluster shown in *C* and *D* of both neutral PF-5215786 (*E*) and charged PF-5215786 (*F*) is shown as red licorice, with the bromine atom shown in a brown sphere, the chlorine atom shown as a green sphere, and the protonatable amine highlighted in blue. The channel is shown in gray cartoon, and surrounding residues (T176, L177, E178, T207, and L211) are shown in licorice, with the threonines shown in light blue, the leucines in green, and the glutamatic acid in pink.

Binding of other protonatable compounds. To see if the different binding modes of the neutral and charged forms of PF-5215786 are also seen for other compounds, two more compounds with a protonatable amine group (lidocaine and PF-6305591) were simulated by using REST. Lidocaine is a commonly used local anesthetic, and PF-6305591 is a newer compound reported to display selectivity for Nav1.8 and suspected to also bind inside the pore. Both lidocaine and PF-6305591 have a protonatable amine group, similar to PF-5215786, and both displayed similar behaviors as PF-5215786 in our simulations. The energy landscapes of the neutral states of all protonatable compounds (*SI Appendix*, Fig. S6 *A*, *C*, and *E*) displayed preferred binding modes lower in the pore than the charged states. For the charged states (*SI Appendix*, Fig. S6 *B*, *D*, and *F*), all three had multiple binding positions, but included one at the base of the selectivity filter ($z \sim 8$ Å), as seen for charged PF-5215786. When these different binding modes were compared between compounds, the neutral compounds preferred to bind in approximately

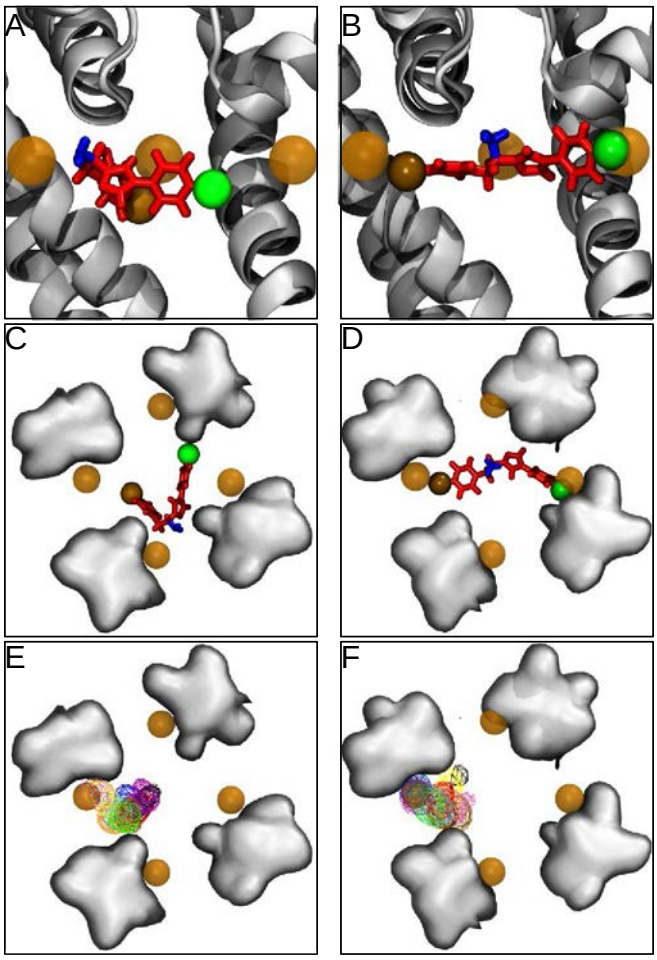

Fig. 4. Binding modes of neutral and charged PF-5215786 compared with the crystal structure. (*A*–*D*) Side views of a representative snapshot from the most populous clusters of neutral PF-5215786 (*A*) and charged PF-5215786 (*B*), along with top views of neutral PF-5215786 (*C*) and charged PF-5215786 (*D*), when aligned with PDB ID code 4p9o. PF-5215786 is shown in red licorice, with the bromine atom as a solid brown sphere, the chlorine atom as a solid green sphere, and the protonatable amine group as blue licorice. NavMs is shown in cartoon (light gray for the simulation structure, dark gray for the crystal structure). Bromine atoms from the crystal structure are shown in brown transparent spheres. (*E* and *F*) Average occupancies of the bromine atoms for each cluster is shown for the neutral (*E*) and charged PF-5215786 (*F*), shown in wire mesh and colored according to cluster. For *C*–*F*, the positions of the S6 helices are shown by the gray surface, with the fenestrations occupying the space between these surfaces.

the same place in the pore (Fig. 6*A*) and occluded the pore, although a relatively large density of sodium ions was still present in the selectivity filter. In contrast, some clusters in the charged compounds were seen entering the selectivity filter site, with a noticeably smaller density of sodium ions in the selectivity filter (Fig. 6*B*). A plot of the average interaction energy of each compound with each protein residue also highlighted the difference between the neutral and charged compounds (Fig. 6 *C* and *D*). While both compounds interacted with S6 and the selectivity filter, the strength of inter- action with the filter was much larger for the charged compounds than the neutral ones. As seen in *SI Appendix*, Fig. S8, while the neutral forms of each compound had a small influence on the number of Na+ in the filter, the effect was much greater for all of the charged compounds. In the latter case, the charged protonated portion of the compound effectively replaced a sodium ion in the filter. Overall, these data reinforce that there are distinct binding modes for neutral and charged compounds, with the latter interacting more strongly with the selectivity filter.

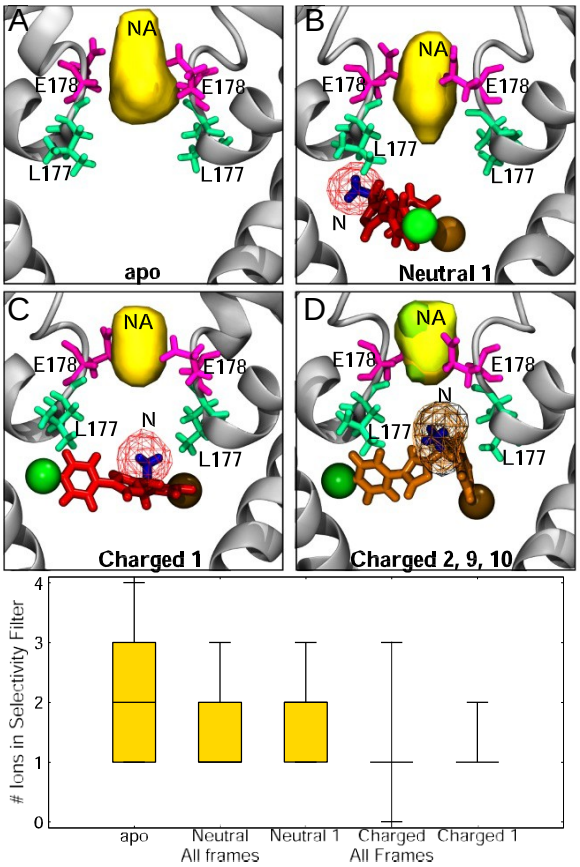

Fig. 5. Drug binding influences Na^+ in the channel. (*A*–*D*) Densities of Na^+ (yellow) and the protonatable amine (mesh) are shown in both the absence (*A*) and presence (*B*) of neutral PF-5215786 and charged PF-5215786 cluster 1 and 2, 9, and 10 (*D*). The protein is shown in gray cartoon, L177 is shown in green licorice, and E178 is shown in pink licorice. (*E*) The average number of sodium ions present in the selectivity filter are shown for simulations containing no drug (apo) as well as neutral and charged compounds using box-and-whiskers plots. Results are shown for all frames of each compound as well as for just those in the most populous cluster.

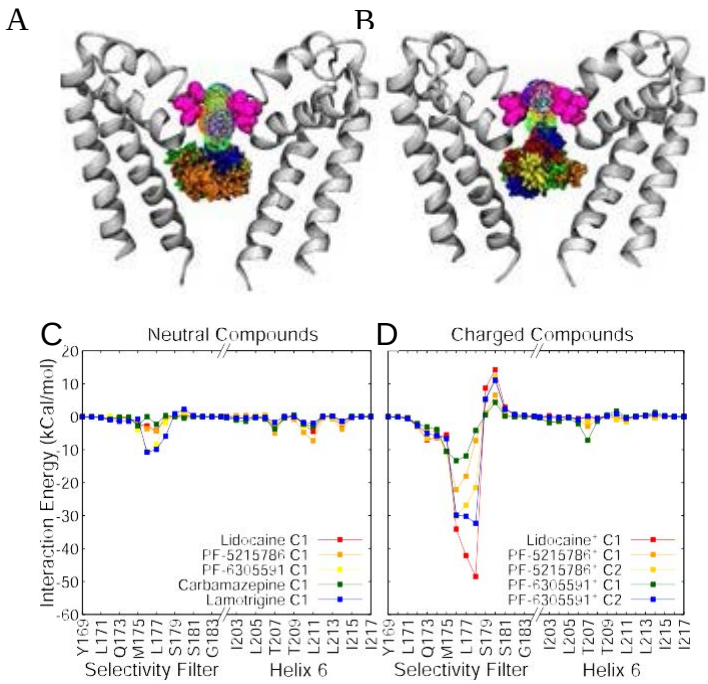

Fig. 6. General behavior of neutral and charged anesthetics. (*A*) Most populous clusters of neutral lidocaine (red licorice), neutral PF-5215786 (orange licorice), neutral PF-6305591 (yellow licorice), carbamazepine (green licorice), and lamotrigine (blue licorice). (*B*) Clusters of charged lidocaine (red licorice), charged PF-5215786 (orange licorice), and charged PF- 6305591 (yellow licorice). For both structures, the channel is shown in silver cartoon, and residue E178 is shown in pink spheres for reference. (*C* and *D*) Drug–protein interaction energies of neutral (*C*) and charged (*D*) com- pounds. For both *C* and *D*, energies are in kcal/mol, and each cluster is shown as a separate line, and only residues from the selectivity filter (left half of each graph) and the S6 helix (right half of each graph) are shown.

Perpetually neutral drugs. As a comparison with the protonatable compounds, two drugs which are always in a neutral state were simulated with NavMs by using REST: carbamazepine and lamotrigine. Both had a relatively similar energy landscape to the neutral protonatable compounds (protonatable: *SI Appendix*, Fig. S6; and carbamazepine/lamotrigine: *SI Appendix*, Fig. S9 *A* and *B*), in that they had preferred binding modes at a *z* coordinate between 4 and 8 Å. However, lamotrigine was more likely to venture toward the selectivity filter than carbamazepine. This could also be seen by snapshots of the most likely positions of each compound (*SI Appendix,* Fig. S9 *C* and *D*). Carbamazepine appeared to have a preference of binding to helix 6, but the most populous cluster of lamotrigine sat higher, with the nitrogen- containing moieties pointing toward the selectivity filter. Despite these differences, both compounds interacted with a similar set of protein residues: M175, T176, L177, 207T, and L211 (*SI Appendix,* Fig. S9 *E* and *F* and Fig. S12). These are the same residues that the protonatable compounds in this study prefer to bind to in their neutral state, and compounds that are perpetually neutral that have been previously studied (benzocaine and lidocaine).

Extension to eukaryotic channels. Although we have identified distinct binding sites for a range of neutral and charged com- pounds in bacterial channels, it is not obvious whether these same sites will be present in eukaryotic channels. In particular, the amino acid sequence of the selectivity filter is distinctly different in prokaryotic and eukaryotic sodium channels, so it is important to confirm if charged molecules can interact with the filter of eukaryotic channels in the same way we found for the bacterial channels. To determine this, we ran REST simulation of both protonation states of lidocaine inside the pore region of the most recent structure of a sodium channel from the American cockroach, denoted NavPas (23). When inside the pore, the neutral form of lidocaine did not interact with the selectivity filter of NavPas and preferred to interact lower in the pore with the S6 helix of DIII and DIV (Fig. 7*A*), in a relatively similar position to the one seen in the bacterial channels. However, the charged form of lidocaine preferentially interacted with the selectivity filter of NavPas, specifically the aspartic acid residue in the "DEKA" ring (Fig. 7*B*). The difference in the binding pose of the neutral and charged forms of lidocaine was highlighted by the fact that there was a large interaction energy between the charged version of lidocaine and the aspartic acid residue, whereas there was virtually no interaction when the neutral state of lidocaine was in the pore (Fig. 7 *C* and *D*). This was a remarkable result, as charged lidocaine displayed the same binding position in NavMs, which has a glutamic acid "EEEE" ring, rather than the DEKA ring present in eukaryotic channels. Charged lidocaine's affinity for the aspartic acid residue in NavPas resulted in a binding pose where the compound blocked ion conduction by both physically occluding the filter and by electrostatic repulsion, as seen by the difference in the number of sodium ions in the selectivity filter (Fig. 7*E*) in the presence of charged lidocaine. The results from simulations with both eukaryotic and prokaryotic channels point to two different binding sites for local anesthetics, depending on their charge state (Fig. 8*A*). The neutral compounds preferred to bind in the central cavity close to helix S6 and had their primary interactions with the hydrophobic or aromatic residues in this region (neutral binding site, Fig. 8 *A*, *Left*). The charged compounds could also have the same hydrophobic interactions with S6, but preferentially sat higher in the pore, exhibiting strong interactions with the negatively charged selectivity filter residues (charged binding site, Fig. 8 *A*, *Right*). Both the neutral and charged compounds shared common interacting residues, but only the charged compounds ventured toward the selectivity filter (Fig. 8*B*).

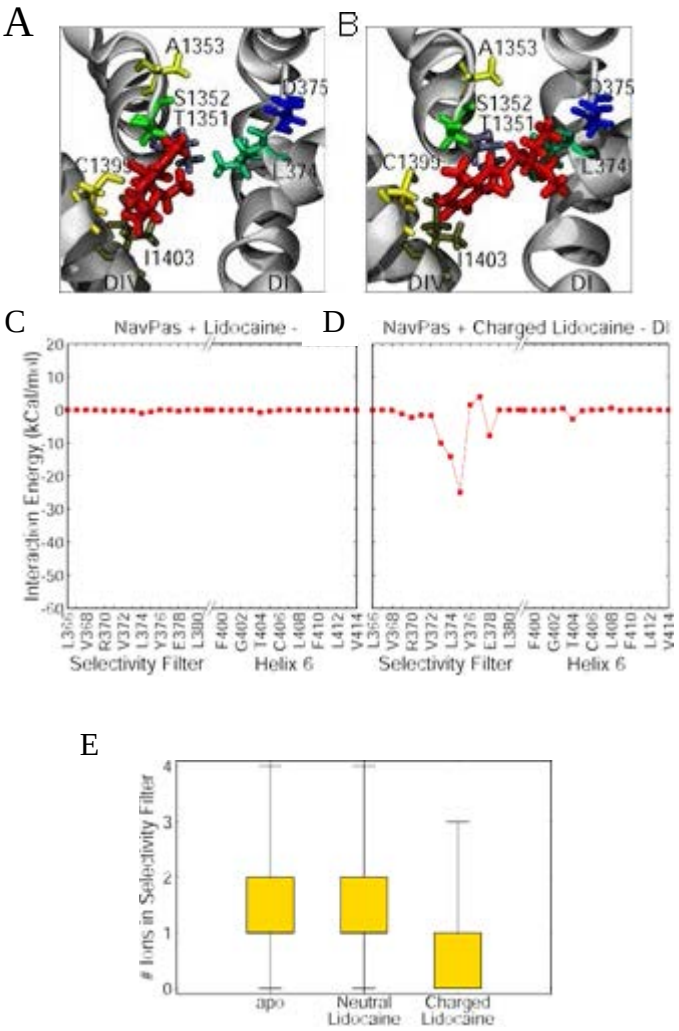

Fig. 7. Binding of lidocaine to NavPas. (*A* and *B*) Representative snapshots from the most populous cluster with neutral (*A*) and charged (*B*) lidocaine. Lidocaine is shown in red licorice, the backbone of the protein is in gray cartoon, and relevant residues are shown in licorice of varying colors. (*C* and *D*) Associated drug–protein interaction energies for neutral (*C*) and charged (D) lidocaine for DI are shown. (*E*) The number of ions present in the selectivity filter for apo, NavPas + neutral lidocaine, and NavPas + charged lidocaine REST simulations are shown as a boxplot.

Discussion

In this study, we have taken a number of steps to improve our understanding of sodium channel inhibition. First, we have rigorously parameterized and characterized a range of pore-blocking compounds and were successful in replicating trends seen in drug-solvent partitioning experiments. Such parameterization is essential before using these compounds in MD simulations with the protein. Second, we determined the energy profiles for partitioning of each compound from the aqueous phase into the lipid bilayer. This indicated that even the charged compounds were likely to move into the membrane from the aqueous phase, but, as expected, the neutral forms were more likely to pass across it. Third, we used an improved sampling method to determine binding positions of the compounds in the pore (REST) and demonstrated that this approach improved the sampling of benzocaine in a previously characterized sodium channel system. This knowledge allowed us to be more confident that we could find the most likely binding position for the newly simulated compounds in the pore. Finally, we characterized the binding of a range of sodium channel inhibitors inside the sodium channel pore. As an MD study investigating a large number of both neutral and charged compounds, we are able to compare our results with a large amount of previous experimental and computational data, and determine trends within our own data, to shed light

on the characteristics of different sodium channel inhibitors. Notably, we show that neutral and positively charged compounds have distinct binding modes in the pore.

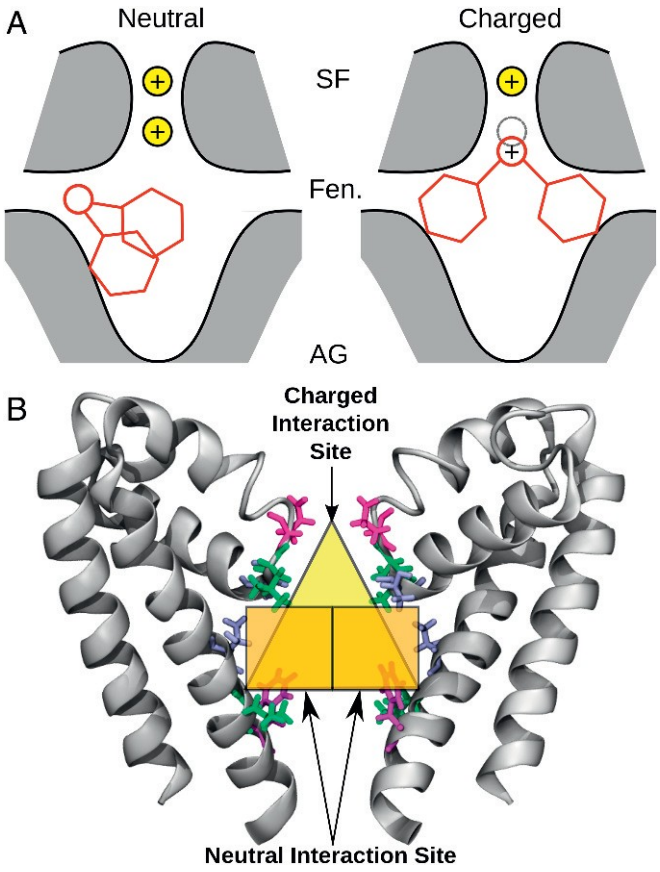

Fig. 8. (*A*) Schematic of the binding modes of neutral (*Left*) and charged (*Right*) compounds. The protein is shown in gray shading with black outline, the drug is shown in red lines, and the sodium ions are shown as yellow spheres, with a gray dotted line outlining a sodium ion site. (*B*) Neutral and charged interaction sites of local anesthetics. The sodium channel pore is shown in gray cartoon. Select residues involved in binding are shown in blue, green, and pink licorice, and each binding site is highlighted by orange (neutral site) or yellow (charged site) regions. AG, activation gate; Fen, fenestration; SF, selectivity filter.

Interactions between all inhibitors in this study and the NavMs sodium channel pore involved both polar and nonpolar interactions, with pore-lining S6 residues and residues at the base of the selectivity filter. This was mainly with key leucines and threonines (T176, L177, T207, and L211), and, in a few cases, there were hydrophobic interactions with F214. These residues were the dominating interaction partners of both the neutral states of PF-5215786, PF-6305591, and lidocaine, as well as the perpetually neutral compounds, carbamazepine and lamotrigine. All of the neutral compounds remained in the central pore chamber, interacting primarily with residues on helix S6. Only benzocaine entered the fenestrations, and none of the compounds ventured into the selectivity filter. Carbamazepine and lamotrigine had more significant interactions with the selectivity filter, with some clusters having their amine groups interacting with T176 and L177. This behavior was also seen in a docking study (51) in the presence of a sodium ion in the filter. This could be because the amines in carbamazepine and lamotrigine made them more strongly attracted to the positive charge of the sodium ion, encouraging the amines to point up toward the selectivity filter. The interaction of carbamazepine and lamotrigine with this sodium ion would prevent sodium currents, with the ion playing a similar role to the protonatable amine of the charged compounds.

In contrast to the neutral compounds, the protonated states of PF-5215786, PF-6305591, and lidocaine had much stronger inter- actions with residues in the selectivity filter. This was clearly seen in the most populated poses of PF-5215786. In these, the compound spanned the width of the pore, with the halides on the ends of the molecule occupying either opposite or adjacent fenestrations, and the protonated amine in the center of the pore pointed up toward the selectivity filter. These positively charged amines effectively replaced a sodium ion at the bottom of the filter and formed strong interactions with residues T176 and L177. The compound thus prevented a positive sodium ion from passing through the filter by both physical occlusion and electrostatics. The binding position seen for this compound agreed with the position of the bromine atoms seen in the crystal structure in which PF-5215786 was co-crystallized with NavMs. However, the specific binding pose predicted by Bagneris et al. (16), in which the chlorine occludes the selectivity filter, was not seen in any of the frames of our simulation. As chlorine is electronegative, we think it is unlikely that chlorine would be attracted to the negatively charged selectivity filter. It is more likely that PF-5215786 spans the pore with both halides occupying fenestrations, providing density for the halide seen in the crystal structure, while the positively charged protonated amine points toward the selectivity filter, altering the sodium ion density.

Similar binding poses were seen for the other protonated compounds, charged PF-6305591 and charged lidocaine, as described above for PF-5215786. For all these compounds, the protonated amine formed strong interactions with residues in the selectivity filter: T176, L177, and E178. In addition to this, all these compounds interacted strongly with T207 on the S6 helix, one of the key interaction partners for the neutral compounds. As suggested in a recent docking study, it is possible that charged compounds form aromatic interactions in the center of the pore while extending their positively charged ends into the selectivity filter (51). The other residues that were implicated in binding neutral compounds (L211 and F214) did not have such significant interactions when the compounds were charged. Interestingly, T207 in NavMs corresponds to one of two residues that were seen as being important for tonic and use-dependent block in a rat sodium channel: F1764 (30, 31). Taking into account this present work, and Ragsdale's previous study (31), this residue at this particular region could act as a scaffold and/or major interacting partner for the compounds in this study, and this residue could be key in understanding sodium channel block. The other residue highlighted by Ragsdale et al. (31), as important for use- dependent block, Y1771 (corresponding to F214 in NavMs), was not found as a major interacting partner in this study. Given that we have not used the structure of an inactivated channel, it could be that this residue becomes available due to conformational changes happening as the channel changes from the resting or open state studied here, to the inactivated state which is the principle target of use-dependent blockers. Indeed, the limited interactions of the compounds studied here with F214 agree with the observation of Ragsdale et al. (30, 31) that mutations of this residue had little influence on tonic block.

Our study used the crystal structures of two bacterial channels (NavAb and NavMs) and one eukaryotic channel (NavPas). While previous studies have indicated that binding of compounds to NavMs (16) and NavAb (52) can reflect binding in eukaryotic channels, there is potential for significant differences to arise. Remarkably, in light of potential differences, lidocaine bound in a similar fashion in both prokaryotic (NavMs) and eukaryotic (NavPas) channels. The neutral state of lidocaine bound lower in the pore and interacted with polar residues, while the charged state used these polar residues as a scaffold to position the charged end of lidocaine next to the aspartic acid residue in the selectivity filter of NavPas, which occluded the pore. Since lidocaine behaved the same way in both prokaryotic and eukaryotic channels, it is likely that the other two protonatable compounds (PF-5215786 and PF-6305591) would exhibit similar behavior when simulated with eukaryotic channels. As charge seems to be important in determining the interaction sites within the pore, it is important to consider whether the compounds will be protonated while in the pore. At physiological pH, all three protonatable compounds studied here will largely be in their protonated state when they are in the aqueous phase. While our lipid-partitioning data indicated that these compounds are much more likely to cross the bilayer in the neutral state, at physiological pH,

we can expect a majority of each compound to return to the charge state in the cytoplasm. If these compounds enter through the open activation gate as suspected, then we would expect that they can enter and bind while protonated. This claim is supported by the close agreement of the halide positions with those observed in a recent crystal structure for the charged state (16) of the charged state of PF-5215786, but not the neutral state. The pKa of the compounds may change inside the pore, but the net negative charge on the pore walls used to attract Na+ into the channel may make it more likely for the compounds to protonate in this environment.

While this study has helped to highlight two distinct binding positions for charged and neutral pore blockers, there are a number of questions it cannot address. First, while the crystal structure of NavMs used in this study is claimed to be in the open state, in the absence of the C terminus (not resolved in the crystal structure), the S6 helices rapidly closed the pore during our simulations. Although it is difficult to be sure exactly what functional state this represents, it is unlikely to be the inactivated state to which most of the compounds studied bind most strongly. As such, we believe that these binding positions most likely represent tonic-like binding, something backed up by the lack of inter- action with F214. Further studies will be required to ascertain the differences in binding of these compounds to the inactivated state. Second, this study only hints at why most of the compounds are use-dependent rather than tonic blockers. Benzocaine, the primary tonic blocker examined here, was the only compound in our study seen to enter the fenestrations. Thus, it is likely that it was simply the small size of benzocaine that allowed it to pass through the fenestrations to block the resting state of the channel. Previous work has concluded that compounds with the maximum width of a benzene ring (i.e., benzocaine) are able to fit through the fenestrations (49, 53). Apart from benzocaine, all of the drugs studied here are wider than the width of a benzene ring, making it unlikely that the compounds would pass through the fenestrations on their way to block the pore. Finally, future work could use the NavPas structure as a basis for studying human sodium channels, to further understand the interactions of pore- blocking compounds with eukaryotic sodium channels and open the door for more targeted sodium channel therapies.

Materials and Methods

Parameterization of Compounds and Validation. Parameterization of the drug models was performed with the aid of Force Field Toolkit (FFTK)(54) implemented in VMD (55). FFTK does not support the parameterization of Lennard–Jones parameters and the Urey–Bradley term, and these parameters were obtained by analogy from the CGenFF program (https://cgenff.paramchem.org/) (56, 57).

The Gaussian09 package was used for all quantum mechanical (QM) calculations. Force-field parameter optimizations were done after the molecular geometries of drugs were optimized at the MP2/6-31G∗ (for drugs with ≤40 atoms) or B3LYP/6-31G** level of theory (for drugs with >40 atoms). Atomic charges were assigned based on the minimum interaction energy and distance between one water and all hydrogen-bond donors and acceptors, optimized at the HF/6-31G* level of theory. Of note, aliphatic and aromatic nonpolar hydrogens in CHARMM have predetermined charges of 0.09 and 0.15, while aliphatic and aromatic carbons not adjacent to a heteroatom are assigned -0.18 (or -0.27 for terminal CH3 group) and −0.15 respectively (58). Equilibrium bonds and angle values were optimized through comparison with the QM-optimized drug geometries, while the force constants were derived from the Hessian calculated in Gaussian. The optimizations of dihedral parameters were done by using a simulated annealing protocol developed by Guvench and MacKerell (59) and implemented in FFTK.

To validate the parameters for each drug, the octanol–water and cyclohexane–water partitioning free energies for neutral drugs were determined by using the Bennett acceptance ratio approach (60), as implemented in GROMACS4.5.4 (61), and compared with available experimental logP values. The

simulations were carried out at a constant isotropic pressure of 1 atm and a temperature of 300K. A series of 51 windows with the λ equally spaced between 0 and 1 were constructed for both water and octanol or cyclohexane phases. Each window was simulated for 1 ns, and the first 0.2-ns simulation was discarded for equilibration. A soft core potential with α = 1, σ = 0.3 nm and λ-power of 1 was used to avoid singularities. Results presented used the CHARMM general force field (58) for the octanol and cyclohexane partitioning simulations. The TIP3P model (62) was used for water. Further details are given in *SI Appendix*.

Bilayer Partitioning. The method of umbrella sampling was used to deter- mine the PMF to move the compounds from bulk aqueous solution into the interior of a DPPC bilayer. Starting configurations for umbrella sampling were generated by using steered MD. The drug was pulled along the *z* axis (perpendicular to the bilayer/water interface) at a rate of 0.01 nm/ps and with a force constant of 1,000 kJ/mol·nm^{-2}. Starting points for umbrella sampling were selected every 0.1 nm along the *z* axis. In each umbrella sampling window, we carried out 50-ns umbrella sampling simulations. During umbrella sampling simulations (63), a biased harmonic potential with a force constant of 3,000 kJ/mol·nm^{-2} was used to confine the drug within the sampling window. The FEPs and error estimates were determined by using the weighted histogram analysis method (64). The first 20 ns within each window was discarded to allow for equilibration at the new solute position, and the FEP was determined from the remaining 30 ns. Further details of the simulation methods are described in *SI Appendix*.

Pore and Drug Simulations. NavAb (PDB ID code 3RVY), NavMs (PDB ID code 4P9O), and NavPas (PDB ID code 5XOM) proteins were embedded into pure POPC bilayers, with explicit TIP3P solvent and 150 mM NaCl. One drug molecule was randomly placed at the center of the pore, and constraints were placed on the protein backbone with a force constant of 0.1 kcal/mol·Å^{-2} to prevent excessive conformational changes in the high-temperature REST simulations. The compound was prevented from exiting the pore by a spherical potential with a radius of 37 Å from the pore center, and force constant of 20 kcal/mol·Å^{-2}. For the REST2 (46) simulations, the patch was compiled with NAMD2.10 (65). A total of 21 parallel simulations for NavMs (25 for NavPas) were run in an NPT ensemble, with 21 and 25 being the number of replicas predicted by a temperature generator for REMD simulations (66) (folding.bmc.uu.se/remd/). Only the protein and the drug were used as the "hot" parts of the system, while the lipids, waters, and ions were used as the "cold" parts of the system, with a temperature range from 310 to 410 K. Exchanges were attempted every 2 ps, yielding an average swap acceptance ratio of ∼0.4. All other simulation details are described in detail in *SI Appendix*. Cluster analysis was performed to identify distinct binding poses and to select representative snapshots. This was done by using the measure cluster tool in VMD (55), with 10 clusters and a 2.5-Å cutoff used for all compounds in the study. Energy landscape analysis and volume sampling were performed with locally written scripts.

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References:

1. Meisler MH, Kearney JA (2005) Sodium channel mutations in epilepsy and other neurological disorders. J Clin Invest 115:2010–2017.

2. Roden DM, George AL (1996) The cardiac ion channels: Relevance to management of arrhythmias. Annu Rev Med 47:135–148.

3. Ruan Y, Liu N, Priori SG (2009) Sodium channel mutations and arrhythmias. Nat Rev Cardiol 6:337–348.

4. Waxman SG, Dib-Hajj S, Cummins TR, Black JA (1999) Sodium channels and pain. Proc Natl Acad Sci USA 96:7635–7639.

5. Waxman SG, Hains BC (2006) Fire and phantoms after spinal cord injury: Na+ channels and central pain. Trends Neurosci 29:207–215.

6. Catterall WA (2000) From ionic currents to molecular mechanisms: The structure and function of voltage-gated sodium channels. Neuron 26:13–25.

7. Fozzard HA, Sheets MF, Hanck DA (2011) The sodium channel as a target for local anesthetic drugs. Front Pharmacol 2:1–6.

8. Jarvis MF, et al. (2007) A-803467, a potent and selective Nav1.8 sodium channel blocker, attenuates neuropathic and inflammatory pain in the rat. Proc Natl Acad Sci USA 104:8520–8525.

9. Kort ME, et al. (2010) Subtype-selective Nav1.8 sodium channel blockers: Identification of potent, orally active nicotinamide derivatives. Bioorg amd Med Chem Lett 20:6812–6815.

10. Scanio MJC, et al. (2010) Discovery and biological evaluation of potent, selective, orally bioavailable, pyrazine-based blockers of the Nav1.8 sodium channel with efficacy in a model of neuropathic pain. Bioorg Med Chem Lett 18:7816–7825.

11. McCormack K, et al. (2013) Voltage sensor interaction site for selective small molecule inhibitors of voltage-gated sodium channels. Proc Natl Acad Sci USA 110:E2724– E2732.

12. Corry BA (2017) Physical basis of specificity and delayed binding of a subtype selective sodium channel inhibitor. Sci Rep 8:1356.

13. Payandeh J, Scheuer T, Zheng N, Catterall WA (2011) The crystal structure of a voltage-gated sodium channel. Nature 475:353–358.

14. Ahuja S, et al. (2015) Structural basis of Nav1.7 inhibition by an isoform-selective small-molecule antagonist. Science 350:aac5464.

15. Bagnéris C, et al. (2013) Role of the C-terminal domain in the structure and function of tetrameric sodium channels. Nat Commun 4:2465.

16. Bagnéris C, et al. (2014) Prokaryotic NavMs channel as a structural and functional model for eukaryotic sodium channel antagonism. Proc Natl Acad Sci USA 111:8428– 8433.

17. McCusker EC, et al. (2012) Structure of a bacterial voltage-gated sodium channel pore reveals mechanisms of opening and closing. Nat Commun 3:1102.

18. Naylor CE, et al. (2016) Molecular basis of ion permeability in a voltage-gated sodium channel. EMBO J 35:820–830.

19. Payandeh J, El-din TMG, Scheuer T, Zheng N, Catterall WA (2012) Crystal structure of a voltage-gated sodium channel in two potentially inactivated states. Nature 486:135– 139.

20. Shaya D, et al. (2014) Structure of a prokaryotic sodium channel pore reveals essential gating elements and an outer ion binding site common to eukaryotic channels. J Mol Biol 426:467–483.

21. Tsai Cj, et al. (2013) Two alternative conformations of a voltage-gated sodium channel. J Mol Biol 425:4074–4088.

22. Zhang X, et al. (2012) Crystal structure of an orthologue of the NaChBac voltage- gated sodium channel. Nature 486:130–134.

23. Shen H, et al. (2017) Structure of a eukaryotic voltage-gated sodium channel at near- atomic resolution. Science 355:1–12.

24. Hille B (1977) Local anesthetics: Hydrophilic and hydrophobic pathways for the drug- receptor reaction. J Gen Physiol 69:497–515.

25. Ahern CA, Eastwood AL, Dougherty DA, Horn R (2008) Electrostatic contributions of aromatic residues in the local anesthetic receptor of voltage-gated sodium channels. Circulations Res 102:86–94.

26. Gingrich KJ, Beardsley D, Yue DT (1993) Ultra-deep blockade of Na+ channels by a quaternary ammonium ion: Catalysis by a transition-intermediate state? J Physiol 471:319–341.

27. Kimbrough JT, Gingrich KJ (2000) Quaternary ammonium block of mutant Na+ chan- nels lacking inactivation: Features of a transition-intermediate mechanism. J Physiol 529:93–106.

28. McNulty MM, et al. (2007) Charge at the lidocaine binding site residue Phe-1759 affects permeation in human cardiac voltage-gated sodium channels. J Physiol 581:741–755.

29. Pless SA, Galpin JD, Frankel A, Ahern CA (2011) Molecular basis for clas Ib anti- arrhythmic inhibition of cardiac sodium channels. Nat Commun 2:351–359.

30. Ragsdale DS, Mcphee JC, Scheuer T, Catterall WA (1994) Molecular determinants of state-dependent block of nal channels by local anesthetics. Science 265:1724– 1728.

31. Ragsdale DS, Mcphee JC, Scheuer T, Catterall WA (1996) Common molecular determinants of local anesthetic, antiarrhythmic, and anticonvulsant block of voltage-gated Na + channels. Proc Natl Acad Sci USA 93:9270–9275.

32. Zamponi GW, Doyle DD, French RJ (1993) Fast lidocaine block of cardiac and skeletal muscle sodium channels: One site with two routes of access. Biophys J 65:80–90.

33. Raju SG, Barber AF, Lebard DN, Klein ML, Carnevale V (2013) Exploring volatile general anesthetic binding to a closed membrane-bound bacterial voltage-gated sodium channel via computation. PLoS Comput Biol 9:e1003090.

34. Martin LJ, Corry B (2014) Locating the route of entry and binding sites of benzocaine and phenytoin in a bacterial voltage gated sodium channel. PLoS Comput Biol 10:e1003688.

35. Boiteux C, et al. (2014) Local anesthetic and antiepileptic drug access and binding to a bacterial voltage-gated sodium channel. Proc Natl Acad Sci USA 111:13057– 13062.

36. Smith NE, Corry B (2016) Mutant bacterial sodium channels as models for local anesthetic block of eukaryotic proteins. Channels 10:225–237.

37. Hille B (2001) Ion Channels of Excitable Membranes (Sinauer Associates, Inc., Sunderland, MA).

38. Porasso RD, Bennett WFD, Lo JJ (2009) Study of the benzocaine transfer from aqueous solution to the interior of a biological membrane. J Phys Chem B 113:9988–9994.

39. Cascales JJL, et al. (2011) Thermodynamic study of benzocaine insertion into different lipid bilayers. J Chem Phys 135:135103.

40. Martin LJ, Chao R, Corry B (2014) Molecular dynamics simulation of the partitioning of benzocaine and phenytoin into a lipid bilayer. Biophysical Chem 185:98–107.

41. Högberg Cj, Maliniak A, Lyubartsev AP (2007) Dynamical and structural properties of charged and uncharged lidocaine in a lipid bilayer in a lipid bilayer. Biophys Chem 125:416–424.

42. Högberg CJ, Lyubartsev AP (2008) Effect of local anesthetic lidocaine on electrostatic properties of a lipid bilayer. Biophysical J 94:525–531.

43. Mojumdar EH, Lyubartsev AP (2010) Biophysical chemistry molecular dynamics simulations of local anesthetic articaine in a lipid bilayer. Biophys Chem 153:27–35.

44. Barber AF, Liang Q, Amaral C, Treptow W, Covarrubias M (2011) Molecular mapping of general anesthetic sites in a voltage-gated ion channel. Biophys J 101:1613–1622.

45. Wang L, Friesner RA, Berne BJ (2011) Replica exchange with solute scaling: A more efficient version of replica exchange with solute tempering (REST2). J Phys Chem B 115:9431–9438.

46. Jo S, Jiang W (2015) A generic implementation of replica exchange with solute tempering (REST2) algorithm in NAMD for complex biophysical simulations. Computer Phys Commun 197:304–311.

47. Walczewska-Szewc K, Deplazes E, Corry B (2015) Comparing the ability of enhanced sampling molecular dynamics methods to reproduce the behavior of fluorescent labels on proteins. J Chem Theory Comput 11:3455–3465.

48. Corry B, Thomas M (2012) Mechanism of ion permeation and selectivity in a voltage- gated sodium channel. JACS 134:1840–1846.

49. Boiteux C, Vorobyov I, Allen TW (2014) Ion conduction and conformational flexibility of a bacterial voltage-gated sodium channel. Proc Natl Acad Sci USA 111:1840–1846.

50. Furini S, Domene C (2012) On conduction in a bacterial sodium channel. PLoS Comput Biol 8:e1002476.

51. Tikhonov DB, Zhorov BS (2017) Mechanism of sodium channel block by local anaesthetics, antiarrhythmics, and anticonvulsants. J Gen Physiol 149:1–17.

52. Lee S, Goodchild SJ, Ahern CA (2012) Local anesthetic inhibition of a bacterial sodium channel. J Gen Physiol 139:507–516.

53. Kaczmarski JA, Corry B (2014) Investigating the size and dynamics of voltage-gated sodium channel fenestrations. Channels 8:264–277.

54. Mayne CG, Saam J, Schulten K, Tajkhorshid E, Gumbart JC (2014) Rapid parameterization of small molecules using the Force Field Toolkit. J Chem Theory Comput 34:1–28.

55. Humphrey W, Dalke A, Schulten K (1996) VMD: Visual molecular dynamics. J Mol Graph 14:33–38.

56. Vanommeslaeghe K, Mackerell AD (2012) Automation of the CHARMM general force field (CGenFF) I: Bond perception and atom typing. J Chem Inf Model 52:3144–3154.

57. Vanommeslaeghe K, Prabhu Raman E, MacKerell AD, Jr. (2012) Automation of the CHARMM General Force Field (CGenFF) II: Assignment of bonded parameters and partial atomic charges. J Chem Inf Model 52:3155–3168.

58. Vanommeslaeghe K, et al. (2010) CHARMM general force field (CGenFF): A force field for drug-like molecules compatible with the CHARMM all-atom additive biological force fields. J Comput Chem 31:671–690.

59. Guvench O, MacKerell AD, Jr. (2008) Automated conformation energy fitting for force-field development. J Mol Model 14:667–679.

60. Bennett CH (1976) Efficient estimation of free energy differences from Monte Carlo data. J Comput Phys 22:245–268.

61. Hess B, Kutzner C, van der Spoel D, Lindahl E (2008) GROMACS 4: Algorithms for highly efficient, load-balanced, and scalable molecular simulation. J Chem Inf Model 4:435–447.

62. Jorgensen WL, et al. (1983) Comparison of simple potential functions for simulating liquid water. J Chem Phys 79:926–935.

63. Torrie GM, Valleau JP (1977) Nonphysical sampling distributions in Monte Carlo free- energy estimation: Umbrella sampling. J Comput Phys 23:187–199.

64. Kumar S, Bouzida D, Swendsen RH, Kollman PA, Rosenberg JM (1992) The weighted histogram analysis method for free-energy calculations on biomolecules. I. The method. J Comput Chem 13:1011–1021.

65. Phillips JC, et al. (2005) Scalable molecular dynamics with NAMD. J Comp Chem 26:1781–1802.

66. Patriksson A, van der Spoel (2008) A temperature predictor for parallel tempering simulations. Phys Chem Chem Phys 10:2073–2077.

Going Small to make it Big – How Designing New Drugs at The Molecular Level can help replace Animal use in Pharmacology

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A large proportion of new disease therapeutics rely on the discovery and implementation of new drugs. Yet 19 out of 20 of promising pharmaceuticals developed by scientists end in phase II drug trial failure and are never utilised [1]; phase II clinical trials representing the transition of animal model data on the efficacy of the drug to human patients [1]. This unproductive usage of animals presents concerns for those who have ethical concerns about the sacrifice of laboratory animals that ultimately fails to deliver usable drugs [2]. The aim of, what is known broadly as, rational drug discovery is to increase the specificity and potency of potential drugs so that they have a much better chance of remaining viable through the phased testing stages. The more successful this process is the fewer animals will ultimately be used.

That is where molecular scientists and fundamental research enters the picture. I want to take you on a journey and explain how looking at most chemically fundamental processes of life are invaluable for understanding how drugs work and how we might improve their success rate in phased trials and, as a consequence, reduce the use of animals in drug testing. I will give you an understanding of what it means to be a molecular scientist and what is vital for designing novel drugs. Then I will introduce you to some of the techniques we utilise to study the proteins which are the molecular targets of the vast majority of commercial drugs.

I will give an overview of a class of proteins known as membrane transporters, and why they are amongst the most frequently targeted protein classes for current drugs and will continue to be so. I will also give a brief summary of some membrane transporters that we work on and why, including some of our previous fundamental discoveries. In particular, I will focus on several human membrane transporters for chemicals known as amino acids, which are essential for human life, and which we investigate as potential drug targets for type II diabetes and multiple carcinomas.

Lastly, I will show how some current funding from the Medical Advances Without Animals (MAWA) Trust is allowing us to implement a cutting edge technique in protein science that helps increase the specificity of drug design and reduces the usage of animals in fundamental research [3]. This technique, known as Cell-Free Synthesis, will be implemented to directly replace a widely used animal model in novel drug screening, the oocytes of the South African Clawed Frog *Xenopus laevis* [4].

1. Petsko, G.A. *Using Phase II Failures*. American Society for Biochemistry and Molecular Biology Feature Story 2010 August 2010 [cited 2018 27-5-2018]; Available from: http://www.asbmb.org/asbmbtoday/asbmbtoday_article.aspx?id=8668.
2. Akhtar, A., *The flaws and human harms of animal experimentation.* Camb Q Healthc Ethics, 2015. **24**(4): p. 407-19.
3. Ezure, T., et al., *A cell-free translocation system using extracts of cultured insect cells to yield functional membrane proteins.* PLoS One, 2014. **9**(12): p. e112874.
4. Broer, S., *Xenopus laevis Oocytes.* Methods Mol Biol, 2010. **637**: p. 295-310.

A large proportion of new therapeutics rely on the discovery and implementation of new drugs. Yet 19 out of 20 of promising pharmaceuticals developed by scientists end in phase II drug trial failure and are never utilised [1]; a far greater proportion of drugs tested in the laboratory never make it to clinical trials. The use of laboratory animals for both pre-clinical and fundamental pharmacological which underpins much drug development is widespread. This usage presents ethical concerns about the invasiveness and sacrifice of laboratory animals that may ultimately fail to deliver usable drugs [2]. One animal widely used in fundamental pharmacological research over the past 40 years is the South African Clawed frog or *Xenopus laevis* [3]. The eggs, or oocytes, of the frog have proven to be an extremely useful tool for the testing of new drugs on isolated targets (usually proteins) and for elucidating the fundamental properties of these targets – both essential prerequisites for validating pre-clinical compounds (e.g. see [4]). A simple search of the medical science database PubMed with the terms 'Xenopus' and 'oocyte' locates 22257 publication hits [5], indicating the ubiquity of their use.

The class of potential drug targets our laboratory regularly express in *Xenopus* oocytes are known as membrane transporters. Membrane proteins are amongst the most frequently targeted protein classes for current drugs and will continue to be so for the foreseeable future. This is largely because they are easily accessible on the surface of cells and are over-represented as the mediators of essential nutrient acquisition or cell signalling. Xenopus oocytes are a particularly adapted to the versatile study of membrane proteins as they possess a very large surface area, a high signal to noise ratio for experimentation, and produce large amounts of protein per egg. However, for all these benefits they have some serious limitations: they are quite expensive to import and maintain and the surgery required to gather the oocytes is invasive and can be harmful to the long-term survival of the frogs (**Figure 1**).

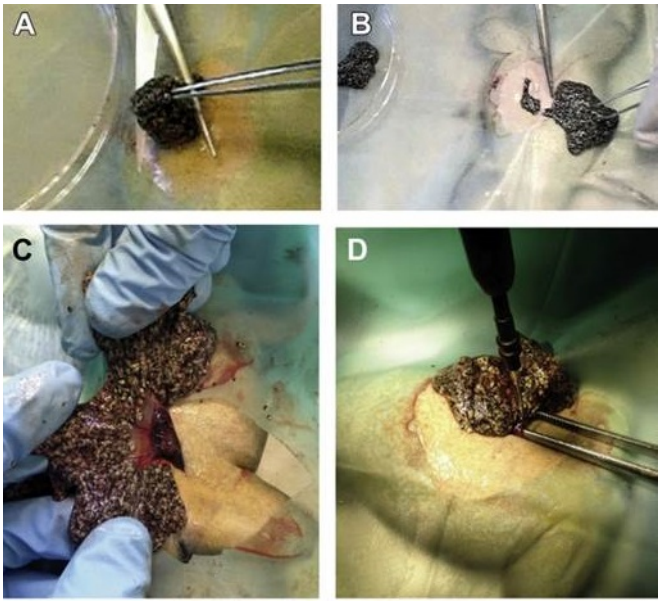

Figure 1: Surgical Procedure to remove oocytes (ovariectomy) from Xenopus laevis frog
(**A, B**) Shows a portion of oocyte mass following excision by surgical procedure. Oocytes are used for a variety of research purposes in the expression of membrane transporter proteins for both pharmacology and fundamental research (**C, D**) The surgery (ovariectomy) uses a small (~1 cm) incision in the abdomen of a anesthetised frog either side of the lateral midline and removal of the oocyte portions with tweezers. Courtesy of Norin Chai, DVM, MSc, PhD, DECZM, Paris, France [6].

Current funding from the Medical Advances Without Animals (MAWA) Trust is allowing us to implement an advanced technique in protein science that helps increase the specificity of drug design and reduces the usage of animals in fundamental research [7]. This technique, known as Cell-Free Synthesis (CFS), will be implemented to directly replace the use of *Xenopus laevis* oocytes, and, hence, the need to use the animals in this field of research [3].

The basic concept underlying CFS is that we can produce the membrane proteins (the drug targets) we wish to study by using the components of living cells but without the actual requirement of using cells or live animal themselves. These essential components include a DNA (which provide the template for the membrane protein), RNA (the intermediate between DNA and protein) T7 Polymerase (a molecular machine which makes DNA from RNA), ribosomes (which synthesises the protein from RNA), and the synthesised membrane protein (**Figure 2**).

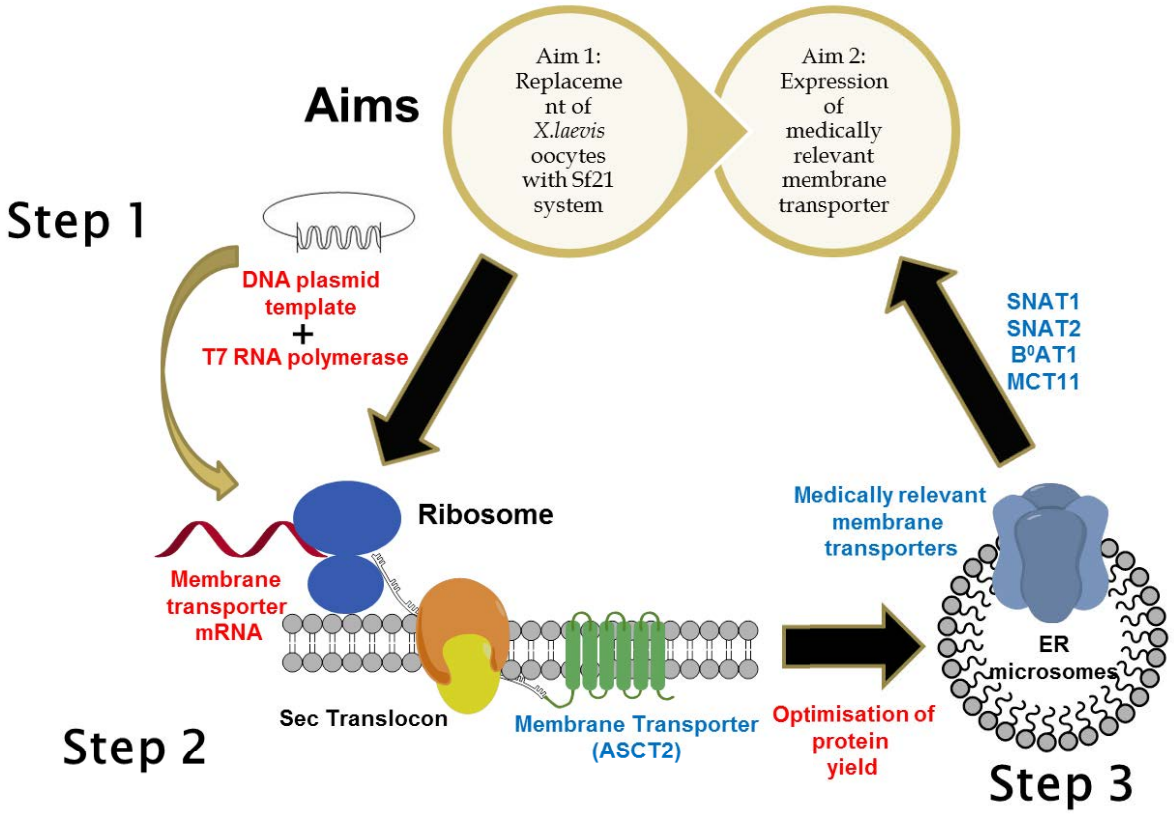

Figure 2. Overview of the Cell-Free Synthesis (CFS) of medically relevant membrane transporters.
Step 1: following the isolation of the DNA encoding the membrane transporter using genetic engineering, RNA of the transporter is produced using a T7 viral polymerase. RNA being the intermediate between DNA and protein. **Step 2**: Using ribosomes and Sec Translocon isolated from an immortalised cell line to produce the required membrane transporter protein from the RNA. **Step 3**: The medically relevant membrane transporter protein can then be studied in isolation and used for the screening of many potential drugs targeting that transporter.

The chemically synthesised membrane transporter protein is then tested for full functionality and can be isolated for pharmacological screening. These are the normal molecular components used by all living cells to translate the DNA code of genes into the functional machines of life, which proteins represent.

The medical significance of this project is the introduction of cheaper and more efficient expression system for membrane transport proteins that are of high clinical importance. This is the first time *Sf*21 cell-free system will be utilised for the synthesis of membrane transporters. Specifically, we will attempt the CFS of transporters for the essential biochemical glutamine, including ASCT2 (SLC1A5, protein name followed by gene name in brackets), SNAT1 (SLC38A1) and SNAT2 (SLC38A2), which are potential targets for certain glutamine-dependent carcinomas [8, 9]. Additionally, we will attempt the CFS of B^0AT1 (SLC6A19) [10] and two poorly characterised transporters of the SLC16 family, MCT11 (SLC16A11) and MCT13 (SLC16A13) [11], all considered current drug targets to treat type II diabetes. Cell-free expression will allow for easier scalability of protein expression than oocytes – scalability which makes high-throughput drug screening on these transporters easier. If successful, Cell Free Synthesis promises to provide a future, widespread replacement method for the use of a currently ubiquitously used animal in pharmacological research.

Acknowledgements: This research is funded by a Medical Advances Without Animals (MAWA) Fellowship.

References

1. Petsko, G.A. *Using Phase II Failures*. American Society for Biochemistry and Molecular Biology Feature Story 2010 August 2010 [cited 2018 27-5-2018]; Available from: http://www.asbmb.org/asbmbtoday/asbmbtoday_article.aspx?id=8668.
2. Akhtar, A., *The flaws and human harms of animal experimentation.* Camb Q Healthc Ethics, 2015. **24**(4): p. 407-19.
3. Broer, S., *Xenopus laevis Oocytes.* Methods Mol Biol, 2010. **637**: p. 295-310.
4. Papke, R.L. and C. Smith-Maxwell, *High throughput electrophysiology with Xenopus oocytes.* Comb Chem High Throughput Screen, 2009. **12**(1): p. 38-50.
5. Information, N.C.f.B., *PubMed database search*. 2018.
6. Chai, N., *Surgery in Amphibians.* Vet Clin North Am Exot Anim Pract, 2016. **19**(1): p. 77-95.
7. Ezure, T., et al., *A cell-free translocation system using extracts of cultured insect cells to yield functional membrane proteins.* PLoS One, 2014. **9**(12): p. e112874.
8. Broer, A., F. Rahimi, and S. Broer, *Deletion of Amino Acid Transporter ASCT2 (SLC1A5) Reveals an Essential Role for Transporters SNAT1 (SLC38A1) and SNAT2 (SLC38A2) to Sustain Glutaminolysis in Cancer Cells.* J Biol Chem, 2016. **291**(25): p. 13194-205.
9. Marshall, A.D., et al., *ASCT2 regulates glutamine uptake and cell growth in endometrial carcinoma.* Oncogenesis, 2017. **6**(7): p. e367.
10. Cheng, Q., et al., *Identification of novel inhibitors of the amino acid transporter B(0) AT1 (SLC6A19), a potential target to induce protein restriction and to treat type 2 diabetes.* Br J Pharmacol, 2017. **174**(6): p. 468-482.
11. Rusu, V., et al., *Type 2 Diabetes Variants Disrupt Function of SLC16A11 through Two Distinct Mechanisms.* Cell, 2017. **170**(1): p. 199-212.e20.

Training Our External AEC Members: Can you, should you, will you?

Arnja Dale

Chief Scientific Officer for the SPCA NZ and

ANZCCART (NZ) Board Member

External animal ethics committee members play a critical role in the integrity of ethical consideration of the use of animals in research, testing and teaching. The roles are meant to bring independence, public representation, fresh perspective and to advocate from the animals' point of view (NAEAC, 2007). In order to have meaningful input, a level of understanding of how to review an AEC application is imperative, rather than simply correcting punctuation and spelling. To review animal ethics applications effectively, some basic knowledge of the relevant legislation, monitoring and animal welfare are crucial. This baseline information could be obtained through a training programme. There are numerous training programmes that exist but they vary widely in content, accessibility, relevance and cost. The results of a review of the available training programmes and key themes that have emerged will be presented.

Reference:

National Animal Ethics Advisory Committee (2007) Guide for Lay Members of Animal Ethics Committees.

No Manuscript was received for this presentation

Presentations given

on

Thursday 26th July

What do opinion polls really tell us about public attitudes to animal research?

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Opinion poll results have become a staple in contemporary media reporting and are also a popular device for bolstering arguments in debates on contentious social issues. The ethical debate over animal research is far from immune to this phenomenon with poll results featured in material ranging from brazen propaganda through to peer reviewed articles in scholarly journals. This presentation will illustrate the variable quality of opinion polls into animal research and will outline how polls can be assessed for potential sources of bias. It will then review findings from some of the more robust polls. These findings suggest that while animal research continues to be ethically acceptable to the majority, this acceptance is conditional and is affected by individual respondent variables and context. Higher acceptance is correlated consistently with male gender, older age and education level, and correlations have also been found with the species of animal used, the goals of the research, attitudes to science, vegetarianism, pet ownership, and the GDP of the country in which the survey was conducted. Findings that warrant further study include an apparent decline in support for animal research, perceptions of a lack of transparency in animal research, and the shortage of robust survey data from Australia and New Zealand.

Would it be beneficial for Australian institutions to align their training requirements and share educational resources in order to promote some degree of standardisation of training in Australia.?

This session will provide the opportunity for trainers to discuss the resources they have used and offer critique on their content, value and limitations, and also to offer details of training resources that they have developed and which could be shared or integrated into a collective training resources.

Introduction

Opinion poll results have become a staple in contemporary media reporting and are also a popular device for bolstering arguments in debates on contentious social issues. The ethical debate over animal research is far from immune to this phenomenon. This presentation outlines how polls can be assessed for potential sources of bias and then reviews findings from some of the more robust polls. It is shown that even within a small selection of polls, support for animal research can appear to range from as low as 23% to as high as 71% thereby creating a slightly absurd situation in which polling results can be used to support either side of the debate. Rather than being a true reflection of public attitudes, these sort of data are more likely to reflect methodological differences and a lack of broader context. Of the more consistent findings, higher acceptance of animal research is usually correlated with male gender, older age and education level; other factors found to influence levels of support include species of animal used, the goals of the research, attitudes to science, vegetarianism, pet ownership, and the GDP of the country in which the survey was conducted. Findings that warrant further study include an apparent decline in support for animal research, and perceptions of a lack of transparency in animal research.

It seems that every day we are presented with the results of yet another opinion poll. And of course some of these polls capture world attention. In 2016 – which one media pundit has dubbed “the year of the polls” – we saw polling results published at possibly unprecedented levels.

Perhaps this was not surprising because 2016 was, after all, the year of the Trump election, the Brexit vote, the election of President Duterte in the Philippines and – easily my personal favourite – a poll conducted

in Britain to decide the name of a new Antarctic research vessel. Who could forget that from a field containing such worthy proposals as the 'Sir David Attenborough' and various famous explorers, the winning name by a sensational margin was…"Boaty McBoatface". And I'm proud to say that my vote contributed to that winning margin.

While many of the polls we see relate to politics, some look at social issues and from time to time we come across one that seeks to gauge public attitudes towards animal research. Most of these don't get much coverage in the mainstream media. Instead, we are more likely to encounter them in other forums where they are supporting one side or other of the ethical debate.

The fact that we see poll results being used to support both sides of the animal research debate is interesting. It's also something that hasn't escaped the notice of scholars. One study looked at this by interviewing 50 stakeholders in the debate ranging from senior researchers on one side to leaders of anti-vivisection organisations on the other[1]. It reported that both sides presented what could be regarded as predictable arguments: scientists stressing the importance of animal research along with reassurances of high welfare standards and a strict regulatory regime, and animal protectionists making claims of animal suffering, questioning the reliability of animal models and advocating stricter regulations. No surprises so far.

But what did come as a surprise was the extent to which both sides used opinion polls to justify their respective positions. This prompted the author to state that public opinion is used as "a crucial route to legitimacy" and "a key battle-ground in the animal research debate".

Another study by reviewed reports from 56 opinion polls that had canvassed animal research[2]. This found that public support for animal research ranged from a mere 27% in one poll up to an impressive but rather implausible 100%. As I will be attempting to show, this probably says far more about the pitfalls of polling and poll reporting than it does about public opinion.

The polls in the review that I've just mentioned were all one offs – that is they represent what is essentially a snap shot at the time the poll was taken. Occasionally, however, polls are undertaken on a repeated basis with a view to plotting trends over time. Known as successive independent sampling, this sort of polling has been used to track public attitudes to animal research in both the US and the UK since the early 2000s.

A graphic summarizing these polls was published in an article in *Science* last year [Fig. 1][3]. We can assume that the apparent decline in support in both the US and the UK was met with concern by the scientific community but with cautious optimism among animal protectionists.

[1] Hobson-West P (2010) The role of 'public opinion' in the UK animal research debate. J Med Ethics 36(1):46-9
[2] Hagelin J *et al* (2003) An overview of surveys on how people view animal experimentation: some factors that may influence the outcome. *Public Understand. Sci*. 12:67–81
[3] Wadman, M (2017) To woo public, Europe opens up on animal experiments, but U.S. less transparent. *Science*, 14 July 2017 doi:10.1126/science.aan7108

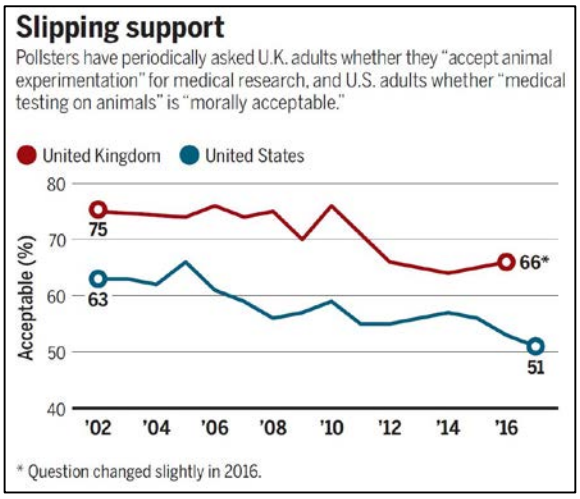

Fig 1: UK and US polling on the acceptability of animal research over time (successive independent sampling).

Graphic: J. You, Science from data by Ipsos MORI (UK) and Gallup (US)[3]

But how much weight should we give to these sorts of trends? I will return to that point later but first let me point out a subtler feature of the graphic.

We often think of the British as a nation of animal lovers. How can it be, then, that the UK line on the graphic is above the US line? If this was a valid difference, it would mean that Britons are considerably more comfortable with animals being used for research than Americans. I don't believe this says anything about real cultural differences – I believe the explanation lies in polling methodology.

Polling methodology

Usually when we encounter the results of an opinion poll, we are only presented with minimum detail – generally just enough to make a newsworthy story or perhaps to bolster an argument in a scientific journal or animal rights website.

Unfortunately, this means that what we see has been filtered. Which is a shame. Conducting a rigorous poll is a lot of work and the results deserve more than just a cursory – and often highly selective – account.

So what should we do? How can we know what a poll is really saying and whether it is valid?

To answer these questions, it's important to touch on some of the methodological considerations that go into a properly conducted poll. These are usually found in a report that any reputable pollster should be willing to supply along with the results.

The report should tell you a number of things:

Random sampling

Firstly, the sampling method. Of course it is rarely possible to poll an entire population, so a sample of the population has to be selected. Usually, the crucial thing to look for here is the word "random". Polls that do not employ some sort of random sampling technique are likely to yield biased results and cannot be regarded as representative of the general population.

Sample size

The sample size is important because of its influence on the margin of error. Based on statistical theory that I don't pretend to understand, a sample of about 1,000 seems to be the magic number these days.

Method of data collection

The method of data collection – whether by mail, phone, face to face or online – can be an important source of bias if not properly controlled.

For example, people may be reluctant to participate in face to face polls if they are worried that their opinions might be unpopular. This has been shown to cause a bias away from more conservative points of view – a phenomenon first documented in political polling in the UK where it is still sometimes referred to as the 'shy Tory effect'. On the other hand, face to face polls administered by a trained interviewer tend to be superior when polls are lengthy or feature complicated concepts.

Polls conducted by phone or face to face are still regarded as the most reliable. Predictably, however, they have been greatly outstripped in popularity by the speed and low cost of online methods. Unfortunately, online polling presents significant challenges if bias is to be avoided and the sort of polls we see daily on social media are usually catastrophically flawed.

Professional pollsters of course work hard to minimize bias in online polls. One of the main ways they do this is to use specially recruited online panels in which participants have been screened and weighted against census data. However, caution is still necessary to avoid bias: panel membership is usually self-selecting, the screening process relies on the judgement of the pollster and panel members may become conditioned.

Even more vexing (at least to my mind) is that online panel members are often given an incentive such as points that are later redeemable for cash. As one expert put it to me, there is a risk that online panel surveys just survey people who like doing surveys.

Wording of questions

Needless to say, the wording of the questions is crucial in obtaining a valid result. The power of even a single word to change polling outcomes is well-established and emotional language, leading questions, and excessive complexity should all be avoided.

Who commissioned the poll?

Many polls are commissioned by organisations that have a vested interest in the outcome. Commissioning a poll is an expensive exercise so it's not an investment organizations would rush into unless the outcome was likely to support an agenda. This does not mean the poll itself is biased, but it does mean the way the results are presented could be.

Topics covered

Finally, some polls cover a single topic in depth whereas others may cover multiple topics of which only one may be of interest to a particular discussion. In the latter case, there is usually less opportunity for respondents to develop their thinking on any one topic and so there is a risk that responses are less considered or informed.

And this brings us back to the *Science* graphic. Both polls represented here were conducted by reputable polling companies employing rigorous methodology. But what the graphic doesn't show is that each poll was vastly different in the topics covered.

The results from the US come from an annual poll which poses questions on more than 20 social issues ranging from marriage to medical ethics. Only three of these are animal-related: one was on animal research while the others were on animal cloning and clothing made of animal fur. Intriguingly to me, the results found that all three of these were rated as less ethically acceptable than the death penalty.

By contrast, the results from the UK come from a biennial poll where all questions relate to only one issue, namely animal research. At the very least, this gives respondents a hint that there are multiple facets to the issue which in turn may encourage a more nuanced position.

Thus, despite being presented in the same graphic, the UK and the US polls are quite different. The data which have been selected from each one make a nice graphic and perhaps do say something about support for animal research over time, but they mustn't be regarded as a valid comparison between countries.

Findings

Trends

Moving on from the process of polling, what might polls be telling us about attitudes to animal research?

The US and UK polls are the only examples that I am aware of which have tracked questions on animal research at frequent intervals over an extended period.

However, some 'one off' polls have been repeated several years apart and so can still give us a rough indication of possible trends.

One such pair of polls was led by the US's Pew Research Center (a not-for-profit social research organisation) in collaboration with the American Academy of Sciences in 2009[4] and 2015[5]. These polls included one question on animal research among a broader range investigating attitudes to science and society. Their 2009 poll put support for animal research at 52% but this had dropped to 47% in 2015.

Another pair of polls was conducted by the European Union across all member countries in 2005[6] and 2010[7]. Once again, attitudes to animal research was just one issue among several aspects of science covered by the polling. Independent analysis of these data confirmed a statistically significant decline in support in 9 countries (Germany, Finland, Estonia, Hungary, Latvia, Lithuania, Poland, Slovenia and Bulgaria) but no change in the remaining 19[8].

Closer to home, polls have been conducted on three occasions over 10 years by Human Research Australia, a not-for-profit advocacy organization that challenges animal research. The polls were conducted by an independent polling company, Nexus Research, and I am very grateful to the CEO of Humane Research Australia, Helen Marston, who willingly supplied me with the full reports from the two most recent polls.

Of the questions that gauged aspects of support for animal research, only one showed a clear downward trend. This asked whether respondents believed that "animal experiments are necessary for the

[4] Kohut, A et al (2009) Scientific Achievements Less Prominent Than a Decade Ago. Pew Research Center in collaboration with the American Association for the Advancement of Science.
[5] Funk, C et al (2015) Public and Scientists' Views on Science and Society. Pew Research Center in collaboration with the American Association for the Advancement of Science.
[6] Special Eurobarometer 224 (2005). Europeans, Science and Technology. European Commission.
[7] Special Eurobarometer 340 (2010). Science and Technology. European Commission.
[8] Crettaz von Roten (2012), F. Public perceptions of animal experimentation across Europe. *Public Understanding of Science* 22(6) 691–703

development of medicines for humans". Responses in the affirmative to this question declined from 59% to 45% over the three polls.

Overall then, a number of polls have suggested that public support for animal research is on a downward trend. And while this is not the case across all countries surveyed, there was little in the polls I reviewed to suggest an increase in support.

Comparing polls

Methodology and demographics can have a major influence on polling outcomes. This means that results from polls that canvass superficially similar topics can vary widely.

To illustrate this point, I have collated results from a small selection of six polls that canvassed views on animal research. One of the difficulties in making this comparison was that some polls focused entirely on animal research whereas others covered several topics of which animal research was only one. In an attempt to address these differences, I have chosen a single question from each poll that I felt best conveys an overall attitude to animal research. It is doubtful whether this sort of comparison is valid in a strict methodological sense. But if nothing else, the exercise serves as a warning about the hazards of drawing firm conclusions from a single poll, especially if taking a single result in isolation.

As seen in Table 1, the level of support for animal research in the six polls extends over a remarkably wide range from a low of 23% to a high of 71% - a finding consistent with that of Hagelin *et al* (2003)[2]. If taken in isolation, these results could be used to argue that public support for animal research is either very weak or very strong. Surely both can't be right.

But looking a little more deeply into the polls, it becomes clear there are some important differences between them (see Table 1). Perhaps most striking is the apparent effect of including information that takes account of measures to minimize suffering. This was provided in both polls where support was highest (the UK IpsosMORI and a New Zealand government poll) but was lacking in the remaining polls where support was lower.

In two of the polls (those by Gallup and advocacy organization Humane Research Australia – HRA), the wording of the question made reference to the morality of animal research. But despite this superficial similarity, the two polls reported substantially different responses: 54% support in the Gallup poll and only 23% in the HRA poll. Again, however, there are prominent methodological differences between the polls themselves: the Gallup poll was conducted by phone and animal research was only one of several topics canvassed while the HRA poll was conducted with an online panel and focused entirely on animal research.

It is also possible that responses in the HRA poll could have been influenced by elements in other questions. These included, for example, a statement about total animal usage that didn't distinguish between biomedical research and the large number of animals counted in non-invasive wildlife or field studies, a lack of contextual information in questions relating to adverse outcomes, use of the emotive term "traumatised", and a question on animal testing of cosmetics even though this does not take place in Australia.

Another of the polls (the 2017 ANU poll) was designed to be based closely on an earlier poll (the 2015 Pew Research Center poll) to allow for international comparison. Although both only contained one question on animal research, the level of support recorded in each was somewhat similar (41% and 47% respectively). We can only guess how these responses would have turned out if respondents had been given more background information.

Support for animal research	Question selected for comparison	Other information included in question	Topic(s) covered	Approx proportion of questions relating to animal research (excl. demographics etc)	Interview method	Primary organisation conducting survey	Country	Poll publication
71%	I can accept the use of animals in scientific research as long as there is no unnecessary suffering to the animals and there is no alternative.	"As long as there is no unnecessary suffering…and no alternative"	Animal research only	100% (66/66)	Face to face	Government	UK	IpsosMORI 2016[a]
68%	Animal research and testing [is] acceptable if no unnecessary suffering.	Provided an outline of the role of Animal Ethics Committees including minimising animal numbers and suffering.	Animal research only	100% (38/38)	Phone	Government	New Zealand	New Zealand Veterinary Journal 2007[b]
54%	Regardless of whether or not you think it should be legal...please tell me whether you personally believe that in general…medical testing on animals...is morally acceptable or morally wrong.	None	Social values and beliefs	5% (1/21)	Phone	Commercial polling organisation	USA	Gallup 2018[c]
47%	All in all, do you favor or oppose the use of animals in research?	None	Science and society	2% (1/43)	Phone	Independent social research organisation	USA	Pew Research Center 2015[d]
41%	[Do you] favour or oppose the use of animals in scientific research (five point scale).	None	Beliefs and attitudes to science	3% (1/35)	Phone	Government	Australia	Australian National University 2017[e]
23%	Do you believe that humans have the moral right to experiment on animals?	None	Animal research only	100% (29/29)	Online	Not for profit advocacy organisation	Australia	Humane Research Australia 2018[f]

Table 1: A comparison of questions relating to support for animal research from a selection of polls.

Polls referenced in table:

[a] Clemens, M & Leaman, J (2016) Public Attitudes to Animal Research in 2016. IpsosMORI.

[b] Williams, V et al (2007) Public attitudes in New Zealand towards the use of animals for research, testing and teaching purposes. New Zealand Veterinary Journal 55(2), 61-68

[c] Jones, J & Saad, L (2018) Gallup Poll Social Series: Values and Beliefs.

[d] Funk, C et al (2015) Public and Scientists' Views on Science and Society. Pew Research Center in collaboration with the American Association for the Advancement of Science.

[e] Lamberts, R (2017) The Australian Beliefs and Attitudes Towards Science Survey. The Australian National University. Canberra, Australia

[f] Nexus Research (2018). National Public Opinion Poll on Humane Research Issues. Humane Research Australia.

Demographic correlations

Polling often includes the collection of background data which can be analysed to look for demographic correlations with the responses.

In polls relating to animal research, this has consistently demonstrated a positive correlation between acceptance of animal research and male gender. Other positive correlations with acceptance of animal research include older age groups, level of education, scientific background, conservative political ideology, rural background and patients with chronic disease[9].

Negative correlations have included include vegetarian diet, an interest in environmental issues, and citizenship of countries with a higher GDP. The last of these is surprising given the otherwise positive correlation between acceptance of animal research and higher levels of education.

Other negative correlations are found when research involves the use of charismatic species (such as dogs and monkeys)[10, 11] or genetically modified animals[12] .

Transparency

Declining support for animal research in opinion polls was one of the triggers for a major effort in the UK to make animal research more transparent to the public. At the centre of this is a pledge to increasing transparency called the 'Concordat on Openness to Animal Research in the UK'[13]. Over 120 research organisations have now committed to the Concordat leading to initiatives such as the open publishing of figures on animal usage and even online virtual tours of animal facilities.

A slight increase in the acceptability of animal research has been recorded in the latest UK polling and this has given hope to some that the new strategy of transparency is working. I suspect, however, that this trend requires support from subsequent polling before any firm conclusions can be drawn. It is also worth noting that the same poll also reported no less than 44% of respondents regard animal research institutions as secretive.

Polls in Australia have also indicated distrust of animal research scientists and the HRA poll reported that 81% of respondents don't think the government provides enough information about animal research.

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[9] Dancet, EA *et al* (2011) Acceptability of preclinical research on nonhuman primates in reproductive medicine: the patient perspective. *Reproductive Sciences* 18(1):70-8. Also spinal cord injury patients Kwon et al.

[10] See for example: Ormandy, EH and Schuppli, CA (2014) Public Attitudes toward Animal Research: A Review. *Animals* 4:391-408

[11] Eurobarometersxx

[12] Schuppli CA and Weary DM (2010). Attitudes towards the use of genetically modified animals in research. *Public Understanding of Science*. 19(6):686-97

[13] See http://concordatopenness.org.uk/

While on the topic of transparency, it is worth mentioning a survey conducted specifically to assess the impact of transparency on acceptance of animal research[14]. This presented two groups of participants with a hypothetical scenario in which animal research was being performed in an institution that was either open about its animal research or deliberately concealed it with a lack of signage, heavy security and lack of publicly available information. I think there is an important lesson in the poll's finding that when presented with the low-transparency scenario, respondents were less accepting of animal research.

Conclusions

I said earlier that 2016 has been dubbed the year of the polls. I will finish with a story about another event that year where polling results were again prominent in the media coverage.

Many will remember the ABC TV documentary that exposed entrenched animal cruelty and dishonest practice among some elements in the greyhound racing industry. This prompted the then premier of NSW, Mike Baird, to order a Special Commission of Inquiry.

The results of the Inquiry were handed down in mid-2016 and the findings were so shocking that Premier Baird made a spectacular announcement: he declared that within 12 months, a permanent ban would be placed on all greyhound racing in NSW – the entire industry was to come to an end. Premier Baird's announcement was greeted with elation by many in the animal protection community and polling suggested it was widely supported in other quarters too.

A mere three months later, however, Premier Baird made an equally spectacular announcement: the government had changed its mind – greyhound racing had been granted a reprieve. Another three months later there was a third bombshell: Mike Baird announced his resignation not just as Premier but from politics altogether. While there were important and well-documented family considerations behind his resignation, it was inevitable that it would also be linked to fallout from the greyhound racing ban.

A quite extraordinary sequence of events. So what were the polls saying about the greyhound saga of 2016?

When the ban was first announced, the media were publishing polls that suggested it enjoyed widespread support. One poll that claimed 82% of people were in favour of the ban. But if we look a little more closely, we discover the figure came from a self-selecting social media poll and would therefore be heavily biased.

Another poll reported a slightly narrower margin yet still suggested that the Premier was on the money: it found 51% in favour of the ban compared to 31% opposed. Unfortunately, we are left to speculate on the validity of this result because no detailed report on the methodology was available.

But what if we look at a poll that we know would have been conducted with rigorous methodology by a professional polling company? Probably the most crucial poll in this story was one conducted by Newspoll. It didn't ask whether respondents supported the ban. It was just part of the ongoing polling that monitors voter support for the Premier and the Opposition Leader.

Prior to the ban, Premier Baird's support was at an almost record high. But the first poll after the ban was announced, showed his support plunging to a level that would end up below that of the opposition leader.

[14] Mills KE *et al* (2018) Institutional transparency improves public perception of lab animal technicians and support for animal research. *PLOS ONE* https://doi.org/10.1371/journal.pone.0193262

Predictably, this poll was given a great deal of weight by the media and it illustrates dramatically the perceived value of polling as a measure of public opinion.

So how much weight should we place on opinion polls about animal research?

As we have seen, some widely reported polls attempt to canvass the ethical complexity of animal research with just a single question. In most polls there is little or no context and the respondent is presented with a simple binary choice or five-point scale at best. This in no way compares to the detailed evaluation of animal research mandated under the Animal Ethics Committee system in Australia and New Zealand in which the experience of diverse stakeholders will have been devoted collectively to several hours scrutinizing a lengthy proposal supplemented by additional discussion and clarification from the applicant.

Those of us with an interest in the ethical debate over animal research should actively encourage a more critical look at opinion poll results. Whatever our ethical position, we should be prepared to ask for and critically evaluate the pollster's report. And we should also be ready to engage publicly in discussions if we feel that poll results are presented in a way that has the potential to mislead.

Unfortunately, the public reporting of an opinion poll is often irritatingly superficial or biased, even when the polls are methodologically sound – and frequently they are not. But the instant appeal of polls means they are here to stay. And perception is everything. In an age of information overload, polls tempt us with a promise to distill complex issues into a simple percentage. They are quick and easy to read, and by their numerical nature, appear to provide the ultimate in objectivity.

Several of the polls I reviewed suggest a decline in public support for animal research and this is accompanied by evidence of troubling shortfalls in public understanding and trust. I am forced to conclude that without more informed input and a proactive approach to challenging poll results reported out of context, the stage is set for poor decisions by policy-makers.

Promoting behavioural change in people working with animals in research and teaching: Challenges and opportunities.

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The 8th Edition of the *Australian code for the care and use of animals for scientific purposes* was published in July 2013, and included definitions of 'must' and 'should' to clarify 'obligatory' versus 'strongly recommended' components of the Code, respectively. However, there is still evidence of hesitation to meet appropriate standards. In order to stimulate behavioural change and "lift the bar" in terms of the governing principles (clause 1.1), that is, respect for animals, responsibility, ensuring activities have scientific or educational merit, are conducted with integrity with minimal animal numbers and with animal wellbeing being supported, key behaviours had to be mandated. In spite of this, and five years on, resistance to change by some animal users still exists. This presentation hopes to stimulate discussion by identifying the most common areas that resistance to change is occurring, why it occurs and how to manage the change process.

The 8th Edition of the *Australian code for the care and use of animals for scientific purposes* (the Code) was published in July 2013, and included definitions of 'must' and 'should' to clarify 'obligatory' versus 'strongly recommended' components of the Code, respectively. The governing principles (clause 1.1) of the Code encompass respect for animals, personal responsibility, scientific or educational merit, integrity, minimal animal numbers and optimal animal wellbeing. To ensure compliance it appears that key behaviours had to be mandated. However, there is still evidence of hesitation to meet appropriate standards and resistance to change by some animal users. In order to stimulate behavioural change and "lift the bar" the most common areas of resistance need to be identified, the reasons for the resistance addressed and the change process managed.

The regulatory framework in Western Australia that underpins the use of animals in research and teaching includes the "*Veterinary Surgeons Act 1960* (WA)" and regulations, 1979; the *"Animal Welfare Act 2002 (WA)"* and regulations and the *Australian code for the care and use of animals for scientific purposes, 8th Edition 2013*.

The "*Veterinary Surgeons Regulations 1979 (WA)"*, §26(3)(e))[1] states that "*veterinary surgery"* includes:

- examination of any animal for the purpose of the diagnosis of disease or injury and the conduct of physiological or pathological tests for diagnostic purposes.
- provision of advice based upon diagnosis.
- provision of surgical or medical treatment of any animal.
- administration of anaesthesia and the performance of surgical operations.
- performing of any act, matter, procedure, or thing that is prescribed pursuant to section 31 as forming part of the practice of veterinary surgery.

However, the *"Veterinary Surgeons Act 1960"*[2] also allows for people without veterinary qualifications and registration to perform "*vivisection and other experiments or operations on animals (including giving any*

necessary anaesthetic)". That is, undertake activities or procedures which may be considered acts of veterinary surgery without the prerequisite veterinary education or experience. It does state that these activities are to be "performed in accordance with the "*Animal Welfare Act 2002 (WA)"* by a person who is authorised under that Act to do so".

The *"Animal Welfare Act 2002 (WA)"*[3] states that in order to undertake animal based activities each institution must have a licence to use animals for scientific purposes. This license usually contains a list of conditions. For the University of Western Australia (UWA) one of these conditions states that "the licensee must establish and maintain a method of ensuring any person who is to be involved in the conduct of an approved project under the licence has the appropriate veterinary skills for that project."

This licence also states that the named scientific establishment, its staff and students, must use animals for scientific purposes in accordance with the Scientific Use Code or the Code. The final piece of the regulatory framework, the Code, provides the most important and prescriptive information on how to use animal in research and teaching in Western Australia.

The Code includes obligatory and 'strongly recommended' components[4]. In brief, the obligatory components include the following requirements:

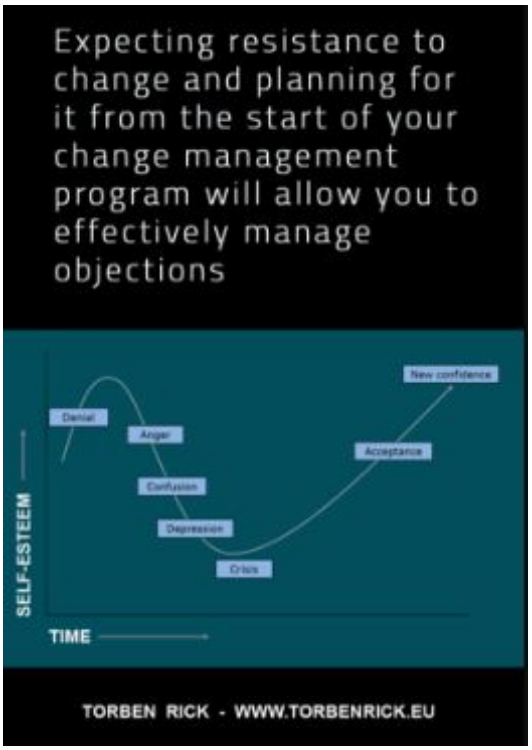
- All those involved in the care and use of animals for scientific purposes MUST be aware of the relevant Commonwealth, State and Territory legislation and act in accordance with the Code (Code §1.31)

- In the Governing Principles, the use of animals for scientific purposes MUST (Code §1.1 & 1.5):
    - Have scientific or educational merit
    - Aim to benefit humans, animals or the environment
    - Be essential to achieve stated aims
    - Apply the 3Rs (Replacement, Reduction, Refinement) (Code §1.18-1.30)
    - Must be conducted with minimum numbers, minimum adverse impact on wellbeing and scientific integrity
    - Must be conducted with minimum numbers, minimum adverse impact on wellbeing and scientific integrity
    - Be subject to ethical review
    - Be undertaken in full knowledge and acceptance of one's responsibilities

        - Practices and procedures MUST be based on current best practice, the assumption that animals have a capacity to feel pain and distress and unless there is evidence to the contrary, that is, assume that this is equivalent to humans (Code § 1.9- 1.14)
        - Prompt action being taken to alleviate pain and distress and that these action take precedent over experimental outcomes (Code § 1.9- 1.14)
        - Animals that have undergone surgical procedures MUST be supported and safeguarded by using aseptic procedures if the animal is expected to recover (Code §3.3.16 (ii))

In spite of this, and five years on, resistance to change by some animal users still exists. Resistance to change may occur because the change is perceived as a threat, whether real or perceived, large or small. Other reasons for resistance may include fear of the unknown or fear of change, insecurity around competence and a lack of understanding of the reasons or benefits for change which may be a result of inadequate consultation and communication (Ref[5], Diagram 1).

Diagram 1. Why do employees resist change? [5.]



Source: Rick, T. (2018). [online] Torbenrick.eu. Available at: https://www.torbenrick.eu/blog/wp-content/uploads/2013/10/change-resistance-e1424767157422.png

Resistance may take many forms, including active or passive, overt or covert, individual or organised, aggressive or timid.[6.] It is likely that about "20% percent of employees will be enthusiastic about organizational change and 60 percent could be persuaded to go along, the remaining 20 percent will resist change no matter what".[7.]

For example, in spite of the fact that aseptic technique is mandated in the Code, and therefore considered 'obligatory', there may be investigators who are resistant to implementation. Objections or barriers often include a perception that there is no or minimal impact, or clinically recognised complications with current practices. In some research groups there is a long held belief that laboratory rodents are resilient and therefore aseptic technique is not required. This observation is supported by Wang-Fischer who stated that the "misconception that rodents have an innate resistance to bacterial infection has not been scientifically substantiated. Infection, which may not be apparent on casual observation, may cause loss of cannulations and numerous changes in physiological parameters."[8.]

Some investigators involved in projects with current AEC approval may hold the view that current practices are AEC endorsed and there is no requirement to improve activities during the approval period. However, the Code (§1.28) states that "the effectiveness of strategies for supporting and safeguarding animal wellbeing must be kept under review during the lifetime of activities" and also states that "the outcome of the review must be implemented in current activities".

Finally, it may be the case that some investigators are simply unaware that their current practices do not accord with aseptic technique, as they have not been adequately educated and trained or object in terms of having additional resource and staffing costs.

Managing behavioural change requires support and resources from senior level organisational decision makers, the Animal Ethics Committee, facility managers and animal care staff. It requires good planning, which includes engagement with all stakeholders in the first instance and a good communication strategy. There is substantial staff resources required for 'on the ground' project reviews and assessments. Where there are identified deficiencies, an education and skills training programme is essential to ensure clear understanding of why the change is required. Furthermore, during the implementation phase, ongoing technical support and mentoring is required until investigators are comfortable, confident and competent in the new practices. It is likely that mentoring will be a key component to preventing relapse to previous 'custom and practice' in procedures and beliefs. And it has been suggested that having an influential 'convert' to best practice may accelerate the uptake within the animal user community.

The Code clearly defines principles and practices that are compulsory. A part of the animal user community will be resistant to change for a variety of reasons or just as a principle. However, with persistence and adequate resources focusing on collaborative planning and communication, education and training and on-going mentoring, behavioural change can be implemented.

References

1. *Veterinary Surgeons Regulations 1979* (WA) (Jan 2018 update). Available at: https://www.legislation.wa.gov.au/legislation/prod/filestore.nsf/FileURL/mrdoc_40318.htm/$FILE/Veterinary%20Surgeons%20Regulations%201979%20-%20%5B05-g0-00%5D.html?OpenElement
2. *Veterinary Surgeons Act 1960* (WA) Available at: https://www.legislation.wa.gov.au/legislation/statutes.nsf/law_a855.html
3. *Animal Welfare Act 2002* (WA) Available at: https://www.legislation.wa.gov.au/legislation/statutes.nsf/main_mrtitle_50_homepage.html
4. *Australian code for the care and use of animals for scientific purposes*, 8th edition (2013) National Health and Medical Research Council. Available at: https://www.nhmrc.gov.au/guidelines-publications/ea28

5. Rick, T. *Change Resistance* [online] *Torbenrick.eu*. Available at: https://www.torbenrick.eu/blog/wp-content/uploads/2013/10/change-resistance-e1424767157422.png
6. Changingminds.org. *Resistance to Change*. [online] Available at: http://changingminds.org/disciplines/change_management/resistance_change/resistance_change.htm
7. Hice, J. *20% will resist change no matter what HiceSchool Blog*. [online] Hiceschool.com. Available at: http://www.hiceschool.com/1-of-many/20-will-resist-change-no-matter-what/
8. Wang-Fischer, Y. (2008). *Manual of Stroke Models in Rats*. 1st ed. CRC Press, p.70.

Report on the forum discussion held on Thursday 21st July

Striving for Consistency in Training Across Australia and New Zealand

Following on from the presentation at last year's conference that was delivered by Deirdre Bourke on the desirability of consistency in training across Australia and New Zealand and the advantages that would follow on for researchers and institutions, we felt it would be useful to consider some of the issues in this forum. As we heard earlier this week, the results of the survey Deirdre ran last year and has been analysing, shows the high level of support for the idea of consistency with a view to determining if there is anything that can be done to make progress towards the goal of consistency in training – something that is also consistent with the aspirations of ANZCCART.

Deirdre introduced some of the key issues as she sees them currently. The first and most important being, how do we move forward? The key point being that we need to do something to make sure progress is made, otherwise we will still be arguing about all these issues in another 5 years' time. She also made the point that all the Core Modules of any training package set up must be very translatable with the ability to be freely adapted to allow for the presiding legislation of any particular State or Region (including New Zealand) to be incorporated instantly.
One of the other great advantages of a common training course is efficiency. Everyone is resource poor in terms of time, so we need to be able to use this as an opportunity to share resources rather than having to reinvent the wheel in each institution. Ideally, we should be able to use opportunities like ANZCCART to set up sharing arrangements. There are also some important conversations that will need to be had about the accreditation of any and all training modules that are produced. Equally, the question of assessment of those who complete their training to determine competency is an important issue that will go to the heart of credibility for any training.

The forum of all delegates was then asked to indicate by show of hands, how many of their institutions had some form of training programme in place and the great majority did. Of those, a clear majority had some form of on-line training package in place. Slightly more than 50% indicated that their institution offered face to face training for investigators and / or AEC members and of those, a significant portion offered both face to face and on-line training options. So there was clear indication that most if not all institutions were working to meet their responsibilities under the Code by providing some training to their people. At this point the discussion was opened to the floor for questions, comments or suggestions.

It was noted that there will be some barriers to setting up a training package that crosses jurisdictions, with the seven different legislative frameworks across Australia and a separate one again for New Zealand, even when it comes to the 'simplest' aspects such as the definition of an animal, but none should be seen as a barrier to progress. One of the big questions considered by the group was who should or could play key roles in setting up training packages and this and other related topics then became the subject of a broad and fairly comprehensive discussion, notes from which are presented below:

- ANZCCART has a very clear and vital role to play in setting up a consistent training programme for the region. We should give serious consideration to both setting up and accrediting the training modules, but serious concerns were raised about the ability to set up any form of quality assessment of people completing the training (essential), largely due to the lack of resources.

- Have a TAFE related training for a Cert 2 or Cert 3 qualification and run online with online materials dealing with the principal as this is fundamental and then have that supplemented with practical training. They would then have a qualification. This was discussed previously at AAWS.
- Developing modules for different legislations is not a barrier. Regardless of who it is at an institution, researchers will be conducting research and moving across multiple jurisdictions and all people coming into training need to know about different legislation that operates in Australia and New Zealand and need to know there are differences and they need to know right from the beginning. We need just one module around legislation and it needs to cover it all.
- We have some researchers that are already at standard and have no qualifications so we may need something that recognises these people so they don't have to sit the whole test.
- Things change and just because we have a qualification it doesn't mean we have registration for the rest of our life and legislation changes so even if they have passed the module, legislation may have changed and so perhaps there needs to be some type of time frame. Also, surgical skills need to stay current.
- With regard to different legislation, eg fish as an animal, should we be treating animals under the Code? Applications for funding have to adhere to the Code and the Code defines an animal in a way which is encompassing.
- There are 3 major components of training. 1. The organisation providing training needs to be accredited. 2. The trainers need to be accredited. 3. The course needs to be at a competency based standard. We already have all that in Australia but we are missing the competency standards and that is what we should be focussed on.
- We could also put together a course and pass that across to a RTO which may be a simpler way of moving forward once we get the framework in place.
- At this early stage, we may be looking at this slightly backward and we should get something down on paper which you can start to work with and the rest will naturally fall in place. The accreditation is the cherry on the top.
- Get the principles, the basics, online or face to face, practical skills started first. Need online support and ongoing reassessment and it has to be resource intensive.
- Check with ANZLAA and see if they are doing anything similar.
- Have a list of subjects which people think should be compulsory.
- There is strong support from the research and teaching community so they do want it and they want to be told what to do.
- Recognising training is very complicated and a broad area so aligning with ARMS could be considered and doing a module on legislative requirements and they provide accreditation and it's ongoing. They don't have a module on that. It is a good model and is an industry based system.
- We should be looking at collaboration with these like-minded organisations, and particularly with hands-on training and practical skills, ANZLAA is better placed to take the lead role. Likewise for higher level administrative skills, ARMs would be good.
- There are already competency standards in nursing, animal technicians with courses for diplomas, certificates and as there is a huge amount of work already done, we could take 1 or 2 competencies as a starting point and put them into a module.
- Talk to ARMS and ask how they started the process.
- How long does accreditation last before it is reassessed? It is an ongoing process. Refresh every 3 years seems to be the right way.
- The courses need to be reassessed and then the people could take a refresher course.
- ANZCCART does not have the resources. Rely heavily on volunteers.

- Rick asked if ANZCCART had funding support would the delegates be in favour of ANZCCART taking charge of accrediting and approving a framework for training programs for animal users? There was a unanimous vote and the delegates endorsed ANZCCART to take a key role in this. .
- Training meeds to be available anytime as research won't wait for your accreditation. Being online is important as some people have a limited time to complete the research project and so can't wait for a course to be available.
- AEC training is not competency training
- ANZCCART is more AEC training and ANZLAA is more hands on training.
- Need 3 modules. Competency. Legislative requirements and ethics ie replacement. (if we are serious about replacing).
- Need more than 3 but start small. Build on as we go.
- Need a supervisor available at time of competency training – Institution to ensure that.
- Perhaps Ethics training should be first module.
- Code re training and assessment in an institution. Open to interpretation.
- We need resources – approach local MP and ask for support.
- Push for more rigid requirements for competency.
- Re replacement - work with groups such as MAWA Trust.
- Mentoring is also important
- The modules should be available to all people involved in the care and use of animals

Summary

- Focus on Australia and New Zealand
- Modules need to be applicable to AEC members, animal care staff, investigators
- Working collaboratively with ARMS, ANZLAA, MAWA
- ANZCCARTs role is along the lines of accrediting courses rather than running them
- There are 3 modules to focus on: Ethics, Legislation, the AEC System

Intraperitoneal telemetry devices: A new approach to temperature monitoring in experimental ferrets

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Background: In ferret studies conducted at PC3/4, subcutaneous temperature-sensing microchips are often used to monitor body temperature. Subcutaneous microchips measure peripheral rather than core body temperature (CBT), only provide time-point data and still require operators to enter the PC3/4 environment. Intermittent and inaccurate CBT monitoring can delay recognition of disease and reduce the scientific value of the data collected. Telemetry systems that provide remotely accessible real-time CBT data may overcome these limitations.

Aim: To investigate the suitability of a novel intraperitoneal telemetry implant as a refinement to study design for experimental studies utilising the ferret model.

Materials and Methods: Four female ferrets were surgically implanted with a temperature-sensing telemetry device (Anipill®, DSI) via flank laparotomy and simultaneously administered a subcutaneous temperature-sensitive microchip. Animals were monitored for an 18-day period following surgery. All ferrets were then challenged with 10^6 EID_{50} Highly pathogenic avian influenza H5N1 via the oronasal route. Subcutaneous microchip temperatures were collected twice daily and the implants recorded temperature at 5 minute intervals throughout the experiment. Data was accessed remotely once daily. Bland-Altman limits of agreement analysis was performed to compare microchip and implant temperature data.

Results: No clinical complications were recorded in the post-operative period, with surgical wounds healing successfully within 7 days. At necropsy, 2 implants had adhered to the incision site in a superficial position, whilst the remaining 2 implants were found free and deep within the abdominal cavity. The telemetry system provided remotely accessible temperature profiles for all ferrets throughout the experiment. Fever (≥40.1°C) was first recorded by the implants 13-18 hours post-challenge in all 4 animals, and despite some fluctuations temperatures remained consistently high until euthanasia. Subcutaneous microchip data demonstrated a fever in two animals 21 hours post-challenge, and in one animal at 28 and 47 hours post-challenge. Two ferrets never recorded a fever based on microchip temperature, despite 53% of their implant temperature readings being ≥40.1°C during this period. All ferrets were humanely euthanased at 48 hours post-challenge. Intraperitoneal implant temperatures were 0.74°C (95% CI 0.60°C-0.87°C, $p = 2.7 \times 10^{-16}$) higher than subcutaneous microchip temperatures, with limits of agreement of -0.32°C to 1.72°C (Bland-Altman analysis).

Discussion: Devices were implanted without clinical complications and provided rapid detection of fever following experimental challenge when compared to subcutaneous microchips. Deep abdominal implants provided significantly higher temperature readings compared to peripheral microchips. Bland-Altman analysis indicates that microchip temperatures may be up to 1.72°C lower than intraperitoneal implant temperature, and thus may not provide an acceptable measure of CBT.

Conclusion: The study has demonstrated proof-of-concept for the use of a novel telemetry system for the monitoring of CBT in experimental ferrets.

Introduction

The CSIRO Australian Animal Health Laboratory is a high-containment diagnostic and research facility. Much of the research conducted at AAHL utilises high risk group pathogens, including *Filoviruses (Ebolavirus)* and zoonotic influenza viruses. These agents pose a significant zoonotic risk to those involved in conducting the research, and as such this work is conducted at high levels of physical containment (PC). Ferrets are a commonly utilised model for infectious disease research at AAHL, due to their natural susceptibility to infection with many zoonotic viruses.

Core body temperature (CBT) monitoring of animals during infectious disease challenge studies is an important tool for monitoring animal health and wellbeing. Additionally, temperature monitoring pre- and post- infection can provide vital supplementary data for assessing study outcomes, such as time to infection and vaccine efficacy.

Monitoring of CBT in ferrets at high PC levels poses some logistical challenges. Traditional methods of rectal probing are not acceptable due to the need to handle the animals, which poses an unacceptable risk to animal staff. In place of rectal probing, current practice at AAHL is to monitor temperature through the use subcutaneous temperature-sensing microchips.

The use of subcutaneous microchips has a number of limitations. Subcutaneous microchips measure peripheral rather than core body temperature, which is potentially subject to changes in environmental temperature, animal behaviour and changes in peripheral blood flow. Additionally, subcutaneous microchips only provide time-point data and still require operators to enter the PC3/4 environment.

Such intermittent and inaccurate CBT monitoring could potentially delay recognition of disease. This may in turn reduce the scientific value of the data collected and could potentially impact on animal welfare outcomes. Telemetry systems that provide remotely accessible, real-time, continuous CBT data may overcome these limitations.

The Anipill® System

One such system (Anipill®, DSI) was recently investigated as a potential refinement for temperature monitoring in ferret studies at AAHL . Anipill® implants were traditionally developed as an ingestible CBT monitor for human patients, however some previous work in mice has demonstrated that they may be suitable as a surgically implantable device for temperature monitoring in experimental animal subjects.

The devices record temperature at predefined intervals (30 seconds, 2 minutes, 5 minutes or 15 minutes) and transmit this data in real time via radiofrequency to a monitor placed within the animal room. The monitor can display the most recent temperature reading or it can be connected to a laptop computer via a USB cable to display a real-time graphical representation of temperature trends. The laptop computer can be remotely accessed from outside the animal room, provided an internet connection is available.

In order to examine the suitability of this system for monitoring temperature in experimental ferrets, a proof-of-concept study was conducted.

Functionality in the Animal Room Environment

Prior to implanting the devices into live animals, their functionality in the AAHL research environment was assessed. Implants and the monitor were placed in various positions within the room and the ability of the device to communicate with the monitor was assessed. This analysis revealed that wire caging significantly impaired the functionality of the system. In contrast, Perspex panels on cages appeared to allow adequate communication to occur. These findings shaped the room configuration selected for the animal study, in which monitors were placed only along the Perspex sides of the cages.

Surgery (Day 0)

Four female ferrets were surgically implanted with an Anipill® device via flank laparotomy (2 x left approach, 2 x right approach). Full aseptic technique was used, and the surgery was performed under an intramuscular general anaesthetic regime of Medetomidine 0.03 mg/kg, Ketamine 5 mg/kg and Buprenorphine 0.02 mg/kg. A three-layer closure was performed using a simple continuous pattern of 4/0 PDM with staples or intradermal sutures applied to the skin layer. All animals were simultaneously administered a subcutaneous temperature-sensitive microchip. Anaesthesia was reversed with atipamezole. The average surgery time was 18.5 minutes (range 15-20) and perioperative medications included Meloxicam 0.2 mg/kg SC, Procaine Penicillin G/Benzathine Penicillin G 15mg/kg IM and an additional dose of Buprenorphine 6-8 hours post induction.

Post-operative period (Day 0-7)

Animals were monitored for a period of 7 days following surgery to document any complications arising from device implantation. Anipill® temperature data was reviewed daily and subcutaneous microchip temperature data collected twice daily. No clinical complications were recorded in the post-operative period, with surgical wounds healing successfully within 7 days. In the immediate 12 hours post-surgery, higher temperatures (39.0-40.0°C) were recorded by the Anipill® implants, however these rapidly stabilised to fluctuate between 37.0 – 39.3 °C by 24 hours post-surgery. No perceptible change in behaviour was noticed following surgery, however one implant was noted to be palpable in a superficial location close to the surgical site of implantation.

Normal Temperature Monitoring (D8-17)

Following the post-operative period, animals were monitored for a further 10 days to gather data on normal temperature patterns in ferrets housed under experimental conditions. Once again, Anipill® temperature data was reviewed daily and subcutaneous microchip temperature data collected twice daily. This revealed 6-10 significant fluctuations of +/-2°C in body temperature

throughout the day, with fluctuations of 1.5°C commonly occurring within a 2 hour period. During this period, no animals reached the traditional fever threshold used at AAHL of 40.1°C and it was identified following visual examination of the data that microchip temperatures trended lower than Anipill® implant temperatures. No discernible circadian rhythm was identified.

Challenge Phase D18-20

On day 18, all four ferrets were anaesthetised and challenged with a 10^6 EID_{50} dose of Highly Pathogenic Avian Influenza H5N1 (A/Vietnam/1203/2004) via the oronasal route. The purpose of the challenge phase was to assess the performance of the implants during infection and to determine if the implants could detect fever earlier than traditional temperature sensing microchips.

Following challenge, all four animals recorded a fever (≥40.1°C) within 12-19 hours based on the readings of the Anipill® implant. In contrast, only 2 animals demonstrated a fever at 24 hours post-challenge based on a subcutaneous temperature-sensing microchip reading, whilst 2 animals never recorded a fever using this monitoring method despite 53% of their implant temperature readings being ≥40.1°C during this period. The Anipill® data suggests that fever was sustained in all animals (despite some fluctuations), whilst the microchip data suggested that fever was transient in some cases. Importantly, over 600 data points were captured per animal within a 48 hour period from the Anipill® system, whilst only 4 data points were captured using the traditional microchip scanning system.

All animals reached humane end point at 48 hours post challenge, displaying clinical signs consistent with those seen in previous studies with this model.

Necropsy and Histopathology

At necropsy two implants had adhered to the incision site in a superficial position, whilst the remaining two implants were found free and deep within the abdominal cavity.

Histopathological examination of the implantation site was performed and revealed a mild, locally diffuse infiltration with lymphocytes, plasma cells and eosinophils in the mesentery around the implant. This is consistent with a mild foreign body reaction, as would be expected with any device implantation. There was no evidence of any extensive inflammatory response.

Comparing Simultaneous Anipill and Microchip Temperatures

A Bland-Altman limits of agreement (LOA) analysis was conducted to assess whether subcutaneous microchip temperatures 'agree' with the intraperitoneal Anipill® implants. The acceptable limits of agreement was set at +/-0.5°C. If subcutaneous microchips temperatures agreed with intraperitoneal Anipill® temperatures, then would represent an appropriate means of measuring core body temperature.

Deeply implanted intraperitoneal implant temperatures were on average 0.74°C (95% CI 0.60°C-0.87°C, $p = 2.7 \times 10^{-16}$) higher than subcutaneous microchip temperatures, with limits of agreement of -0.32°C to 1.72°C (Bland-Altman LOA analysis). This demonstrated that subcutaneous microchips provide both a lower and a highly variable estimate of body temperature when compared to deeply implanted Anipill® implants. As such, they may not provide an appropriate means of measuring core body temperature in experimental ferrets.

Advantages of intraperitoneal telemetry for temperature monitoring

The study demonstrated that intraperitoneal telemetery can provide improved animal monitoring in ferrets during challenge studies at high levels of biocontainment. The implants can provide an earlier and more sensitive recognition of fever, and provide a more accurate measure of core body temperature when compared to traditional means of temperature monitoring. Improved data quantity and quality allows the investigator to have a more complete of temperature trends and fluctuations in their experimental subjects, which may lead to improved decision making regarding humane end points and a better understanding of the animal model in use. Ultimately, this refinement may result in improved animal welfare outcomes and more efficient use of animals in research.

Limitations

The system does have some limitations, including:

- The surgeon must ensure deep implantation of the implant in order to achieve an accurate core body temperature estimate;
- The research environment must be suitable and not interfere with the implant signalling;
- Implantation is labour intensive and requires significant planning, especially in facilities in which surgery is not regularly performed;
- Implants require sterilisation following activation, which can be achieved with the use of cold sterilants;
- There is a significant capital investment required (Monitors cost approximately $7000).

Conclusion

The study has demonstrated proof-of-concept for the use of a novel telemetry system for the monitoring of CBT in experimental ferrets.

Welfare Management of Pigs Undergoing Gastrointestinal Surgery

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The University of Auckland recently ran its first chronic porcine study. This occurred as a pilot study on three 12-18 week old gilts that underwent gastrointestinal surgery, followed by a two-week recovery and monitoring period. Various recordings needed to take place over the monitoring period, and cooperation from the pigs was essential. We did not want to give pain relief in an injectable form, and ideally wanted to make use of a transdermal patch. After reviewing the existing literature on pain relief in laboratory pigs (of which very little was available that used transdermal patches) and speaking with experts in the field of human gastrointestinal surgery, we eventually decided on a multimodal pain relief regime, using oral acetaminophen, oral meloxicam and a fentanyl transdermal patch. Due to the inherent risk of stomach ulcers in growing pigs and potential stress from the surgery, we gave omeprazole to reduce the chance of this occurring. Bupivacaine was used to infiltrate the body wall incision sites, at the time of surgery. A specially tailored post-gastrointestinal feeding regime was instituted and refined over the course of the pilot study. Enrichment and behavioural training were implemented, to decrease the chance of stress in the first place. Behavioural training was carried out several times daily, commencing one week prior to the surgery. Behavioural training was accomplished using positive reinforcement techniques. This allowed recordings to occur with minimal restraint and a restraint sling that was purchased did not need to be used. Many enrichment items (both food and non-food items) were trialled, creating a hierarchy of food enrichment items and favourite toys for each animal. Training went well, and the pigs learned quickly. Individual pigs expressed personal preferences for certain foods and toys. However, the pilot cohort unanimously enjoyed apples, broccoli, and fruit-flavoured gelatine as food-based enrichment, and inflatable balls as toys. The end result was three pigs with well controlled pain who successfully contributed the fields of human and veterinary medicine. Completion of this pilot study is encouraging for the success of the main study. Due to its success, additional recording types will be able to occur, further increasing the knowledge gained. The multimodal anaesthesia used here was well tolerated by the pigs and was effective for pain control, as evidenced by objective and subjective parameters. The training and enrichment implemented worked well, as evidenced by various parameters. All aspects of welfare management for the pigs will continue to be refined, as the main study progresses.

[i] NHMRC's 2018–2019 Corporate Plan https://nhmrc.gov.au/about-us/publications/nhmrc-corporate-plan-2018-2019

No Manuscript was received for this presentation