anzccart
HUMANE SCIENCE

2016 ANZCCART Conference

"Man or Mouse"

Conference Proceedings

Tuesday 19th to Thursday 21st July

Melbourne, Victoria

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Dr Geoff Dandie
Mr Peter Maley
Mr Phil Franchina
Ms Amanda Errington
Ms Faye Bulled
Ms Chris Wadey

We would also like to acknowledge the support of the following organizations that have helped make this conference possible:

2016 ANZCCART Conference Programme

Tuesday 19th July 2016

9.00am	**Conference Registration Desk Opens**
9.00 – 10.00 am	Tea and Coffee available for all delegates in the conference foyer area
10.00am	**Conference Opening**
Session Chair	*Geoff Dandie (CEO, ANZCCART)*
10.30am	**James Bourne**- The Future of non-human primate research in Australia
11.00am	**Trichur Vidyasagar –** The deep ethics of animal use
11.30am	**Dani Maver / Mandy Errington** – Implementation of Section 6 of the Australian Code for the Care and Use of Animals for Scientific Purposes
12.00 – 1.00pm	**Lunch**
Session Chair	*Geoff Dandie (CEO, ANZCCART)*
1.00pm	**Marika Ley** – Compassionate animal care using dogs as the demonstrative animal
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	*Marc Rands, (Executive Officer, ANZCCART New Zealand)*
3.30pm	**Megan Wallace** - Seeing is believing; visualising the first breaths of life
4.00pm	**Paloma White** - Man or Mouse? Dualities and hierarchies in animal research ethics
4.30pm	**Nita Harding** - A Good Death in Field Trials
5.00pm	**Pete Hodgson** - Development of a Concordat on Openness on Animal Research in Australia/New Zealand
6.00pm – 8.00pm	**Cocktail Function**

Wednesday 20th July

Session Chair *Phil Franchina (Member, Local Organizing Committee)*

9.00am **Aeron Hurt** – Influenza and other viruses in Antarctica – who knew that penguins 'flu?

9.30am **Ding Oh** – A novel video tracking method to evaluate the effect of influenza infection and antiviral treatment on ferret activity

10.00am **Morning Tea**

Session Chair *Amanda Errington (Member, Local Organizing Committee)*

10.30am **Tony Hannan** – Environmental enrichment and experience-dependent plasticity in mouse models of brain disorders

11.00am **John Moody** – Improving Animal Health and Welfare in the Production of Snake Antivenom in Myanmar

11.30am **Andrea Britton** – Rabies vaccine developments and use in the global elimination of dog-mediated human rabies by 2030.

12.00 – 1.00pm **Lunch**

Session Chair *Geoff Dandie (CEO, ANZCCART)*

1.00pm **Rob Gration** – What if drones could be used for good instead of evil?

1.30pm **Workshop 2 - Mixed Group Discussions by Topics**

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

2.30pm **Workshop Reports to Delegates**

3.00pm **Afternoon Tea**

Session Chair *Peter Maley (Member, Local Organizing Committee)*

3.30pm **Yugeesh Lankadeva** - An ovine model for studying the pathophysiology of cardiovascular and renal failure in septic shock

4.00pm **Jess Nithianantharajah** - Mice and touchscreens – advancing rodent behavioural testing

4.30pm **Deirdre Burke & Melissa Lindeman** - Let's discuss: Standardisation of training in Australia

5.00 pm End of formal sessions for day 2

7.00pm **Pre-Dinner Canapes and Drinks & Conference Dinner** at Encore

Thursday 21st July

Session *Pete Hodgson (Board Chair, ANZCCART New Zealand)*

9.00am **Steve Petrou** – The beginning of the end for the lab mouse? Computational and stem cell approaches to modelling neurogenetic disorders.

9.30am **John Schofield** – Gatekeeper benchmarking for AEC animal advocacy

10.00am **Morning Tea**

Session Chair *Faye Bulled (Member, Local Organizing Committee)*

10.30am **Paul Adlard – Model Systems for Alzheimer's disease research**

11.00am **Joel Huang** – Organization and management of ethics data

11.30am **Justine Felix** – The role in Zoos in Compassionate Conservation.

12.00 – 1.00pm **Lunch**

Moderator *Geoff Dandie (ANZCCART CEO)*

1.30pm **The Great Debate**

2.00pm **The Great Debate**

2.30pm **Conference summary and conclusion.**

3.00pm **Update on 2017 Conference**

3.30pm **Conference Ends**

3.30 – 4.00pm **Afternoon Tea**

Presentations given

on

Tuesday 19th July

The Future of Non-human primate research in Australia.

James Bourne
Australian Regenerative Medicine Institute
Monash University

Associate Professor James Bourne is currently a Group Leader at the Australian Regenerative Medicine Institute and NHMRC Senior Research Fellow. James completed his undergraduate training in Biochemistry (Hons) at Imperial College of Science, Technology and Medicine, London. Following this, he pursued a PhD in the field of Neuropharmacology at King's College, London. Subsequent to this he came to Australia to undertake a Postdoctoral position at the Vision, Touch and Hearing Research Centre at UQ and subsequently moved to Monash University (Clayton) in 2000. In 2009, James accepted a position at the newly founded Australian Regenerative Medicine Institute and in 2014 was recipient of a NHMRC Senior Research Fellowship. James' research focuses on brain plasticity and development, in an attempt to alleviate the symptoms of stroke and other brain injuries and disorders. Finally, he has published over 50 papers, is on the Editorial Board of 5 journals and is a Member of the NHMRC Research Committee.

For his presentation, James will discuss his recent foray into the ethical debate surrounding the use of nonhuman primates in biomedical research. This was sparked following the introduction of a Private Members Bill in the Australian Senate late last year by the Green's Senator, Lee Rhiannon and resulted in James presenting at the Public Enquiry. This has subsequently resulted in James writing pieces for The Guardian newspaper, and presenting at the Ethics Centre IQ2 Series debate in Sydney, amongst others. While being an advocate for nonhuman primate research, James will also discuss the ethical challenges raised in the context of his own research and present the argument as to why research involving primates is still necessary for many areas of biomedical research for understanding human health and disease processes.

No Manuscript was provided for this presentation

The deep ethics of animal use

Trichur Vidyasagar
Department of Optometry and Visual Sciences at University of Melbourne and
Melbourne Neuroscience Institute, The University of Melbourne

Professor Trichur (Sagar) Vidyasagar heads up the Visual & Cognitive Neuroscience Unit in his department. Sagar completed his undergraduate studies and internship in Medicine at the University of Madras, India. His research into vision during these studies aborted a lucrative career as a doctor and he went on to pursue a PhD in neuroscience at the University of Manchester, England. Subsequently he worked at the Max-Planck Institute for Biophysical Chemistry, Goettingen, Germany and at the Australian National University before moving to the University of Melbourne in 2002. Sagar's research into the neural basis of visual perception, attention and memory has involved using a number of species – cats, rats, monkeys, wallabies, tree shrews and humans, depending upon the suitability of the species for the particular scientific question. His basic research into visual attention has also led him to identify crucial neural mechanisms that have possibly enabled some unique human abilities such as reading. He has published around 80 peer-reviewed articles.

Sagar will discuss in his talk the overarching ethical principle of "least harm" that should underpin all animal use, scientific and non-scientific, and which can be consistently applied across the different purposes for which they are used. Why is animal research, including a small amount of non-human primate research, an ethical imperative? Why are alternatives, though very useful, still limited without use of whole animals in research? Why is basic research necessary? What are the ethics of not using the great apes and humans for invasive research while we continue to use monkeys and other animals? Finally, he will contrast the use of animals in research with their use for food and argue that reducing meat and dairy consumption is also an ethical and environmental imperative.

No manuscript was provided for this presentation

Implementation of Section 6 of the Australian code for the care and use of animals for scientific purposes 8th Edition (Independent external review of the operation of institutions) in Victoria.

Mandy Errington
Senior Project Officer - Licensing and Audit
Agriculture, Energy & Resources | Agriculture and Rural Division
Department of Economic Development, Jobs, Transport and Resources
475 Mickleham Road ATTWOOD Vic 3049

The Department of Economic Development, Jobs, Transport and Resources (DEDJTR) regulates the use of animals in scientific procedures and the breeding of specified animals in Victoria. Organisations or individuals wanting to use animals in research, testing or teaching in Victoria must hold a scientific procedures premises licence or scientific procedures fieldwork licence. Breeding of specified animals (guinea pigs, rats, mice and rabbits other than rats, mice and rabbits bred in their native habitat, and non-human primates) for use in research or teaching must be conducted under a specified animal breeding licence.

In late 2016, DEDJTR is proposing to introduce a revised audit program that will allow Victorian institutions to meet their obligations for independent review in line with the *Australian code for the care and use of animals for scientific purposes, 8th edition.*

The audit program will assess:

i. the licence holder's application of the governing principles of the Australian Code, including the resourcing and effectiveness of the institutional program to promote and monitor compliance;
ii. how participants, including the organisational representatives, AEC members, animal carers, investigators and teachers involved in the care and use of animals for scientific purposes are meeting the responsibilities identified in:
    - the licence conditions and mandatory codes;
    - AEC processes, approvals and conditions;
    - internal policies and procedures;
iii. competency, training and competency assessment of all people involved in the care and use of animals for scientific purposes; and
iv. animal care and facilities.

This presentation will give a brief overview of how DEDJTR proposes to encourage best practice through communication, benchmarking, audit ratings and by introducing an expert audit advisory panel.

No manuscript was submitted for this presentation

"Compassionate animal care using dogs as the demonstrative animal. "
Dr Marika Ley (Animal Behaviour) with assistant Jess Busuttil as "Dog"
Principal Veterinarian, Caroline Springs Veterinary Hospital.

All animals respond to stress. Reptiles, birds and mammals all have the same stress response pathways in the brain involving the autonomic nervous system (note how this 'dog' is startled and jumps when it hears a loud noise) and the hypothalamic pituitary axis (cortisol levels increase). Ultimately the stress response is a survival mechanism and allows an animal to prepare to fight or flee to keep alive. Stress is modulated by negative feedback to the brain so that an animal does not maintain this state for extended periods. Extended, chronic stress or distress is detrimental to the long term health of an animal. Blood glucose levels, blood pressure and heart rate are all elevated. The stress hormone cortisol circulates at higher levels and can shut down the negative feedback loop. These can all contribute to health problems and affect an individual's behaviour.

An important part of animal welfare is to minimise stress. As a vet of 21 years and having worked with many species, it has been essential to reduce stress and care compassionately with all the species. My "Dog" and I will give a demonstration of the way we would attempt to minimise stress in a research facility drawing on experience in the veterinary clinic. Using the beagle dog breed as an example, we will show how we would attempt to make "Dog's" life in research less stressful.

During this dramatized demonstration we will show how animals can be conditioned to their environment in various ways that will help reduce stress during the time leading up to their entry into a research trial and while they are part of that work. Such factors as regular exercise, food and accommodation can all play a part in that acclimatisation process, as can familiarisation with the people will whom they will be working.

We will also show how important it is for those people working with animals to become familiar with the natural behaviours exhibited by each animal, so they can be aware of changes that may indicate stress or changes in the wellbeing of their animals.

We have found in the vet clinic that by reducing animal stress this, in turn, reduces staff and handler stress and improves the experience and animal-human bond for all involved.

How can the handling of animal species you work with become more compassionate? Take some time, reassess techniques, allow animals a choice where possible and reward with high value motivators- usually food.

All animals respond to stress. Reptiles, birds and mammals all have the same stress response pathways in the brain involving the autonomic nervous system (note how this dog is startled and jumps when it hears a loud noise) and the hypothalamic pituitary axis (cortisol levels increase).

Ultimately the stress response is a survival mechanism and allows an animal to prepare to fight or flee to keep alive. Stress is modulated by negative feedback to the brain so that an animal does not maintain this state for extended periods.

Ask DOG to sit.

Extended, chronic stress or distress is detrimental to the long term health of an animal. Blood glucose levels, blood pressure and heart rate are all elevated. The stress hormone cortisol circulates at higher levels and can shut down the negative feedback loop. These can all contribute to health problems and affect an individual's behaviour.

An important part of animal welfare is to minimise stress. As a vet of 21 years, and having worked with many species, it has been essential to reduce stress and care compassionately with all the species I come across. This includes gerbils, mice and rabbits as common pets when practicing in the UK to ostriches and alpacas, along with cattle and horses in mixed practice to now predominantly cats and dogs in small animal general practice.

My "Dog" and I will give a demonstration of the way we would attempt to minimise stress in a research facility drawing on experience in the veterinary clinic.

Understand the species you are working with.

Is it a predator or prey species?
Rabbit or mouse- prey
Cat-predator and prey
Dog-predator

Is the animal a social species or evolved as solitary? Dogs social groups are people or other dogs. Cats have smaller social groups and can live solitary.

How does the animal use its senses? The species perception of their environment will determine how they react. With rodents and canines their sense of smell is supreme. Vision is the key sense for birds.

Using the beagle dog breed as an example, we will show how we would attempt to make "Dog's" life in research less stressful.

Let's assume "Dog" is born in the facility to a bitch chosen for her calm temperament. She has an uncomplicated pregnancy and has been living in a comfortable environment with positive enrichment, good nutrition and has regular exercise. "Dog" is one of 8 puppies born in an uncomplicated whelping.

From birth to 2 weeks (neonatal period) the pups are reliant on the bitch and use their sense of smell and touch. They benefit from receiving mild physical stressors such as being handled

away from the litter for a few minutes a day. Pups handled early in this way are more likely to handle stress and learn more quickly later in life.

From 2-3 weeks of age rapid behavioural and physical changes occur with improved locomotion and vision and hearing senses developed. Taste improves and ingestion of solids starts. Pups begin to explore away from the nest and interact with litter mates. Novel stimuli should be introduced during the few minutes of handling for each puppy. Create an area of environmental enrichment with various hung up objects and different surfaces for the pups to explore taste, texture and sensations.

Canine socialization from 3-7 weeks occurs with the pups learning bite inhibition, play and body language communication from the bitch and other litter mates. Continue to expose pups to handling, a variety of noises, objects and floor surfaces.

Human socialization from 7-12 weeks occurs within this period of rapid learning. Pups would be fully weaned from 8 weeks and taken away from the bitch. Remember dogs are social animals and need to learn to cope for periods alone. Provide some bedding material smelling of the bitch or use *Adaptil* calming pheromone (synthetic form of dog appeasing hormone from bitch mammary secretions), comfortable rest area and motivating food dispensers during short times pups are away from people and other dogs.

"Through a dog's ear" classical piano music has been useful in our clinic to calm dogs.

Positive experiences during this time have lasting effects. Consider all forms people could take from a dog's perspective. Expose pups to people walking slowly, fast, leaning over, staring, wearing a hat, glasses, backpack, carrying a bag etc.

Use portions of the pups daily food calories as food rewards fed and therefore associated with each of these experiences.

At 12 weeks old "Dog" enters to start a research project of expected 12m duration. She is comfortable around other dogs, loves people and is used to the noises, smells, lights and floor surfaces around the facility.

Despite all best efforts to breed even temperament dogs there is genetic variability. After all, we are not the same as our sisters and brothers. Is there a selection criteria or temperament test pups must pass to be accepted into a project? Would a timid puppy be better suited placed into a home pet environment?

Consider what environmental conditions "Dog" will live in and what procedures will be performed during the project. Verbal praise goes a long way low soothing tones versus high pitched fast. Watch how "Dog is tense and alert with a loud fast noise and then relaxes with a soothing tone. Teaching new things use strong motivators-food. Phase out food and use verbal rewards when a cue is well learnt. Predictability and routine reduce stress. Try to establish schedules for feeding, exercise, play and procedures. Handlers could always walk on dog's right side and use the same cue word to start walking and stop etc. Demonstrate heel on left walk and turn then sit when stop and reward.

Teach "Dog" to sit and be still for a blood collection. Consider breaking this down into small steps over multiple training sessions if time permits and for more complex behaviours taught.

Observe dogs body language.

Note how "Dog" is unsettled and hypervigilant, licks the tip of her nose and yawns often and has her ears back. These are subtle ways she is trying to communicate to us she is uncomfortable. This is different and new to her and she is unsure.

She may have had 2 people near her before but not holding her so closely. Stop, observe, reassess, continue.

I give her space, invite her to choose to come over for a treat and asking her to perform a "sit" reassures her as she understands what that means. Note her ear position changes to forward and her body is not as tense. Giving dogs a choice can reduce anxiety.
Note how I approach "Dog" squatting down and ask her to approach, taking time and then approach her side.

We use calming caps in the vet clinic and this could be used when initially teaching dogs to calm for a procedure. Note that when I place the cap and reduce the visual sense this settles "Dog".

The music "through a dogs' ear could play in the background to counteract harsh noises that often startle dogs.

Take care to support dogs well ensuring they are balanced and do not slip. Use the soothing low tone of voice. Observe how "Dog" settles when approached and is held confidently and sits for her leg to be clipped. She rests and recovers before a tourniquet is applied and the blood sample is taken.

After the procedure, "Dog" is allowed to settle at the end of her lead in loose restraint and praised. Note how "Dog" shakes her body. Dogs often perform this shake off behaviour when they are recovering from a period of stress.

"Dog" can then get a treat when asked to sit and can then be walked back to her pen.

We have found in the vet clinic that by reducing animal stress this, in turn, reduces staff and handler stress and wellbeing, improving the experience and animal-human bond for all involved.

How can the handling of animal species you work with become more compassionate? Take some time, reassess techniques, allow animals a choice where possible and reward with high value motivators- usually food.

Useful websites for dogs

ThroughADogsEar.com -calming music for dogs.

drsophiayin.com -information on low stress handling techniques and understanding dog body language.

Ceva.com.au -*Adaptil* is a preparation of calming pheromones for dogs

Body Language of Fear in Dogs

Slight Cowering

Major Cowering

More Subtle Signs of Fear & Anxiety

Licking Lips
when no food nearby

Panting
when not hot or thirsty

Brow Furrowed, Ears to Side

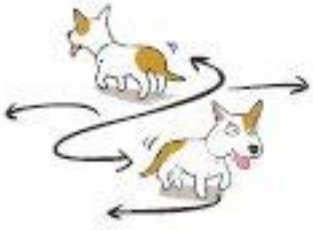
Moving in Slow Motion
walking slow on floor

Acting Sleepy or Yawning
when they shouldn't be tired

Hypervigilant
looking in many directions

Suddenly Won't Eat
but was hungry earlier

Moving Away



Pacing

Dr. Sophia Yin, DVM, MS | *The Art and Science of Animal Behavior* | (888) 638-9989 www.drsophiayin.com

Seeing is believing; visualising the first breaths of life

Megan J. Wallace
The Ritchie Centre, Hudson Institute of Medical Research, and Department of Obstetrics and Gynaecology
Monash University, Melbourne, Australia
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Background: The time of birth is considered to be the largest physiological challenge that most of us will ever experience during our lifetime. The lungs undergo the most significant change. Before birth, the placenta performs the role of gas exchange and the lungs of the fetus are completely filled with liquid. At birth, babies must clear the liquid from their lungs and fill their lungs with air in order to take on the role of exchanging gases, a role the lungs have never performed before. They must perform this new role efficiently within minutes, or the baby will be at risk of brain injury, lung injury and injury to other organs. Babies that are born prematurely or with immature lungs, have difficulty taking on this role so they often require respiratory support. This is a life-saving intervention, but it can damage the lungs and lead to long-term lung disease. We have been using synchrotron X-ray imaging to "see", how the lungs fill with air at the time of normal birth and to compare how effective different types of respiratory support are for aerating immature lungs. This is a process that cannot be mimicked in cell culture and cannot be simulated because we do not yet understand enough about the fundamental processes involved.

Methods and Results: All experiments have been performed at the Spring-8 synchrotron in Japan. We use pregnant rabbits for these experiments and we deliver kittens either close to term (31 days gestation) or preterm (~28 days of gestation) by caesarean section. Rabbits are used because: the lungs are at a similar stage of development as human lungs at the time of birth, they are small enough that we can image the entire lung at a high resolution, and they are large enough to ventilate using strategies similar to those used in humans. Rabbit kits are either allowed to breathe spontaneously or are sedated, intubated, delivered and ventilated. Data will be presented from a number of different ventilation scenarios demonstrating how effectively (or ineffectively) different ventilation strategies are for aerating the lung at birth.

Conclusion: This imaging technique allows us to identify which resuscitation techniques most effectively aerate the lung at the time of birth. The video's that have been produced as a result of these experiments have already changed clinical practice all around the world and are currently being used to train paediatricians and midwives how to effectively resuscitate premature babies at the time of birth.

Funding Sources: National Health and Medical Research Council of Australia, Australian Research Council, International Synchrotron Access Program managed by the Australian Synchrotron, Victorian Government's Operational Infrastructure Support Program and the Spring-8 synchrotron, Japan Synchrotron Radiation Research Institute, Japan

No manuscript was submitted for this presentation

Man or Mouse? Dualities and hierarchies in animal research ethics

Paloma White
PhD Candidate, Department of Anthropology and Development Studies, The University of Adelaide, South Australia

The theme of this year's conference, 'Man or Mouse', is undoubtedly intended to provoke delegates to think critically about the Three Rs – Reduction, Refinement, and particularly, Replacement. Replacement is often a hard principle to enforce, given animal studies are often required by regulatory bodies as an intermediary step before human clinical studies. Replacement is also complicated in the contexts of wildlife and veterinary research where the research being done is for animal rather than human benefit, therefore necessitating the use of animals for meaningful outcomes.

To complicate matters, the term 'animal' is socially constructed through not only the community's understanding of what an animal is, but also what The Code defines an animal to be, along with other legislative definitions of the term. Many of these socially constructed notions of animals, and our relationships with them, are steeped in history. Aristotle classified animals according to their complexities: with blood or without; quadrupeds or winged; with a skeleton or without. Linnaeus, the forefather of our modern biological classification system, drew on Aristotle when he classified animals as per kingdom, phylum and class.

Implicit, and sometimes explicit, in these understandings of what it is to be an animal (and therefore the inverse; human) are two things: Firstly, a human-animal dualism, bringing with it an inherent tension between humans and animals; each moving in opposite directions; Secondly, a hierarchy, placing humans at the top of the biological order, followed by animals, themselves classed according to their 'status'. Taxonomical arrangements for the human-animal entanglement (including dualities and hierarchies) imply that animals and humans are not only biologically different, but socially and morally different, bringing with it the implication that it is therefore ethical to treat animals differently based on their difference to humans. Meanwhile, animals are used in biomedical research for precisely the opposite reason: their remarkable resemblances to us.

This paper will draw out some of these tensions, to show how they may complicate AEC practice and the implementation of the Three Rs, particularly Replacement. It will argue that until we begin to understand the human animal entanglement as an intricate web rather than a dualistic or hierarchical relationship, giving power to men over mice, Replacement as a goal in the animal research ethics debate will not progress. It is not until we can begin to understand the human animal entanglement as something other than a power dynamic, that animal ethics and welfare will progress for the betterment of man AND mouse.

Introduction

The theme of this year's conference, 'Man or Mouse', is undoubtedly intended to provoke delegates to think critically about the Three Rs: *Reduction*, *Refinement*, and particularly, *Replacement*. Replacement is a hard principle to enforce given animal studies are often required by regulatory bodies as an intermediary step before human clinical studies. Replacement is also complicated in the contexts of wildlife and veterinary research where the research being done is for animal rather than human benefit, therefore necessitating the use of animals for meaningful outcomes.

To complicate matters, the term 'animal' is socially constructed through the community's understanding of what an animal is and what The Code defines an animal to be, along with a number of legislative definitions of the term across the country. These definitions are socially constructed and steeped in history. Aristotle classified animals according to their complexities: with blood or without; quadrupeds or winged; with a skeleton or without. Linnaeus, the forefather of our modern biological classification system, drew on Aristotle when he classified animals as per kingdom, phylum and class.

Implicit in these understandings of what it is to be an animal (and therefore the inverse; human) are two things: firstly, a human : animal dualism, bringing with it an inherent tension between humans and animals; and secondly, a hierarchy which places humans at the top of the biological order followed by animals classed according to their 'status'. These taxonomical arrangements suggest that animals and humans are not only biologically different but socially and morally different. There is, therefore, an implication that it is ethical to treat animals differently based on these differences. Meanwhile, animals are used in biomedical research for precisely the opposite reason: their remarkable resemblances to us.

This paper draws on data obtained during an extensive period of ethnographic fieldwork within a South Australian research organisation and its animal ethics committee (AEC, henceforth, the Committee). It will show how the tensions posed by the social and biological classification of animals are manifested through the routine practices of the Committee and complicate its work in implementing the Three Rs, particularly Replacement. Using species-specific case examples, this paper will present different ways the human-animal relationship is manifested in the biomedical context. It will show how historical and philosophical assumptions about what it means to be an animal continue to shape the practice of ethics. This paper will argue that until we begin to understand the human animal entanglement as an intricate web rather than a dualistic or hierarchical relationship, which gives power to men over mice, Replacement will not progress. It is not until we can begin to understand the human-animal entanglement as something other than a power dynamic, that animal ethics and welfare will progress for the benefit of man *and* mouse.

The problem with Replacement

In biomedical research where the ultimate beneficiaries are humans, ethics – and what is *ethical* – is largely informed by a utilitarian principle of creating the best possible outcome for the greatest number (Douglas-Jones 2015; Johnson 2013b; Marks 2013;

Rollin 2006). The judgement of ethical acceptability is therefore made on a cost/benefit judgement of whether the cost to each individual animal used as a model for human illness or disease can be justified by the expected benefit to the human population. In South Australia, an explicit cost/benefit judgement forms the first part of the ethical decision-making process (Ideland 2009). Applicants are asked to explain the ethical considerations of their proposed activities:
What is the welfare cost to the animal? In what way is the level of pain/discomfort justified? How does this mesh with the cost/benefit? (DEWNR 2016, p. 8)

The aim of the cost/benefit judgement is to strike a balance between human benefit and the animal welfare cost. However, in many cases, the human benefit is the justification and this places humans in a hierarchical relationship to animals from the outset. Placing a human benefit above the cost to animals risks animals being used as a means to a human end (Kant 1997). Under this framework, animals are often considered instruments: equipment used in the advancement of human health (Dennis 2010; Johnson 2013b). However, this is in stark contrast to my observations of committee-level ethical decision making, where a popular phrase was "animals are not test tubes with tails".

The cost/benefit judgement, although being an effective way to force researchers to justify their use of animals, is not unproblematic because it ultimately places human benefit over the suffering, even if minor, of animals. If there is a benefit to human health, under a utilitarian framework, the question becomes: is it unethical not to use animals in medical research (Houde, Dumas & Leroux 2003, p. 335; Ideland 2009, p. 259)? One of my participants was critical of the cost/benefit analysis, calling it
"that dreaded cost/benefit argument… I don't like the idea of framing things in economic terms… I prefer the anguish/anguish judgment… The judgment of how much anguish is the animal going to suffer in order to alleviate the anguish of another animal?" (Committee member, interview, 2014)

This analysis is a return to an integrated bioethics, an understanding that humans are just one species of animal, doing away with the human : animal dichotomy and the power relationship that it entails. The cost/benefit judgement, using the utilitarian ethic of creating the best possible outcome for the largest number of people, is an anthropocentric approach of using animals as means to human ends. It was unacceptable to the committee members who participated in my research and contravenes the Code's governing principle of Respect.

The rub between animals being used as means to a human end and being viewed as ends in themselves is transcended through the ethics application and committee process through the Three Rs (Russell & Burch 1959). In the first instance, animals should be Replaced with other models (such as *in vitro* cell cultures or computer modelling). Increasingly, however, *in vivo* testing must be conducted as an intermediary step between *in vitro* and human clinical research as mandated by regulatory and funding authorities. In this context, there is an underlying assumption that animal models can not be completely replaced (Houde, Dumas & Leroux 2003; Johnson 2013a; Schuppli, Fraser & McDonald 2004). Nonetheless, the Three Rs underscore an approach which mandates that life, in all its manifestations (human, animal and plant), should never be regarded as means to human ends, but only as ends in themselves (Jahr 1927).

Ethical decision-making at the committee table is only implicitly informed by such philosophical reasoning. These arguments were usually only expressed by my participants outside of the committee context. While the Committee asks whether animals should be used for each specific application (that is, whether animals are needed for this research, or if they can be Replaced), the overarching ethical question of whether animals should be used at all is rarely entered into at the committee table. Instead, the Committee's focus is on the cost/benefit judgement and the Two Rs – *Reduction* and *Refinement* (Johnson 2013b) – as a way to ground the abstract philosophy into everyday practice.

Normative and abstract ethics aside, at the heart of these tensions sits the human tendency to classify the world around us in order to simply understand it. Central to everyday committee practice (and the subject of this paper) is, to borrow from Durkheim and Mauss (1963), a 'primitive' set of classifications: human and animal. The mere fact that research applications are considered by 'animal ethics committees', rather than simply 'ethics committees', demonstrates a divide between human and animal. The divide is culturally and temporally determined and is reinforced through the research community's governing texts, documents, policies and legislation. The framework by which we decide what an animal is (and therefore what a human is) determines the scope of AECs: what work they will and will not consider, let alone approve. Before presenting some instances where this conceptual divide is perpetuated, it is important to firstly address the issue of, 'What is an animal?'

What is an animal? An archaeology of classification

Aristotle's classification system was written in the 4th century BC and is one of the earliest recorded animal classification systems in the western world. A large portion of Aristotle's system is dedicated specifically to humans, but his approach is more integrative than divisive. Aristotle looked not only at the differences and similarities between species of non-human animals, but also the similarities between humans and non-humans. Aristotle's system classified animals into those with blood and those without, those which bore live young and those which lay eggs, those which flew, and those which didn't (Aristotle 2000). This system included humans in the realm of the 'animal', albeit a special kind of animal. For Aristotle, what sets humans apart from animals is an ability to exert free will:

But of all animals, man alone is capable of deliberation. … Many animals have memory, and are capable of instruction; but no other creature except man can recall the past at will. (Aristotle 2000, Book I, 1).

While not providing a hierarchy of beings, Aristotle's explicit detail on the human form, implies humans are not only different, but they are exceptional in some way, allowing humans to dominate animals.

Aristotle's classification system gave rise to The Great Chain of Being, a Judeo-Christian construction of the cosmos, which explained a hierarchical relationship between humans, animals and angels as well as dirt, rocks and plants. According to The Great Chain of Being, God sits at the top of a hierarchy with other categories sitting beneath ranked in order of divinity: angels under God; cherubs under seraphs. Humans sit below angels, but above animals which sit above plants and minerals. Animals are hierarchised according to their unique features: wild or tame; mammal, bird or fish.

Under this classification system, what sets humans apart from animals is that humans have a spirit while animals do not. Once again, humans are exceptional, are higher on the conceptual hierarchy, and therefore have

…dominion over the fish of the sea, and over the fowl of the air, and over the cattle, and over all the earth, and over every creeping thing that creepeth upon the earth. (Genesis 1:26).

Linnaeus is regarded as the forefather of our modern biological classification system of kingdom, genus and species. Building on the Great Chain of Being, Linnaeus' *Systema Naturae* (1758) included animate and inanimate objects from the natural world, where Aristotle stopped at animate life forms. What sets Linnaeus' *Systema Naturae* apart from the Great Chain of Being, however, is that it was a return to the taxonomical classification system that Aristotle observed, rather than the hierarchical system of divinity in The Great Chain of Being. Like Aristotle's system, the *Systema Naturae* was based on observable traits: whether animals swam, flew or roamed the earth; on two or four legs; with wings or hooves. While the formal classification system has done away with the hierarchical structure, elements of the hierarchy at least implicitly remain today. This continues to place animals in a diminutive position if for no other reason than they are 'non-humans' and complicates AEC practice.

What's in a name?

The South Australian *Animal Welfare Act,* draws upon Linnaeus' *Systema Naturae*, instructing us that an animal is 'a member of any species of the sub-phylum *vertebrata* except … a human being; or … a fish…' (1985, p. 3, emphasis in original: henceforth the Act). This definition, despite applying 'equally to all animals regardless of their value or status' (DEWNR 2014), implies vertebrates are more valuable than invertebrates using it as the basis of what it means to be an 'animal'. Because humans are also vertebrates, and because they are more valuable than other vertebrates, they deserve an explicit *exclusion*. Conversely, at the national level, the *Australian Code for the Care and Use of Animals for Scientific Purposes* (2013, henceforth the Code) tells us that an animal is 'any live non-human vertebrate … that is, fish, amphibians, reptiles, birds and mammals … and cephalopods' (NHMRC 2013, p. 3).

What is interesting about these definitions is not what they define as 'an animal', but what they exclude. An animal *is* a vertebrate, but humans are explicitly *not* animals, nor are worms or shellfish simply because they are invertebrates. Equally interesting is the Code's inclusion of one member of Linnaeus' corresponding *vermes* (worms) class: cephalopods. There is growing evidence to support cephalopod intelligence (Low et al. 2012) which is one reason that they are afforded a particular status which places them higher than other *vermes*, molluscs, shellfish, and insects, even though none are vertebrates. Fish are explicitly excluded from the South Australian definition despite technically being vertebrates[1]. Regardless, fish are regarded animals for the purposes of research involving animals simply because the Code is enshrined in the Act through a complex licencing framework which requires all aspects of the Code be complied with, including its definitions.

[1] Both chondrichthyes (i.e. fish with skeletons made of cartilage) and osteichthyes (i.e. fish with skeletons made of bone) are vertebrates. None of my participants were sure why fish were excluded from the Act, but many suspected a number of different social and political reasons.

Man, the Social Animal?

Debates about the ethical issues of using animals in research are often structured around two issues: whether or not animals are sentient and therefore able to feel pain or pleasure; and whether they have the capacity for a morally or socially engaged life complete with emotions, psychological abilities, beliefs and a sense of temporality (Aristotle 2000; Herzog 2002). These themes are analogous to those raised by Agamben (1998) in his discussion of the ancient Greek understanding of bare (*zoē*) and social (*bios*) life. The biological processes of a bare life, a *zoē,* are common across species but a politically and socially engaged life, a *bios,* is unique to humans. This was a point Aristotle himself made: although humans are social creatures, placing them in the same category as 'the bee, the wasp, the ant, and the crane…' (Aristotle 2000, Book I, 1), humans alone are capable of a *bios* because they have a 'sense of good and evil, of just and unjust … [of a] family and a State' (Aristotle 2015, Book 1, II). Humans, for Aristotle, are political animals. It is therefore of interest that he categorises humans among invertebrates, a category which escapes the 'animal' classification in many of our contemporary guiding documents and policies (Animal Welfare Act, 1985; NHMRC 2013), which are so symbolic of our political and social entanglements.

Following, I will present two case studies which will show how these understandings and definitions of 'animal', and what it means to be 'an animal', are socially and politically constructed in a manner which complicates the already fraught task of judging the ethical acceptability of research. In these examples, the Committee navigates between policies and procedures which tell them which species are animals, which are not, and which are more special than others. Although the Committee maintained there is no hierarchy of species in the decision-making process, an implicit hierarchy is evident at the social level which reinforces the human:animal dualism and makes the Replacement of animals difficult.

A Shellfish is not an Animal

In the following example, the Committee has invited a researcher to the meeting to discuss her recent applications to the Committee, for a mouse bioassay to test shellfish for human consumption, so-called 'shellfish toxin testing'.
The researcher entered the room and took a seat next to the Chair. The Committee began its question-answer session, with the researcher giving a verbal lay summary. She said, "We have been proactive in seeking other models [chemical assay], but the animal [mouse] model is still relevant."

Maggie looked very stressed. She was sitting with her head hanging low as she began to rub her temples. When she eventually looked up, she looked deeply troubled by the conversation.
George asked, "Can there be a humane killing endpoint, rather than allowing the mice to die a painful death?"

The researcher explained why this wouldn't be appropriate: "There is no way to detect how much toxin is being injected so a more diluted toxin may take longer to have an effect, but it will kill within one hour…" If the mouse is exposed to the toxin it will die.

The Committee was clearly uncomfortable about this, but they began to discuss pain and suffering. A committee member explained the toxin process: the test induces paralysis. The mice will eventually stop breathing as the lungs and other muscles shut down.

The researcher didn't refute this but said she has "never witnessed severe pain [in the mice]. We've seen the mice hunched in the back of the cage but they are not in pain." The room descended into a cacophony of laughter, and no member made an attempt to hide it. They were astonished at the Researcher's inaccurate assessment of mouse welfare and wellbeing. They didn't find it funny; they were shocked and greatly dismayed by the researcher's apparent inability to recognize signs of pain or distress in mice. At this point, I had only been in the field for a matter of months, but from the little that I knew, a mouse hunched in the corner of a cage is a huge alarm signal for pain and distress.

Later in the meeting, in the researcher's absence, the Committee went about assessing the project. It was duly noted that the researcher, in collaboration with the Committee had been able to Reduce the numbers by over 60%, which the Committee was very happy with. (Field notes, 2014)

Although mouse bioassay testing for shellfish toxins have been a common practice for many years, it is increasingly becoming a contentious issue specifically because the test is categorised as 'death as an endpoint' (Stewart & McLeod 2014). In this example, the researcher was aware that *in vitro* models were favoured by the Committee over the use of any animal as well as becoming a more accepted protocol within the scientific community (Stewart & McLeod 2014). She was able to pre-empt the Committee's concern over the Replacement of animals by stating that although there are alternatives the mouse model is still 'relevant'. The mouse model was therefore taken for granted by the researcher. The Replacement of mice was addressed, the Committee moved on to the 'other' two Rs: George's suggestion that humane endpoints be included in the protocol represented a significant Refinement but this was challenged by the researcher. The Committee instead stipulated more stringent monitoring of the animals and more frequent reporting from the researcher to the Committee, as well as negotiating a Reduction of numbers by over 60%.

This case study exemplifies a human:animal:non-animal trichotomy, which places humans at the top of the literal food chain. Shellfish are not animals under the Act nor the Code, but they are regarded as *food*. They are tested for food safety standards before they enter the human food chain using an animal which is not regarded as food: mice. Shellfish are placed higher than mice in the hierarchy. They are used as tools in research (because they are not animals) with the ability to cause pain, distress and death to mice. In this instance, the benefit of human food safety is placed above the wellbeing of the mice and the human consumption of shellfish is a taken-for-granted assumption. In an interesting twist, however, mice are also placed above shellfish on the hierarchy. The Committee considers the welfare of mice (animals, mammals moreover, and a species we would normally not consider eating) but not the ethical considerations of using another living species because it is a mollusc which is not considered an animal under the Act nor the Code. It is not the role of the Committee to consider the ethical acceptability of eating animals, but the fact that this discussion was not entered into at the committee meeting, nor during my one-on-one interviews on the topic, shows how

ingrained the hierarchy is to Committee practice and decision making. This case also exemplifies how the cost/benefit analysis complicates the implementation of Replacement. So long as the human benefit is adequately addressed (i.e. if toxins enter the food chain, human lives are at risk) and so long as the two Rs are addressed, then Replacement is not pursued.

A marmoset is a special animal

In the following example, a different hierarchy is evident not only humans vis-à-vis animals but also within the 'animal' category itself. In this case, animals are afforded status according to their similarity to humans.

I met Mike at 7:50am at the door to the animal facility. He explained to me that a special feature of this animal house is that it has the ability to hold marmosets which need access to outdoors. He explained the NHMRC guidelines for using non-human primates in research, giving them greater environmental enrichment and special housing conditions,
"Obviously, because they're different…"
He didn't tell me how they were different, or what they were different from.

The NHMRC guidelines on primates afforded the marmosets significantly different housing than I had become accustomed to through my participant observation in a 'sister' facility specializing in rodent research. These enclosures looked like large bird aviaries, each housing one marmoset (one enclosure had two males, a father and son). The cages had large runs which allowed the marmosets to run between cages and to the adjacent outdoor facility. Mike walked me through two doors which were under lock and key. When he opened the final door, it was light and breezy; the morning sun filtered down through the leaves of the large trees which filled the outdoor enclosure.
Mike explained the marmosets hadn't been used for about 12 months. When I asked why they hadn't been humanely killed, like a mouse would have been if it was no longer being used, he said,
"Well, you're right… But I suppose it's different for primates, we can't just euthanise them because they aren't being used, like a mouse". (Field notes, 2014)
Mike didn't articulate how or why the marmosets were different, but in a later interview, he elaborated:
"Because they're primates and people have a different view of primates […] it's a different attachment. It's that these animals have been around for years and although they're owned by a research group, they're more like your pets more so than the research animals." (Mike, interview, 2015)

For Mike, the special place that the marmosets occupied in his mind was brought about by two interrelated things. Firstly, the public's view of primates which means they aren't humanely killed at the end of an experiment. This then meant that he had grown a particular personal attachment to the species such that they could be regarded companion animals or 'pets'. This was interesting given another participant used a different companion species to justify the special place that marmosets had in the biomedical context:
"We can't just have guinea pigs growing out into their old age and dying of natural causes, we'd never get anything done [but] because they're marmosets, non-human

primates. And then they look at you with those little eyes, and you think 'I reckon he's trying to work me out'" (Committee member, interview, 2015)

For my participants, non-human primates were 'different' to guinea pigs and mice: they were viewed as companion animals capable of higher order thinking as they tried to 'work out' the intrinsically human world they were entangled within. This is an idea which is entrenched in national level policies and procedures which work alongside the Code to protect the welfare of non-human primates because, 'special ethical and welfare issues arise because of the cognitive abilities associated with their well-developed forebrains' (NHMRC 2003, p. 4). Non-human primates have their own guidelines because they hold a special place in the public psyche, as per Mike's comment "people have a different view of primates." The special place that non-human primates occupy is supported by species-specific guidelines, policies and procedures which work together to reinforce the idea that non-human primates are special, different to other animals, and therefore can't simply be humanely killed at the end of a protocol. Animal technicians are then able to form more companion or pet-like attachments because of the length of time they spend with the animals. This further entrenches the marmoset's special status and sets them apart from guinea pigs and mice. Further, non-human primates are also granted their own guidelines not only because they are *different* to other animals, but because they are *similar* to humans by virtue of their 'well-developed forebrains' (NHMRC 2003, p. 4). Their biological similarity to humans is another impetus for the special guidelines, as this similarity can pose risks to the human caretakers who are in routine contact with the 'animals'.

This similarity to humans is a delicate line. If non-human primates are *different* to other animals and *similar* to humans, why are they 'animals' and not 'humans', and, therefore, how is it 'ethical' to use them for medical research? My participants always played up the anthropomorphic features of the marmosets; something they never did for any other species. One committee member found marmoset projects problematic but thought the species-specific environmental enrichment provided to them by virtue of the supplementary guidelines, meant they had more fulfilled lives than other species. However, she commented

"I think maybe I'm a species racist, and it's perhaps more normal to think about rats and mice in that sort of experimental [context], than to think about rabbits or cats or dogs or marmosets… I would be really opposed to chimps and apes and things like that… Cos they're human. They're not human but they are a much higher intelligence, … There is a perception thing. You know, maybe they don't suffer any more than any other species, but I think that they are such a higher intelligence, and it just wouldn't feel right for me." (Committee member, interview, 2015)

Amanda's comments reiterate an animal hierarchy. Humans are at the top of the hierarchy because the research is for their benefit, but under the 'animal' category another hierarchy exists. While it may not be morally right, or speciesist, rats and mice are so engrained into the culture of biomedical research that they fall lower on the hierarchy (Dennis 2010, p. 19) than rabbits, cats and dogs. Rabbits, cats and dogs are themselves lower than the marmosets which are lower than great apes because "they're human", or at least similar to humans. Primates are particularly problematic not only because they are 'seen as bearing a metaphorical resemblance to man' (Tambiah 1969, p. 456), but because they are literal representations of humans. This is an idea which

is also reinforced through the NHMRC supplementary document for non-human primates which says 'gorilla[s], orangutan[s], chimpanzee[s] and bonobo[s], are closely related to humans in evolution.' (NHMRC 2003, p. 8), and any research involving these 'other humans', or 'close relatives', in addition to the traditional cost/benefit analysis, should 'benefit the individual animal and the species' (NHMRC 2003, p. 8). It is therefore only when the animal is closely related to humans that we require a direct benefit to the animal. That is to say, their similarity to us means that they then begin to be seen as ends in themselves.

Anthropological classifications

Anthropology has a long tradition of using human-animal relationships as a lens to conduct analyses into the relationships between people (Dennis 2010, 2013; Mullin 1999; 2002, pp. 388-389). Claude Lévi-Strauss is often cited in anthropological analyses of the role of animals in society, with his idea that animals used in totemic social systems are not only chosen because of a magical significance, or because of their natural significance as consumables – because, he says, they are 'good to eat' (Lévi-Strauss 1962b, p. 89) – but because they are a good vehicle for understanding human relationships with each other and the world around them. Animals, according to Levi-Strauss are also 'good to think' with (1962b, p. 89). This notion is extremely relevant to the use of animals in research and teaching, where animals may be analysed as 'consumable' laboratory equipment (Dennis 2010, p. 33), and of course, they are good for researchers to 'think' of new ways of understanding human health and disease. That is, researchers are able to use the physical body of the animal as a model to think, hypothesize and test their understandings of the human body.

In Lévi-Strauss' *Totemism* (1962b), his analysis of totemic cultures showed that clans aligned themselves with elements from the natural world including the broader cosmos such as deities, the sky and earth, much like the Great Chain of Being. However, it was only the celestial and spiritual worlds which represented, and were represented by, a hierarchical relationship with humans. Animal totemic emblems were instead represented as a planar relationship with humans aligned to animals according to the clan's resemblance to the totemic emblem (Lévi-Strauss 1962b, p. 22). In this sense, humans and animals are symbolic analogues (Durkheim & Mauss 1963, pp. 6-7) where animals represent, rather than being dominated by, humans. This is an interesting way to think of the idea of Replacing animals in biomedical research; it is the differences that animals embody in relation to humans which make it *ethical* to use them as scientific instruments (Dennis 2010; Johnson 2013b), but their similarities which make them *valid* models (Dennis 2010). The dual positioning of animals as the same yet different is what facilitates their diminutive position in the human:animal dualism.

Conclusion

These case studies illustrate the ways that our social understandings of animality complicate the implementation of Replacement. Species which are not considered 'animals', e.g. shellfish, are not considered at the committee table. The underlying ethical assumption of the animality of a shellfish was not considered, although Aristotle, Linnaeus, and the Great Chain of Being would have us believe shellfish are

indeed animals. Conversely, the use of species which are classified as 'animals', i.e. mice, *was* questioned by the researcher and the Committee. However, having determined the 'relevance' of the mouse model in the test, and having determined that the human benefit balanced the welfare cost to the mice, the research was justified and the Replacement of the mice was given no further consideration[2]. In the case of non-human primates, the perception of what a non-human primate is – 'human', or close to human; a companion; a pet – has shaped the development of specific guidelines which reinforce their special treatment at the committee level. Non-human primates challenge our understandings of what it is to be human (i.e. not an animal) because they are like us, or we are like them.

The ambiguous placement of animals, as the 'other' to 'human' (Haraway 1991), particularly in the case of the marmosets, posed a problem for the Committee. Non-human primate research generally caused the Committee and its members more concern than other species did because the ambiguity eroded the traditional human:animal binary (Dennis 2010, pp. 14-15; Haraway 1991). Breaching the binary opposition, biomedical research on non-human primates disrupts our ordering of the world, and our place within it: Who are we if we are not different to animals? The assumption that non-human primates are more like humans than other animals means they not only deserve but receive special consideration.

Classifications are not natural. They are human constructs which are socially and culturally determined as a way of ordering the world (Lévi-Strauss 1962a). Classifying things is therefore not only about creating groups of things, but it is also about arranging and representing our relationships with the world around us (Durkheim & Mauss 1963, pp. 6-8; Lévi-Strauss 1962a, p. 135; Tambiah 1969, p. 452). Thus we understand man in relation to woman, tame in relation to wild, human in relation to non-human. What develops out of these constructs are power relationships, where one side emerges as dominant over the other, as we struggle to define which categories we have dominion over (Agamben 1998, p. 9). What is excluded from the conceptual animal category and in arbitrary definitions such as those found in the Code and the legislation, more so than what is included, unveils an underlying tension between humans and animals; excluding some categories or species makes them more significant (Tambiah 1969, p. 453), further entrenching the dominant position that men occupy over mice.

While Aristotle and Linnaeus both included man among the animal kingdom, our current method of classifying and categorizing animals is more specific with explicit inclusions and even more exclusions. The categories human : non-human and animal : non-animal expand and contract, responding to changing social, religious and political environments, in turn restructuring the human : animal dualism. The inconsistencies and slippages in these definitions exemplify the way that our current socio-legal frameworks for understanding what an 'animal' is (and isn't) are arbitrarily decided upon and enforced. While we may rely on objective observations based on the biological sciences as the basis for our animal classifications, we reinterpret these observations through our current social, political and legal frameworks in order to create new taxonomic arrangements (Lévi-Strauss 1962a, p. 137). Re-interpreting the natural categories into socially constructed taxonomies, such as animal : fish : shellfish

[2] It is important to note that throughout my fieldwork the Committee persistently worked with the researcher to actively move towards the use of chemical bioassays, rather than the more familiar animal model.

or human:non-human primate, determines what is possible in the human world by opening up new avenues of ethical science and research in species which biologically speaking *are* animals, but from a socio-legal framework are *not*.

This paper has begun to illuminate some of the ways that animals are increasingly drawn in to what is largely regarded as a 'human world'. Whether or not it can be truly regarded as 'human' is debatable given the inconsistencies and ambiguities regarding the definition of 'animal', the measure by which we judge our contrastive human-ness. Nonetheless, the 'human' trend of not only incorporating animals into our lives but using them for our very existence, for our *zoē*, entrenches animals into a diminutive position under man. The question 'Man or Mouse?' implies that the two are diametrically opposed: one will always dominate the other (Haraway 1991). Because these dichotomies are not natural – that is they are socially constructed and reconstructed by 'man', a human, or non-animal – man will inevitably emerge as dominant over mouse. The very fact that the animal category defies a concrete definition which delineates it from 'man', should serve as the impetus for us to reconsider the man-made construction of the 'man or mouse' dichotomy. It is not until we attempt to reorder our understanding of the human-animal relationship that we will be able to take Replacement seriously, and really begin to treat animals not as means to a human end, but as ends in themselves (Jahr 1927).

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A Good Death in Field Trials

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Trials that have an end point of euthanasia in field situations can pose challenges in terms of balancing animal welfare and the practicalities of the trial site and procedures.

In some situations, it is possible to sedate animals and then use barbiturates for euthanasia, however this usually requires a veterinarian to be present, and if more than a few animals are involved, perhaps more than one veterinarian. Control of the drugs and costs are also important considerations. Carcases from such animals need to be disposed of carefully to prevent any access by scavengers, including dogs, as deaths in animals that have eaten the meat have been recorded.

Firearms can be very effective for euthanasia; however, training is required to ensure that this is carried out correctly. It is important that the right type of firearm and ammunition are used for the class of animal. In both Australia and New Zealand, anyone using a firearm is required to have a firearms licence. Care needs to be exercised to ensure the safety of people and other animals, particularly from the risk of ricochet if the animal is confined in an area with solid wall.

Captive bolts have the advantage of no requirement for a firearms licence (in New Zealand), and no risk of ricochet, however training is still required and it is very important that the captive bolt is well maintained and the right charge is used. In most cases use of a captive bolt does not kill an animal and a secondary method of slaughter is required such as exsanguination or pithing.

A percussive blow (blunt force trauma) to the head can also be used to stun an animal prior to a secondary method of slaughter. In young animals with a soft skull this can be effective if sufficient force is applied to the correct site. However, skill and strength on the part of the operator are required and this method of slaughter has received a significant amount of negative publicity over recent years.

In New Zealand the use of blunt force trauma for on-farm euthanasia of young calves was banned from routine use in June 2014. This meant that farmers have to use a firearm, or captive bolt followed by a secondary slaughter method to euthanase sick, deformed or unwanted calves. The two recommended secondary slaughter methods are exsanguination or pithing; however, there is little information available on pithing in very young calves.

This paper also reports on a pilot study that investigated the use of flexible pithing rods as a secondary method of slaughter following captive bolt use in one to four-day old dairy breed calves. Three different lengths of pithing rod (300mm, 450mm, 1000mm) were compared to no pithing. Measures included immediate collapse after use of the captive bolt, lack of eye reflexes, lack of pedal reflex, absence of respiration and loss of heart function, as well as operator ease of use.

The findings of this work demonstrated that the length of the pithing rod had considerable bearing on the duration of involuntary muscle movement and also the time that rhythmic cardiac contractions could be detected.

Introduction

Trials that have an end point of euthanasia in field situations can pose challenges in terms of balancing animal welfare and the practicalities of the trial site and procedures. Field situations, by their very nature, often mean that the expertise, facilities and equipment that are normally present in a laboratory setting, may not be readily available.

In addition, all trials should have contingency plans for unexpected or unplanned events and in some cases it will be appropriate to have considered how humane slaughter could be carried out if an animal requires euthanasing.

Whatever the reason for euthanasing an animal, the primary aim is to bring about death with the minimum of pain, suffering and distress to the animal concerned.

Preparation

A number of basic requirements should be covered as part of the preparation for euthanasia of animals in field situations. These include:

- Having a plan: establish a policy which outlines who can slaughter animals, when, where and using what method
- Making sure everyone involved is properly trained and they know how to use all equipment safely (including firearms)
- Following the correct processes: bear in mind the needs of different species and different classes of animals within a species will vary
- Minimising stress for all: a quick process is best for both animals and operators
- Choosing the right location: different methods have different requirements to make them safe and effective. Screening activities from public view should also be considered.

Euthanasing animals can be a distressing procedure. The people designated with this task must be willing and physically able to complete the task competently. For some, religious beliefs or ethical positions may prevent them from doing this work. It is recommended that at least two people are trained so that there is always someone available in case an animal needs to be euthanased in an emergency.

Methods of euthanasia

It is important that decisions about euthanasia are made in a timely manner. Emergency situations require animals to be destroyed at the earliest practical opportunity. If a decision is made to discontinue treatment for a sick or injured animal because recovery is unlikely, then euthanasia of the animal is a priority.

Regardless of the method of euthanasia, it should either:

- Cause immediate death, or
- Render the animal immediately insensible (unconscious) and be followed by a suitable process to cause death, such as bleeding out (exsanguination) without the animal regaining consciousness.

Stunning - Signs of an effective stun

Indicators of an effective stun are firstly the immediate collapse of the animal. The animal is likely to be stiff for a few seconds (tonic phase) and then relax (clonic phase) with some involuntary paddling of limbs. At this point there should be no eye reflexes, no pedal reflex, absence of regular respiration, no vocalisation and eventually loss of heart function.

Head righting, or attempting to do so, should be regarded as a sign of returning consciousness and result in repeat stunning.

Secondary slaughter methods must be applied as soon as possible after stunning.

Chemical

Euthanasia can be carried out by the intravenous injection of a product specifically registered for this purpose. As these products are controlled veterinary medicines they must be administered by a veterinarian. Control of the products and licencing requirements are important considerations.

In some situations, especially for large animals, it is recommended that the animals are sedated first and then euthanased. This would usually require a veterinarian to be present, but does make the process safer and less stressful for both the animals and the veterinarian.

Carcases from such animals need to be disposed of carefully to prevent secondary poisoning through ingestion by scavengers, including dogs. Accidental deaths in animals that have eaten the meat from chemically euthanased animals have been recorded.

Firearms

Firearms (shotguns and rifles) can be very effective for euthanasia, however training is required to ensure that this is carried out correctly. It is important that the right type of firearm and ammunition are used for the class of animal. In both Australia and New Zealand anyone using a firearm is required to have a firearms licence.

The correct target must be used. This varies from species to species (see appendix).

Care needs to be exercised to ensure the safety of people and other animals, particularly from the risk of ricochet if the animal is confined in an area with solid wall. Where possible, the animal should be shot outdoors on soft ground. The animal's head must be held still to ensure the shot is effective and this may require some sort of restraint such as a head bail. Whenever possible the animal should be shot from close range, with the muzzle of the gun held 5-20 cm from the head. The muzzle of the gun must not be held against the animal's head.

Captive bolts

In New Zealand, captive bolts have the advantage of no requirement for a firearms licence and no risk of ricochet, however training is still required and it is very important that the captive bolt is well maintained and the correct charge is used. Charges must be kept dry.

Captive bolts are designed to stun animals and when used on their own, may not cause death. A secondary method of slaughter is required such as exsanguination or pithing.

As with firearms, the correct target area on the head or body must be used and this is usually the same target used with firearms.

The captive bolt must be placed against the animal's head which will normally mean some sort of restraint, such as a head bail, will be needed.

Blunt force trauma

A percussive blow (blunt force trauma) to the head can also be used to stun an animal prior to a secondary method of slaughter. In young animals with a soft skull this can be effective if sufficient force is applied in the correct site. However, this method of slaughter has received a significant amount of negative publicity over recent years.

Percussion stunning devices are commercially available and have been shown to be useful for euthanasing kid goats.

In New Zealand the use of blunt force trauma for on-farm euthanasia of young calves was banned from routine use in June 2014. In Australia blunt force trauma can only be used on piglets less than 15 kg in weight and on calves, lambs, fawns, kids and young camelids less than 24 hours of age.

Secondary slaughter methods

For all methods of slaughter that do not cause immediate death a secondary method of slaughter is required. This is commonly bleeding out (exsanguination) and this needs to be completed before that animal shows any signs of recovery from the stun. A sharp knife is needed and the cut must be deep and wide enough to sever all the main blood vessels in the neck. This is particularly important for cattle which have two sets of vessels supplying blood to the brain.

The disadvantage with bleeding out is that large volumes of blood are drained from the animal which must be dealt with from a hygiene and biosecurity point of view, as well as being unsightly. Blood in animal handling areas will also upset any remaining animals and may make handling these more difficult.

The other method of secondary slaughter that is practical for field use is pithing. Here a plastic or metal rod is inserted through the hole made by the captive bolt and pushed into the brain and upper spinal cord. It is important to move the rod around inside the animal to destroy the tissue, and this may result in strong involuntary movements by the animal. The person pithing should stand at the animal's back to avoid being kicked. Pithing is less messy that bleeding out, and also removes the risk of handling sharp knives in confined areas.

A recent pilot study at DairyNZ investigated the use of flexible pithing rods as a secondary method of slaughter following captive bolt use in one to four-day old dairy breed calves. Three different lengths of pithing rod (300mm, 450mm, 1000mm) were compared to no pithing. The findings of this work demonstrated that the length of the pithing rod had considerable bearing on the duration

of involuntary muscle movement and also the time that rhythmic cardiac contractions could be detected. The longer the rod the shorter the duration of involuntary muscle movement.

Confirmation of death

It is essential that animals are checked to ensure that they are dead. This should be done as soon as euthanasia is complete and should be repeated a few minutes later to ensure that no signs of life have been missed. In a field situation, the following should be checked; for the eyes no blink reflex and the pupils are fixed and dilated, no regular breathing, jaw relaxed and tongue floppy, and no heartbeat. The heart beat in deeply unconscious animals can be very slow and soft, and not easy to detect especially when there is other activity in the vicinity.

Further reading

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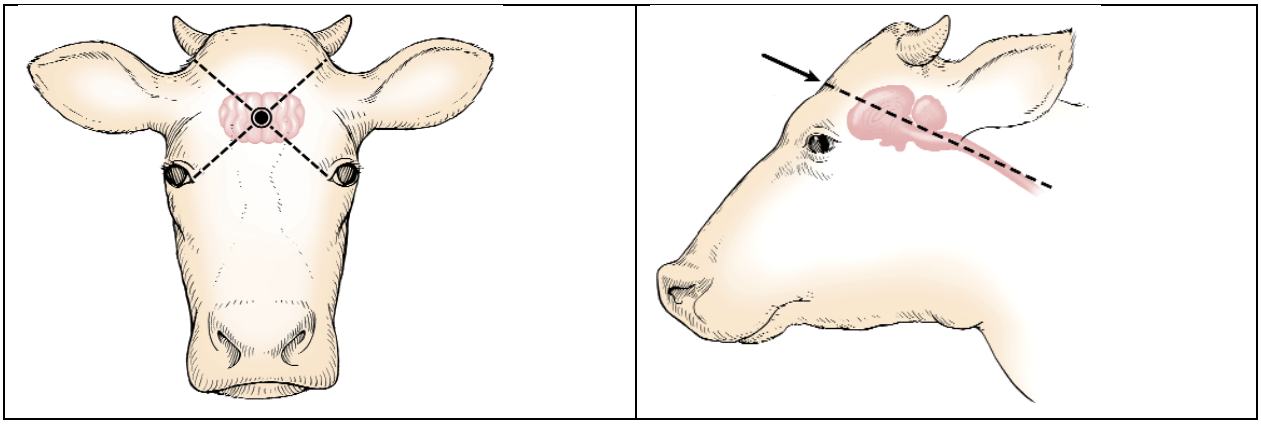
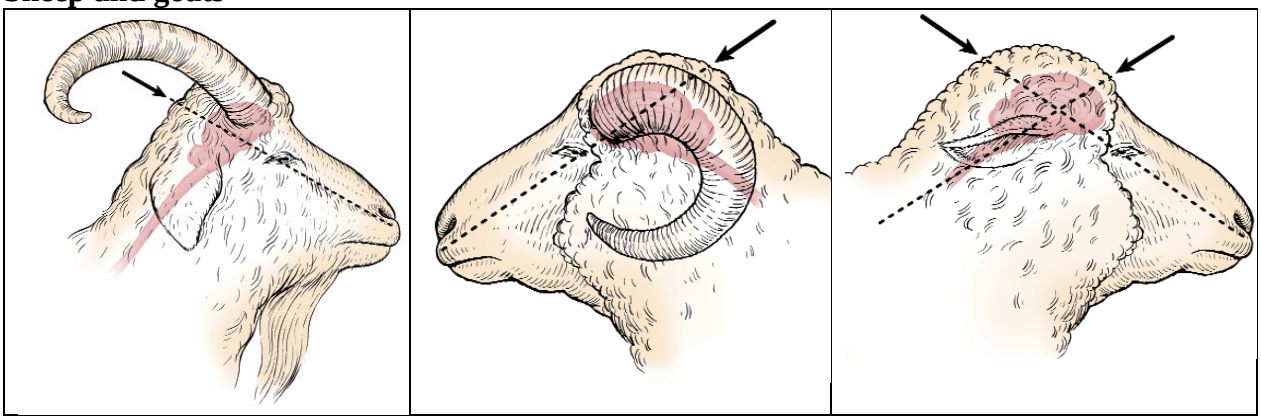
Appendix 1 – Species specific targets
From: Shearer, J.K. and Nicoletti, P. (2002)

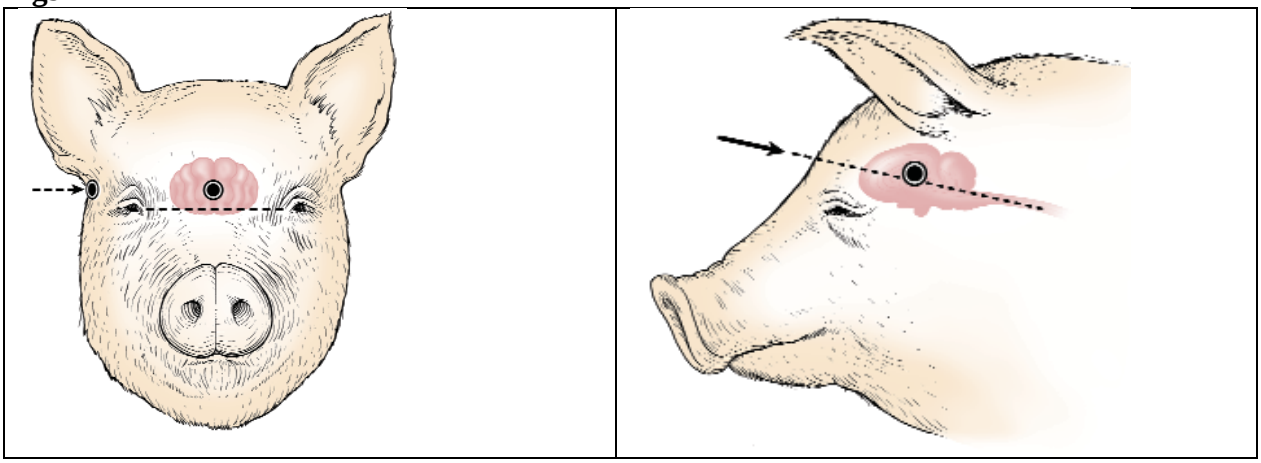
Cattle



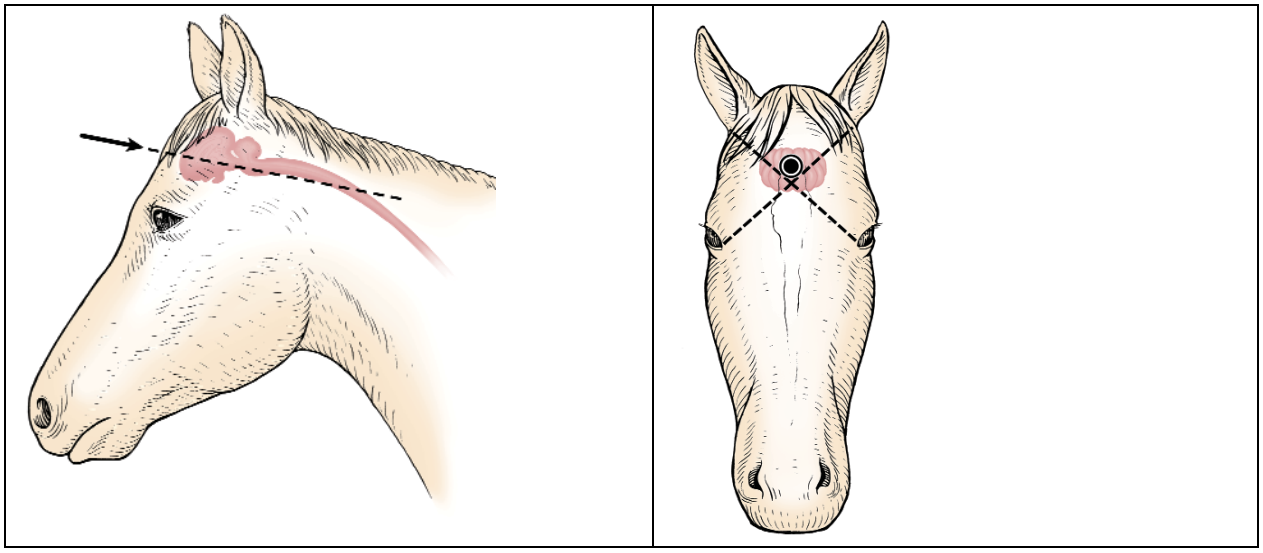
Sheep and goats



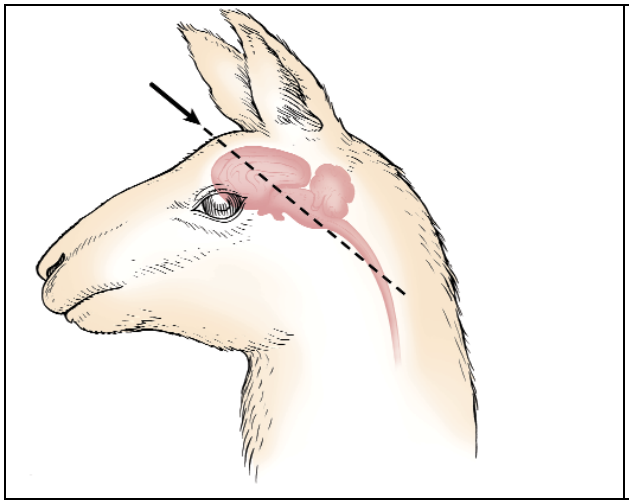
Pigs



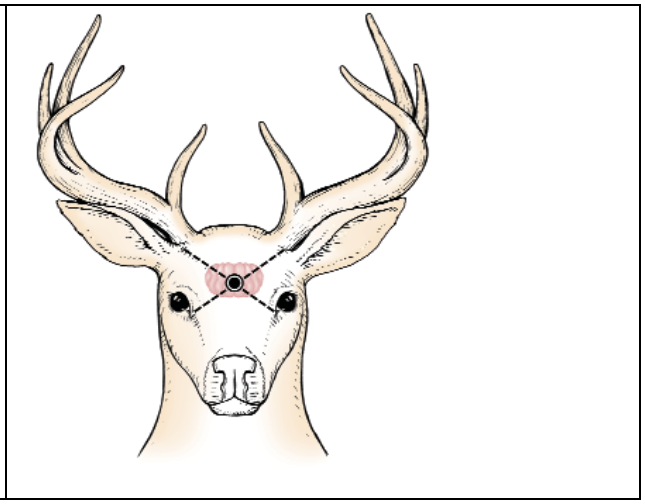
Horses



Camelids



Deer



Development of a Concordat on Openness on Animal Research In Australia/New Zealand

Pete Hodgson
Chair, ANZCCART New Zealand

In October 2012, over 40 organisations involved with bioscience in the UK, including many universities, pharmaceutical companies and research institutions, signed a declaration on openness on animal research[1], with all signatories agreeing to be more open about their use of animals in research and to abide by the following four commitments:

1. We will be clear about when, how and why we use animals in research
2. We will enhance our communications with the media and the public about our research using animals
3. We will be proactive in providing opportunities for the public to find out about research using animals
4. We will report on progress annually and share our experiences

The first overall Annual Report was published in September 2015[2], and examples of statements that organizations have put on their web sites as a result include:

- Babraham Institute (http://www.babraham.ac.uk/about-us/animal-research)
- Cardiff university (http://www.cardiff.ac.uk/research/our-research-environment/integrity-and-ethics/animal-research)
- University of Nottingham (http://www.nottingham.ac.uk/animalresearch/index.aspx)
- University of Portsmouth (http://www.port.ac.uk/research/using-animals-in-research/)

This presentation will offer delegates an opportunity to discusses whether it is now time for a similar Concordat in Australia and New Zealand.

From the outset ANZCCART has strongly supported the responsible use of animals in research and teaching. We do so publically and consistently. We believe the ethically justified use of animals saves lives and advances knowledge. We have simultaneously promoted the highest standards in that use. This includes compliance with New Zealand and Australian law, including the principles of refinement, reduction and replacement in the use of animals to the extent practicable.

The UK concordat was instituted in response to the perception of growing concern in UK society regarding the use of animals in research and teaching. The view of those who promoted the Concordat process was, broadly, that society's trust in the research community was being increasingly called into question, often because there was a dearth of information to counter the incorrect or outlandish assertions mad by those with an ideological or implacable opposition to any animal use. Further, society's implicit consent to the use of animals for research and teaching was accordingly less certain. The most appropriate response, it was thought, was greater transparency.

ANZCCART- NZ would like to discuss the idea of a similar Concordat in Australia and New Zealand now being timely.

[1] www.understandinganimalresearch.org.uk/policy/concordat-openness-animal-research/
[2] www.understandinganimalresearch.org.uk/files/9214/4319/6363/UAR_Concordat_Report_2015.pdf

Presentations given

on

Wednesday 20th July

Influenza and other viruses in Antarctica – who knew that penguins 'flu?

Aeron C. Hurt [1,2]

[1] WHO Collaborating Centre for Reference and Research on Influenza, VIDRL, at the Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria 3000, Australia

[2] Melbourne School of Population and Global Health, University of Melbourne, Parkville Victoria 3010, Australia

Avian influenza viruses (AIVs) are typically maintained and spread by migratory birds, resulting in the existence of distinctly different viruses around the world. However, AIVs have not previously been detected in Antarctica. In this study, we characterized influenza viruses sampled from different penguin species from geographically different sites in Antarctica and show that for one of the viruses that the segmented AIV genome diverged between 49 and 80 years ago from other AIVs, with several genes showing similarity and shared ancestry with H3N8 equine influenza viruses. In addition, we have detected multiple novel paramyxoviruses from this isolated group of birds. This study provides the first insight into the ecology of AIVs in Antarctica and highlights the potential risk of an introduction of highly pathogenic AIVs into the continent.

No manuscript was submitted for this presentation

A novel video tracking method to evaluate the effect of influenza infection and antiviral treatment on ferret activity

Ding Yuan Oh[1,2], Ian G. Barr[1,2], Aeron C. Hurt[1,3]
[1]WHO Collaborating Centre for Reference and Research on Influenza, VIDRL, at the Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria 3000, Australia; [2]School of Applied and Biomedical Sciences, Federation University, Churchill, Victoria 3842, Australia; [3]Melbourne School of Population and Global Health, University of Melbourne, Parkville, Victoria 3010, Australia

Ferrets are the preferred animal model to assess influenza virus infection, virulence and transmission as they display similar clinical symptoms and pathogenesis to those of humans. Measures of disease severity in the ferret include weight loss, temperature rise, sneezing, viral shedding and reduced activity. To date, the only available method for activity measurement has been the assignment of an arbitrary score by an observer based on a pre-defined responsiveness scale which has the potential to be subjective and therefore prone to bias. As an alternative, we developed a novel video-tracking methodology for determining the movement, speed and distance travelled by ferrets to determine the relative effect of influenza infection and antiviral drug treatment. This method eliminates some of the limitations of manual scoring, which includes the need for a sole experienced observer (who is unaware of the different groups and therefore unbiased) and the requirement to recognise the 'normal' activity of ferrets in order to assign relative activity scores. In ferrets infected with an A(H1N1)pdm09 virus, the video-tracking method was more sensitive than manual scoring in detecting ferret activity changes. Using the video-tracking method, we found that ferret activity, speed and distance travelled were all significantly reduced from day 2 to 6 following influenza infections, but that oseltamivir (an influenza antiviral) treatment completely ameliorated the effect on ferret activity. Oseltamivir treatment of ferrets was also associated with reduced inflammatory responses in the upper respiratory tract, lower body weight loss and a smaller rise in body temperature, despite there being no significant reduction in viral shedding.

In summary, this novel video-tracking is an easy-to-use, objective and sensitive methodology for measuring ferret activity following viral infection. From an animal welfare perspective, the better understanding of the ferret's behaviour can facilitate a better management of the animal welfare throughout the infection period. Given that the ferret model has recently been expanded to assess pathogenesis due to other viral infections such as Hendra virus, Nipah virus and severe acute respiratory syndrome-coronavirus (SARS-CoV), this method has broad use to assist in measuring viral pathogenesis, the impact of novel antiviral interventions and better management of animal welfare in these different infection settings.

No Manuscript was submitted for this presentation

Environmental enrichment and experience-dependent plasticity in mouse models of brain disorders

Anthony J. Hannan

Florey Institute of Neuroscience and Mental Health,
University of Melbourne, VIC 3010, Australia

We have demonstrated that environmental enrichment (which enhances sensory stimulation, cognitive activity and physical exercise) can delay onset of disease symptoms in in various mouse models. Our original discovery, that environmental enrichment delays onset of Huntington's disease in a transgenic mouse model, has been followed up by our group and others in a variety of different laboratory rat and mouse models. We have also demonstrated beneficial effects of physical activity, by increasing voluntary exercise through provision of running wheels in home cages. The molecular effects of environmental enrichment and physical activity exhibit temporal specificity, regional selectivity and sexually dimorphism. Our results suggest that the timing and duration of these environmental manipulations are critical in terms of their ability to modify gene expression. We have further characterized the behavioural changes in mice modelling Huntington's disease (including dementia and depression), anxiety disorder, schizophrenia and autism spectrum disorder. Our data demonstrates that environmental enrichment can exert a range of beneficial effects on cognitive, affective and motor symptoms. Our experiments have revealed cellular and molecular mechanisms mediating the therapeutic effects, informing our understanding of experience-dependent plasticity in the healthy and diseased brain. These approaches may inform the development of a new class of therapeutics ('enviromimetics') for brain disorders known to be modulated by enhanced complex mental activity and physical exercise.

No manuscript was submitted for this presentation

Improving Animal Health and Welfare in the Production of Snake Antivenom in Myanmar

Dr John Moody
Veterinarian Seqirus PL (CSL Company)

Australian Government Funded Snakebite Project in Myanmar Department Foreign Affairs and Trade Government Partnerships for Development (DFAT-GPFD): Project Leader: Dr Chen Au Peh (Renal Specialist, Adelaide University), Dr Debbie Eagles (Veterinary Epidemiologist, AAHL-CSIRO, Geelong), Dr Lucy Woolford (Veterinary Pathologist, Adelaide University Faculty of Veterinary Science), Myanmar Ministry of Industry (Dr Aung Zaw and Dr Moe Moe Hliang).

Snakebite is a major public health issue in the developing nation of Myanmar. The main snake envenomation is from the Russell's Viper (Daboia russelii siamensis). Poor/subsistent farmers are the common victims of this snake. Early treatment with effective antivenom is critical to not only the survival of the envenomated patient, but also reducing the risk of developing conditions such as acute renal failure, Sheehan's Syndrome (hypopituitarism).

The Russells Viper (RV) is a common snake of SE Asia (eg Thailand, India). Geographical barriers with neighbouring countries have led to the evolution of a subspecies of the RV in Myanmar having unique bioactive venom components. This is evident by the limited effectiveness of RV anitvenoms produced by other SE Asian countries when used on RV snakebite patients in Myanmar (Myanmar Ministry of Health data, external consultant Toxinologist). Antivenom supply and treatment is funded by the Government of Myanmar, production by the Ministry of Industry and distribution and delivery to patients by the Ministry of Health (MOH).

Snakebite is recognised by the WHO as a major public health issue in developing countries and encourages input from organisations such as CSL/Seqirus in assisting in developing efficient, self-sufficient hyperimmunised plasma production and manufacturing processes to delivery life-saving antivenoms. Currently antivenom production is only achieved by dosing donor animals (mostly horses) with extracted venoms and collecting hyper immunised blood/plasma for processing.

In the early 1970's the then Australian Government owned CSL was involved in advising the Government of Burma (Myanmar) in the production of antivenoms, with the potential of using cattle as donors and importing snake venom from Myanmar. Communication faded, with reconnection with the Government of Myanmar regarding this public health issue initiated by a Human Renal Specialist, Dr Chen Au Peh, from Royal Adelaide Hospital in 2014. Dr Chen Au observed during consulting visits to the country for the MOH, that most acute renal patients requiring dialysis were RV snakebite victims.

The key issue confronting the delivery of snake antivenom to regional health centres and treating patients in Myanmar was supply. The MOI was responsible for the production of hyper immunised plasma from locally derived equines (horses/donkeys/mules) and the manufacturing of antivenoms. A high mortality rate of donor equines (and snakes) threatened supply and required sourcing of antivenoms with inferior efficacy from neighbouring countries.

Scoping of the Project, prior to application to the Australian Department of Foreign Affairs and Trade (DFAT) for funding, involved a visit by an Australian delegation of human medical specialists (Renal /Toxinologist/Public Health Management) and CSL/Seqirus Subject Matter Experts (SME-Veterinarian, Antivenom Manufacturing Manager and Quality/Validation Manager) in July 2014. This provided information to the Seqirus Veterinarian on the extent of the animal health/welfare issues and likely causes of the high donor mortality rate. A follow up visit post DFAT funding approval in Dec 2014 identified other areas that would require engagement of other veterinary specialists in the project (epidemiologist, pathologist) to improve the health and welfare of donor equines.

The Project's Myanmar colleagues from MOI responsible for donor animal (& snake) care advised in a recent update that the mortality rate for 2015-16 had been reduced by over 50% from 2014-2015.

SUMMARY

Australian Government Funded Snakebite Project in Myanmar Department Foreign Affairs and Trade Government Partnerships for Development (DFAT-GPFD): Project Leader: Dr Chen Au Peh (Renal Specialist, Adelaide University), Dr Debbie Eagles (Veterinary Epidemiologist, AAHL-CSIRO, Geelong), Dr Lucy Woolford (Veterinary Pathologist, Adelaide University Faculty of Veterinary Science), Myanmar Ministry of Industry (Dr Aung Zaw and Dr Moe Moe Hliang).

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The Russells Viper (RV) is a common snake of SE Asia (eg Thailand, India). Geographical barriers with neighbouring countries have led to the evolution of a subspecies of the RV in Myanmar having unique bioactive venom components. This is evident by the limited effectiveness of RV Antivenoms (AV) produced by other SE Asian countries when used on RV snakebite patients in Myanmar (Myanmar Ministry of Health data, external consultant Toxinologist). AV supply and treatment is funded by the Government of Myanmar, production by the Ministry of Industry and distribution and delivery to patients by the Ministry of Health (MOH).

Snakebite is recognised by the WHO as a major public health issue in developing countries and encourages input from organisations such as CSL/Seqirus in assisting in developing efficient, self-sufficient hyperimmunised plasma production and manufacturing processes to delivery life-saving AVs. Currently AV production is only achieved by dosing donor animals (mostly horses) with extracted venoms and collecting hyper immunised blood/plasma for processing.

In the early 1970's the then Australian Government owned CSL was involved in advising the Government of Burma (Myanmar) in the production of AVs, with the potential of using cattle as donors and importing snake venom from Myanmar. Communication faded, with reconnection with the Government of Myanmar regarding this public health issue initiated by a Human Renal Specialist, Dr Chen Au Peh, from Royal Adelaide Hospital in 2014. Dr Chen Au observed during consulting visits to the country for the MOH, that most acute renal patients requiring dialysis were RV snakebite victims.

The key issue confronting the delivery of snake AV to regional health centres and treating patients in Myanmar was supply. The MOI was responsible for the production of hyper immunised plasma from locally derived equines (horses/donkeys/mules) and the manufacturing of AVs. A high mortality rate of donor equines (and snakes) threatened supply and required sourcing of AVs, with inferior efficacy, from neighbouring countries.

Scoping of the Project, prior to application to the Australian Department of Foreign Affairs and Trade (DFAT) for funding, involved a visit by an Australian delegation of human medical specialists (Renal /Toxinologist/Public Health Management) and CSL/Seqirus Subject Matter Experts (SME-Veterinarian, Antivenom Manufacturing Manager and Quality/Validation Manager) in July 2014. This provided information to the Seqirus Veterinarian on the extent of the animal health/welfare issues and likely causes of the high donor mortality rate. A follow up visit post DFAT funding approval in Dec 2014 identified other areas that would require engagement of other

veterinary specialists in the project (epidemiologist, pathologist) to improve the health and welfare of donor equines.

OVERVIEW OF ANITVENOM PRODUCTION BY CSL/SEQIRUS

CSL/Seqirus has been producing highly effective/potent AVs since the 1930s. The manufacturing of these lifesaving biotherapies requires the hyperimmunisation of animals against specific venom. Horses, sheep and rabbits are used by CSL/Seqirus for this purpose.
The following is the donor species and respective portfolio of antivenoms produced by CSL/Seqirus.
AV produced from Horse plasma -

- Land snakes –Tiger, Taipan, Brown, Death Adder and Black/King Brown (Elapids)
- Sea snake (Elapid)
- Stonefish
- Redback Spider

AV produced from Sheep plasma -

- Box Jellyfish

AV produced from Rabbit sera -

- Funnel Web Spider

CSL/SEQIRUS COLLABORATION WITH MYANMAR IN ANTIVENOM PRODUCTION

In the early 1970s the then Australian Government entity Commonwealth Serum Laboratories (CSL) was approached by the Myanmar authorities to advise on the production of AVs. Issues that were communicated in the 1970s that risked the continuity of supply of antivenoms appear to be currently confronting the MOI's pharmaceutical division.

A delegation that included CSL/Seqirus subject matter experts visited Myanmar in July 2014 at the request/invitation of the MOI and Ministry of Health (MOH). This visit was to inspect and assess aspects of antivenom production, distribution and clinical use in Myanmar that required improvement. The identified areas for improvement were to be the basis for a submission for DFAT aid. DFAT funding was approved in Dec 2014.

The Project aims are-

1) Improve quantity and quality of AV production with aim of full self-sufficiency

2) Improve distribution/availability of AV to ensure reaches those in need

3) Improve clinical management pathway for snakebite patients- village⇨ regional health centre ⇨major hospital and emphasis on education of rural population & health workers

Aim #1 – IMPROVE DONOR ANIMAL HEALTH AND WELFARE

New Outdoor Facility:Hmawbi

Moving from fulltime stabling at Insein Facility in Yangon to paddock environment at rural facility in township of Hmawbi, north of Yangon, was in the process of implementation at the commencement of the DFAT Snakebite Project. This was an initiative of the MOI, with strong support from Australian participants in the project. A significant reduction in the mortality rate was observed within 6 months of the change of environment for the donor equines.

Equine Donor Health

A contributing factor to the high donor mortality rate was the procurement and introduction of equines with underlying conditions –e.g. Equine Infectious Anaemia (EIA) and Piroplasmosis.
To improve the health of equines entering the AV production process, the following recommendations have been adopted –

1) Pre-acceptance criteria/ health parameters
    - Minimum Body score
    - Maximum age (if known/verifiable)
    - Minimum Packed Cell Volume (red blood cells)
    - EIA negative

2) Ongoing Disease monitoring/surveillance/investigation

There is only one Veterinary Diagnostic Laboratory available in Myanmar with a 2week delay in any test results, so it was recommended to procure an in-house blood analyser for rapid haematocrit and biochemistry results. This was introduced in 2015. The in-house analyser provides rapid information for veterinary diagnosis of sick donors and monitors response to treatment. It also provides data to assist in identifying common causes of sickness/death potentially leading to effective corrective/preventive actions to reduce the mortality rate.

With the introduction of balanced/fortified pelleted feed designed for horses to replace whole grain mixture which was lacking fibre and micronutrients (vitamins and minerals), nutrition was also improved, leading to healthier donor animals.

3) Addition of specific mineral supplements - Oral Iron and Selenium.

The introduction of an electronic donor health and activity record system replaced haphazard hard copy records. The electronic recording system facilitates rapid analysis of donor data to assist in identifying the main factors associated with donor morbidity and high mortality rate. This data system will provide the basis for setting minimum health parameters to identify donors that should be withdrawn from AV production to receive specific veterinary treatment.

4) Dosing protocols and procedures
    - Introduction of disposable collection system - needles, collection lines and blood bags.
    - Modify dosing method - change from intramuscular dosing to subcutaneous.
    - Introduction of adjuvants - improve potency, reduce risk of accidental envenomation of donors with straight venom
    - Introduce the use of rewarding donors (supplementary feeding) post interventions - dosing and blood collection/reinfusion.
    - Monitor PCV/body score/weight regularly to identify donors requiring resting from AV production.

CHALLENGES

- Absence of health data for equines/horses in Myanmar. Equines are only used as a mode of transport not as a food source or in the production of food, hence the absence of disease investigation, monitoring and surveillance data. The social value of equines has reduced with the widespread introduction/affordability of motorbikes for transport in rural areas.

- Training of Veterinary Graduates - equine medicine is not included in undergraduate veterinary training. Equine competency is reliant on post graduate mentoring and clinical experience. Plan is to identify a young veterinarian to gain experience in Australia from private, general equine practitioners and specialist referral facilities (University).

- Prevalence/clinical significance of endemic equine diseases - Elimination of EIA from donor herd: Piroplasmosis - able to treat horses to control/eliminate. Due to the reported incidence adverse side-effects in donkeys & mules to adverse side-effects of the drugs of choice for treating Piroplasmosis, treatment cannot be recommended. Hence as mules are a significant proportion of the donor herd, these animals remain a source / risk of iatrogenic transmission of this blood infection. This situation highlights the importance of using disposable collection system.

DONOR SNAKE HEALTH

Transfer of venom donors to spacious/enriched enclosures at Hmawbi has dramatically improved snake health/survival, delivering superior venom quality and quantity. This change has delivered successful in-house breeding, reducing the need of sourcing venom donors from the environment. Snake health and welfare recommendations provided by herpetologist from South Australian company Venom Supplies.

PROJECT TEAM

Human/ Public Health

- Dr Chen Au Peh-Renal , Adelaide
- Dr Julian White-Toxinologist, Adelaide
- Dr Afzal Mahmood-Public Health Systems, Adelaide
- Dr David Warrell-Professor Tropical Medicine, Oxford University
- Dr Robert Cumming-Public Health Epidemiologist, Sydney University
- Plinio Hurtado-Adelaide
- David Bacon , Project co-ordinator, Myanmar

Veterinary Health & Welfare

- Dr Debbie Eagles-AAHL/CSIRO-Geelong
- Dr Lucy Woolford-Adelaide Uni Veterinary Faculty
- Dr Sarah Van Dyk-Equine Veterinarian
- Nathan Dunstan, Herpetologist, Venom Supplies, South Australia

Seqirus Antivenom Manufacturing SME

- Keiran Ragas

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Rabies vaccine developments and use in the global elimination of dog-mediated human rabies by 2030.

Andrea Britton
Director, Ultimate Efficacy Consulting Pty Ltd and Vets Beyond Borders

Rabies vaccines have been used to prevent the highly fatal viral encephalitis for over 130 years but still tens of thousands of people die annually mostly in developing countries from rabid dog-bites. The rabies vaccine developed by Louis Pasteur was first used back in 1885 to treat a 9-year-old boy, Joseph Meister, using fourteen daily doses of infected rabbit spinal cord suspensions that had been inactivated by drying. Initially this vaccine was fully inactivated but then vaccine containing more virulent virus preparations of spinal cord were injected. Pasteur had developed an animal model for the predictable passage of rabies virus. These vaccines were inconsistent and some recipients developed rabies from the vaccine and others had severe hypersensitivity reactions. Today most countries have stopped manufacturing nerve tissue rabies vaccines and have replaced it with concentrated, purified cell culture and embryonated egg-based rabies vaccines. These vaccines are used both pre and post-exposure prophylaxis (PEP) producing antibodies which neutralise the virus before it enters the central nervous system. Rabies immunoglobulin is recommended for category III exposures to suspect rabid animals, which is injected in and around the bite site. These immunoglobulins are produced in horses or humans and are not widely used in developing countries due to cost and access.

Historically, controlling rabies in humans has been through the use of PEP. Following recent Gates Foundation funded proof of elimination studies, in four WHO demonstration projects in the Philippines, KwaZulu-Natal, Tanzania and Bangladesh, it has been shown dog-mediated rabies can be eliminated by repeat mass dog rabies vaccination campaigns, stopping the virus at the source. The Sikkim Anti-Rabies and Animal Health (SARAH) program in Sikkim, India has also shown dog-mediated rabies can be eliminated by: dog population management, mass dog vaccination with greater than 70 percent dog population coverage and rabies advocacy and education. Additionally, the Global Alliance for Rabies Control have developed valuable tools to support developing countries in planning, implementing and evaluating rabies elimination programs. The World Health Organisation (WHO), World Organisation for Animal Health (OIE) and the Food and Agriculture Organisation (FAO) are now on a mission to eliminate dog-mediated human rabies globally by 2030. By using a One Health approach with human and animal health sectors collaborating including mass dog rabies vaccination and integrated bite case management plus continued stakeholder engagement, dog-mediated rabies can be eliminated world-wide.

No manuscript was submitted for this presentation

What if drones could be used for good instead of evil!

Rob Gration
EcoAerial Pty Ltd

There have been huge advancements in the technology available to improve the quality of the data collected and minimise the impact to wildlife and habitat. The technology available includes; handheld computers for mapping, remote sensing cameras, remote sound recorders and more recently remotely piloted aircraft, commonly referred to as '*drones*'. The media has generally focused on the military use of drones and the perceived indiscriminate destruction they cause. Over the last 5-years the commercial use of drones has grown exponentially as the cost, function, reliability and capability of small drones (>5kg) has improved. It has been estimated that the market for commercial/civilian *drones* will grow at an annual growth rate of 19% between 2015 and 2020. This expected growth presents challenges for regulators with the emphasis on safety and human concerns (i.e. aviation & privacy).

In 2014 a commonwealth government report reviewed the use of drones for scientific research. The report acknowledged that Australian scientific organisations have already found a range of uses for *drones* e.g. crop monitoring in plant breeding experiments, beach surveys, monitoring of bushfire experiments, and test a tracking device. The authors anticipate that the fall in price and increased capabilities that drones will be used in a range of additional scientific survey and monitoring roles. With the increased use of drones for environmental research and monitoring, the interaction between drones and wildlife is going to increase. Notwithstanding the obvious benefits using *drone*s for environmental studies, with the exception of Canada, there have been few studies focusing on the behavioural response of wildlife to *drone* interactions. A quick search of YouTube provides many examples of aggressive responses by wildlife. In light of these examples and my personal experience deploying drones over the last 5-years, is there a need for animal ethic committees and state wildlife agencies to approve their use for environmental studies.

No manuscript was submitted for this presentation

An ovine model for studying the pathophysiology of cardiovascular and renal failure in septic shock

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[1]*Florey Institute of Neuroscience and Mental Health, University of Melbourne, Victoria*

Background: Septic shock remains the main cause of death from infections in Intensive Care Units (ICU) around the world. Sepsis is characterized by cardiovascular failure with persistent hypotension that can reduce blood supply to vital organs. Vasopressors are hence a cornerstone of therapy in sepsis to counteract this low blood pressure. However, a major challenge for clinicians caring for patients with sepsis is reduced sensitivity to vasopressor drugs, leading to severe hypotension that can cause organ failure and death. Vasopressor insensitivity has the potential to contribute to septic acute kidney injury (AKI), which develops in up to 50% of patients, one-third of whom do not leave hospital alive. Despite an increase in the annual incidence in the cases of sepsis, the underlying pathophysiological mechanisms are not well understood. Because of the difficulty of invasive experimentation on critically ill humans, most available information on the causes of organs/systems failure in septic shock comes from animal experiments. Our ignorance of the mechanisms causing cardiovascular and kidney failure in sepsis arises at least partly from the use of animal models which poorly reflect the clinical condition, so hindering translation of basic research into clinical practice. Consequently effective therapeutic strategies have not been developed.

Results: We have an established model of sepsis in conscious sheep induced by the infusion of live *Escherichia coli* that is characterized by hypotension, peripheral vasodilatation, tachycardia, increased cardiac output and AKI. This hemodynamic profile is similar to that commonly reported in patients with septic shock. Using this clinically relevant model of septic shock, we have recently made several important discoveries that we believe will improve management of patients with sepsis. First, treatment with the α2-adrenoceptor agonist clonidine helped maintain blood pressure and restored vasopressor sensitivity in sepsis. Based on our findings, an ongoing clinical trial is evaluating the effects of α2-adrenoceptor agonist treatment in patients with septic shock in the ICU at the Austin hospital. Second, in septic sheep we showed an early onset of reduced tissue levels of oxygen in the inner zone of the kidney, which may play a pivotal role in the development of septic AKI. Third, we showed a close correlation between the oxygen levels in the urine, measured in a bladder catheter, with that in the inner zone of the kidney during development of septic kidney failure. This simple measurement can now be used as the first real-time biomarker to detect risk of kidney injury before it occurs so that treatment can be initiated early to prevent development of kidney injury. Based on our findings, a clinical trial is about to test the efficacy of using bladder urine oxygen levels as a predictor of kidney failure in patients with sepsis at the Austin hospital.

Significance and Clinical Implications: There is no dispute that the development of vasopressor insensitivity and AKI are major unresolved problems encountered during the treatment of patients with sepsis. Our pre-clinical studies in a large animal model of sepsis, that has a similar phenotype to human sepsis, are a vital step towards developing interventional strategies that can revolutionize the way clinicians manage patients with sepsis in ICUs and ultimately improve health outcomes.

Background

Septic shock remains the main cause of death from infections in Intensive Care Units with mortality rates in excess of 40%[1, 2]. The annual incidence of the cases of septic shock continues to rise due to an aging population and the increasing number of drug-resistant infections[1]. Septic shock is characterized by cardiovascular failure with persistent hypotension that can compromise perfusion to vital organs leading to multiple organ dysfunction[3]. Vasopressors are hence a cornerstone of therapy during septic shock to restore blood pressure and maintain adequate organ perfusion. Noradrenaline is currently the first choice vasopressor used to maintain blood pressure in patients with septic shock[2]. A major clinical problem encountered during the treatment of septic shock is that patients become unresponsive to vasopressor drugs, leading to severe uncontrolled hypotension and death. Huge does of vasopressor drugs are therefore used, but they have deleterious effects including the development of localized tissue ischemia and hypoxia.

Vasopressor hypo-responsiveness also has the potential to contribute to the development of septic acute kidney injury (AKI), which develops in up to 50% of patients, one-third of whom do not leave hospital alive[4]. The mechanisms underlying vasopressor hypo-responsiveness and AKI in septic shock are unclear, and consequently there are no treatments to prevent or reverse these detrimental symptoms. Because of the great difficulty of invasive experimentation on critically ill humans, most available information on the causes of the failure of organs in septic shock comes from animal experiments. Our ignorance of the mechanisms causing cardiovascular and kidney failure in septic shock arises at least partly from the use of animal models which poorly reflect the clinical condition, so hindering translation of basic research into clinical practice.

To overcome this setback we have established a large animal model of hyperdynamic septic shock in conscious sheep, characterized by hypotension, peripheral vasodilatation, tachycardia, increased cardiac output and elevated renal and cardiac sympathetic nerve activity[5-8]. This hemodynamic profile is similar to that commonly seen in patients with septic shock[2]. In addition, we demonstrated that in this model of hyperdynamic septic shock, there was an increase in renal blood flow[6-8]; in contrast to the dogma that renal blood flow was decreased. Interestingly, despite this decrease in total renal blood flow, AKI progressively developed[6-8]. We believe our findings have more relevance to human sepsis than studies in anaesthetized rodents where there is hypotension with reduced cardiac output and a decrease in renal blood flow, where renal ischemia is proposed as the cause of the AKI. Using this clinically relevant ovine model of septic shock we have recently made several important discoveries and learnt some valuable lessons that may pave the way to improve management of patients with septic shock.

Results and Discussion

Discovery 1: In conscious sheep with septic shock, infusion of a clinically relevant dose of the α_2-adrenoceptor agonist clonidine, prevented the progressive decline in blood pressure, reduced the high levels of sympathetic nerve activity and improved responsiveness to the α_1-adrenoceptor agonist phenylephrine, and also to the non-adrenoreceptor agonist angiotensin II[9].

Lessons Learned: Clonidine improves vascular responsiveness to both endogenous and exogenously infused vasoconstrictors, suggesting that it may be an effective adjunct therapy for patients with septic shock who are resistant to vasopressor therapy[9]. Clonidine may improve vasopressor sensitivity by reducing the high level of sympathetic nerve activity and noradrenaline release in sepsis, leading to an up-regulation of vascular smooth muscle α_1-adrenoreceptors that were down-regulated in sepsis[10]. However, given that clonidine also restored sensitivity to the

non-adrenoreceptor agonist angiotensin II, it is possible that its actions to improve vasopressor sensitivity result from mechanisms that are either downstream or independent of α_1-adrenoreceptors[10].

Discovery 2: In conscious sheep with sepsis, there is an early onset of ischemia and hypoxia selectively within the inner zone of the kidney (medulla), despite increased total renal blood flow. These reductions in medullary perfusion and oxygenation occurred several hours prior to the detection of AKI, suggesting that these changes may be contributing to the development of the AKI[11, 12].

Lessons Learned: A possible mechanism contributing to septic AKI, in the face of renal hyperperfusion, is microcirculatory dysfunction leading to redistribution of renal blood flow within the kidney resulting in medullary tissue ischemia and hypoxia. In turn, hypoxia can lead to oxidative stress, that can initiate a vicious cycle leading to cellular injury, further kidney injury and reduced function[13].

Discovery 3: In septic shock, restoration of blood pressure with a clinically relevant dose of noradrenaline exacerbated the degree of medullary ischemia and hypoxia[12].

Lessons Learned: The primary vasopressor used clinically to support blood pressure in patients with septic shock may worsen kidney injury. Noradrenaline may further reduce medullary perfusion and oxygenation in septic AKI, either directly by inducing localized renal vasoconstriction, or indirectly by increasing glomerular filtration rate, renal tubular sodium load, and renal oxygen consumption. It is now imperative to develop a new therapeutic approach that can restore blood pressure in septic shock, without enhancing the underlying pathological processes leading to AKI.

Discovery 4: During the development of septic AKI and treatment with noradrenaline, the changes in urinary oxygenation, measured at the tip of a bladder catheter, closely paralleled changes in medullary tissue oxygenation, measured using a probe surgically implanted in the medulla[11]. The similarity between these measurements is not surprising considering the collecting ducts run parallel to, and in close association with, the medullary vasa recta[14].

Lessons Learned: Urinary oxygenation can provide a real-time assessment of medullary oxygenation and be used as a non-invasive, real-time biomarker for risk of AKI and as a guide for therapy in patients with septic shock and other situations such as in patients undergoing cardiac surgery, who also have a high risk of developing AKI. Based on our findings, an on-going clinical trial is examining Urinary oxygenation as a new diagnostic tool on patients with septic shock at the Austin Hospital.

Significance and Clinical Implications

There is no dispute that the development of vasopressor hypo-responsiveness and AKI are major unresolved problems encountered during the treatment of patients with septic shock. Development of therapies to prevent or reverse these detrimental symptoms would be a major breakthrough that would result in improved outcomes for patients. Our pre-clinical studies in a large animal model of septic shock, that has a similar phenotype to human septic shock, are a vital step towards

developing interventional strategies that can revolutionize the way clinicians manage patients in Intensive Care Units and ultimately improve health outcomes. Our additional finding that the non-invasive measurement of urinary oxygenation indicates the level of oxygenation in the renal medulla, suggest that this technique can be used as a real-time biomarker for the early detection of patients who are at risk of developing AKI.

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Mice and touchscreens – advancing rodent behavioural testing

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Development of effective therapies for brain disorders has been hampered by a lack of translational cognitive testing methods. The last decade has seen increasing calls to develop improved, standardised assays for assessing behaviour in animals, to not only advance robustness of scientific practice between laboratories, but the fundamental goal of effective medical translation. Towards this, the recently developed rodent touchscreen testing system is a versatile cognitive assessment tool that contributes to addressing the 3Rs. The technology avoids aversive conditions and instead uses rewards. Touchscreen-based rodent tests are similar to those used in humans, thus greatly increasing the probability of successful translation of treatments from the lab to the clinic, potentially reducing the number of animal experiments required. Additionally, this technology increases the precision of data collected through automation and computerisation, and provides the possibility to conduct multiple tests in the same apparatus, contributing to reduction.

No manuscript was submitted for this presentation

Let's discuss: Standardisation of training in Australia

Deirdre Bourke & Melissa Lindeman
University of Western Australia, Perth, WA.

Proposal and aims:

The aim of this proposed interactive session is to initiate a discussion where audience participants can share experiences of training resources for the education of people involved in the care and use of animals for scientific purposes. The overall aim is to suggest that Australian Institutions align their training requirements and share educational resources in order to promote some degree of standardisation of training in Australia. A common training curriculum could be beneficial as it would ensure consistent standards, provide reciprocal recognition of training between institutions, and promote sharing of resources. There is also the potential that further development could lead to International acceptance of Australian based training in the care and use of animals for scientific purposes.

The session format will be a brief introduction followed by a general discussion. Information will be collected and collated before being submitted to ANZCCART for publication.

Abstract:

The Australian code for the care and use of animals for scientific purposes (8th Edition, 2013) requires institutions to ensure that all people involved in the care and use of animals, including AEC members, have access to appropriate education programs and resources. The Code, however, offers no direct guidance on the minimum training necessary for people undertaking specific roles.

Many other countries set out minimum formal training and curriculum requirements. For example, the UK and the other European Union (EU) Member States now have a common framework for training which will be used throughout Europe. The EU are also considering accepting training undertaken overseas, if it is of an equivalent training standard. This standardisation has led to the general availability of various media resources, self -learning and assessment tools.

This is not the case in Australia where institutional trainers develop and provide a wide range of training options to their own staff and students, using a multitude of different didactic and online platforms.

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

The *Australian code for the care and use of animals for scientific purposes*, 8th Edition, 2013 (the Code) requires institutions to ensure that all people involved in the care and use of animals, including Animal Ethics Committee (AEC) members, have access to appropriate education programmes and resources. However, the Code offers no direct guidance on the minimum education and training necessary for people undertaking specific roles or tasks. Many other countries set out minimum formal training and curriculum requirements or frameworks. This is not the case in Australia, where institutional trainers develop and provide a wide range of training options to their own staff and students, using a multitude of different didactic and online platforms. 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?

Background

Education and training of researchers, teachers, animal carers and AEC members is a regulatory requirement and is an integral part of institutional governance relating to the use of animals in science. The regulatory framework includes, State legislation, the *Australian code for the care and use of animals for scientific purposes*, 8th Edition and the *Australian Code for the Responsible Conduct of Research*. The Code's key statements relating to education and training are listed in Appendix 1.

In addition to the regulatory requirements, an education and training programme forms part of each institution's risk management strategy and provides the opportunity to:

- Promote a culture of care and respect.
- Impart knowledge, practical skills and promote a standard of best practice.
- Promote better science.
- Introduce participants to key contacts.

Education and training review at the University of Western Australia (UWA)

A review of education and training was undertaken at UWA. The aim of this review was to critically examine the current programme for researchers, teachers, students, animal carers and AEC members who planned to use, or oversee the use of, animals for scientific purposes. This was done in the context of the regulatory framework (legislation, institutional and AEC requirements), user feedback and local, national and international programmes. The current programme is described in Table 1.

Table 1. *Current animal user education and training programme at UWA*

Part 1 Regulations and Institutional principles & practices *Core online module that is compulsory for all people working with animals*
Part 2 Workshop (face-to-face; approx. 5 hours) Content includes: Pain and Distress, How the AEC works/Ethics, Anaesthesia, Experimental Design and Facility Introduction (including Health and Safety) *Compulsory for most people (with some exemptions); a generalised approach; not specific to role or species*

Part 3 Additional education and training (offered on basis of need)

- Low stress animal handling and practical skills training (including anaesthesia)
- Completing animal ethics applications
- Designing project specific monitoring programmes

A feedback survey of over 100 participants showed that the overall satisfaction rates were high for both the online and face to face workshop components, and there was widespread support for inclusion of additional training in species-specific handling and procedures, and a desire for greater access to both online and hardcopy resources.

This survey identified some issues and led to discussions about how best (in terms of content and delivery platform) to provide an education and training programme that meets the needs of UWA and its research and teaching community, including AEC members, and also supports the wider Western Australian research community. Some educational opportunities are already shared between institutions in Western Australia and it was considered valuable to enhance these collaborative relationships.

Some of the issues identified at UWA included:

- Provision of education and training for remote campuses and external collaborators (including timely and easy access to online programmes).
- Recognition of previous education and training, and/or experience.
- Utilisation of existing institutional Learning Management Systems, with inherent limitations identified for ready access and rigid registration requirements.
- Course structure (generic, linear, species streaming, modular).
- Reliance on skills training within research groups with identified variation in standards (e.g. sometimes 'custom and practice' rather than 'best practice').
- No specific course for AEC members.

The review then posed the question: Should educational and training resources be shared to an even wider research community within Australia and New Zealand? It was considered that this could be best achieved by designing a national or regional education and training framework that promoted standardisation, as has been done internationally (e.g. in UK, EU, Canada and USA). This could also provide the potential for mutual recognition of equivalent training with the region and internationally.

Education and training in Australia

In our region, individual institutions develop and provide a wide range of education and training opportunities to staff, students and collaborators, using a multitude of different didactic and online educational platforms. Information about the programmes offered by different institutions was investigated using an internet search. Sixteen universities and research institutions in different parts of Australia were accessed. For a further four institutions in New Zealand and one in Australia, information on training content was not freely accessible or was password protected.

All sixteen institutions in Australia provided compulsory education for animal users. In fourteen this was clearly stated as compulsory, and in two institutions it was implicit. It was not clear whether any institutions offered exemptions to personnel with appropriate prior education or

experience. Some institutions were pooling resources by offering common, combined education and training courses. The time allocated to these compulsory programmes varied from one hour to two days (see Table 2), and courses were presented as a variety of online modules and / or face to face seminars or workshops.

Table 2. *Duration of compulsory animal user education programmes in Australia*

	Time Allocation (approx.)			
	Unclear	**1 hour**	**2 to 5 hours**	**1-2 days**
Number of institutions	4	2	7	3

In general, it appears that the core content is primarily oriented to the regulatory framework for use of animals in science. In addition to this, some institutions offer either further compulsory or optional content which appeared to be specific to species, role (e.g. Chief Investigator (CI) versus honours students) or tasks undertaken (see Table 3). Variation occurred in level of training (e.g. theory versus practical) or whether the training was provided by the institution, the CI on the project or another external source. Three institutions indicated that they require refresher courses at intervals of two or three years. Knowledge assessment was part of some content. No institutions promoted courses specifically for AEC members.

Table 3. *Range of education and training topics listed across 16 institutions in Australia*

- Regulations
- Responsibilities
- Institutional principles and practices
- Ethics
- Research Integrity
- How the AEC works
- Completing animal ethics applications
- Pain and distress
- Normal versus Abnormal
- The 3Rs
- Wellbeing
- Monitoring
- Research planning and design
- Impact on scientific integrity
- Analgesia and anaesthesia
- Surgery and aseptic technique (theory)
- Procedures (theory)
- Facilities induction
- Safety
- Animal handling
- Practical skills training and assessment
- Train the trainer

Education and training internationally

Canada, USA, UK and the EU have structured guidelines or legal mandates governing the education and training of personnel working with animals. The general course content is very similar in Canada and the USA, and the EU and UK have recently harmonised their education and training. These international standards have led to the general availability of various media resources as well as self-learning and assessment tools.

CANADA

The Canadian Council on Animal Care (CCAC) is the national regulatory body responsible for standards for the ethical use and care of animals used in science. The CCAC publishes guidelines

on the care and use of animals in science and addresses ethical concerns. In 2015, the CCAC produced new guidelines entitled '*Training of personnel working with animals in science*'. This provides a comprehensive framework for the training, supervision and competence assurance for personnel involved in the care and use of animals in science. It outlines recommended content which can be tailored to institutional needs and categories of personnel working with animals (e.g. animal users and animal care staff). The syllabus is divided into core topics, which are relevant to all animal related staff (whether directly involved with animals or not), and topics within specific knowledge streams that are relevant to those working with particular species or circumstances. The core topics are listed in Table 4. The CCAC has also developed a range of educational resource materials and quizzes to assist institutions deliver these programmes.

USA

In the USA, federal laws mandate educational programmes for personnel who use animals in research, testing, and teaching. To facilitate compliance by institutions, the Committee on Educational Programs in Laboratory Animal Science provides a guide entitled, 'Education *and Training in the Care and Use of Laboratory Animals: A Guide for Developing Institutional Programs'*. This guide has been in place since 1991 and outlines a syllabus containing four course modules that can be customised to fit the needs of the institution's education and training programme. The first of these, the core module (see Table 4), is intended for all personnel involved both directly and peripherally with laboratory animals. The other three modules are intended for select groups of personnel, according to their need-to-know. For example, species-specific sessions with hands-on training for all people in direct contact with animals and additional pain management and surgery modules, as applicable.

EU

The European Union has a common education and training framework to fulfil the legal requirements under Articles 23 and 24 of Directive 2010/63/EU on the protection of animals used for scientific purposes. This framework aims to assure the competence of staff caring for or using animals in procedures, and facilitate the free movement of personnel within the EU. It is based on a modular training structure with a focus on learning outcomes. It establishes minimum recommended training for all personnel. It sets out core modules of basic theoretical training for personnel taking care of animals, carrying out procedures and designing projects (see Table 4). There are also additional modules to meet the minimum training needs for specific defined functions and tasks, as well as requirements for species-specific training.

UK

In 2013, the UK amended the Animals (Scientific Procedures) Act 1986 to incorporate changes brought in by the European Directive (2010/63/EU) on the protection of animals used for scientific purposes. The content of UK training courses was adapted to the EU common framework and recommended modules.

Table 4. *Core module content–International*

CANADA	• Ethics in animal experimentation • Guidelines, legislation and regulations • Occupational health and safety • 3Rs of humane animal experimentation
USA	• Laws, regulations, and policies that impact on the care and use of animals • Ethical and scientific issues • Alternatives

EU & UK	• Responsibilities of the institution, the animal care and use committee and the research and veterinary staff • Pain and distress • Survival surgery and post-surgical care • Husbandry, care, and the importance of the environment • Euthanasia and resources • National legislation • Ethics, animal welfare and the 3Rs • Basic and appropriate biology—species-specific (theory) • Animal care, health and management—species-specific (theory) • Recognition of pain, suffering and distress—species-specific • Humane methods of killing (theory)

Conclusion

Overall, the key components of various international training courses are very well aligned. In view of recent trends towards global collaborative research, it would seem logical for Australia and the surrounding regions to develop more structured training which aligns with global standards and expectations. It is perhaps more appropriate for individual institutions in Australia and the region to avoid the need to reinvent the wheel and consider working toward a common framework and sharing of resources. Not only would this be cost effective, it would also lead to standardisation of education and training and facilitate mutual recognition of standards within the region. Additionally, it would enable animal research personnel moving overseas to meet international requirements.

As a preliminary step, the authors proposed the conduct of a national survey to collate information on current education and training, future aspirations, and to gauge interest in standardisation of education and training in the region and sharing of educational resources. The response from conference delegates to this proposal was positive and supportive, and it is the intention that a voluntary survey will now be conducted through ANZCCART.

Appendix 1. ***Australian code for the care and use of animals for scientific purposes*** **8th Edition (2013) – Key statements on education and training.**

§2.1.8 Institutions must ensure that all people involved in the care and use of animals understand their responsibilities and the requirements of the Code, are competent for the procedures they perform or are under the direct supervision of a person who is competent to perform the procedures, and have access to appropriate education programs and resources, by:
With respect to investigators
(ii) providing adequate resources for appropriate education, training, and assessment of competence of investigators, and certification of such competence to the satisfaction of the AEC.

§2.2.12 Institutions should ensure that AEC members undergo appropriate induction, and have
access to appropriate education programs and resources.

§2.4.4 Investigators must:
(v) undertake education and training, and competency assessment, in
accordance with institutional and AEC policies and procedures.

§2.4.5 A person must be identified who has ultimate responsibility for the care and use of animals in a project. This person must:
(ii) ensure that procedures and resources are in place so that all people involved in the care and use of animals in the project can meet their responsibilities, including their education, training and supervision, as appropriate.

§2.4.8 During planning, investigators must consider the following factors and be satisfied that:
(xix) procedures are performed competently, by people competent for the procedures or under the direct supervision of a person competent to perform the procedures, and provisions are made for the education, training and supervision of people nominated on the application, as appropriate.

§2.5.15 The facility manager, with support as required from the institution and other staff members, and advice from veterinarians, must:
iv) ensure that procedures and resources are in place so that all people involved in the care of animals can meet their responsibilities, including education, training and supervision of staff, as appropriate.

§2.3.28 The AEC must submit a written report on its operations at least annually to the governing body of the institution(s) for which it acts.

§2.3.29 The report should advise on:
(iii) actions that have supported the educational and training needs of AEC members and people involved in the care and use of animals.

§2.4.18 Investigators must take steps at all times to safeguard the wellbeing of animals by avoiding or minimising known or potential causes of harm, including pain and distress, to the animals. Steps include:
(ii) ensuring that procedures are performed competently, and that the investigators are: (a) competent for the procedures they perform, or (b) under the direct supervision of a person who is competent to perform the procedures.
(v) ensuring that people involved in the care and use of animals in the project are knowledgeable about the normal behaviour and signs of pain and distress for the species they will use.

Presentations given

on

Thursday 21st July

The beginning of the end for the lab mouse?
Computational and stem cell approaches to modelling neurogenetic disorders.

Steven Petrou PhD FAHMS.
The Florey Institute of Neuroscience and Mental Health,
The Department of Medicine (RMH),
The University of Melbourne.

Convergent breakthroughs in epilepsy genomics, stem cell technology, genome editing, computational neuroscience and instrumentation have created a perfect storm for the generation of new modelling approaches for studying disease mechanisms and discovery of targeted therapies.

The epileptic encephalopathies, rare yet severe forms of epilepsy, are particularly well poised for major treatment breakthroughs. I will describe some of our recent efforts in using human stem cell based approaches and real time *in silico-in vitro* modelling to begin the task of providing viable alternatives to mouse experimentation.

This is an important first step towards a longer term vision of generating new modelling approaches that not only replicate many of the features of mouse models but also surpass them in many ways.

A copy of the slides used during this presentation is provided below:

THE FLOREY
INSTITUTE OF NEUROSCIENCE & MENTAL HEALTH

THE UNIVERSITY OF MELBOURNE

The beginning of the end for the lab mouse?

Computational and stem cell approaches for modelling neurogenetic disorders.

Steven Petrou

Genomics driving medicine

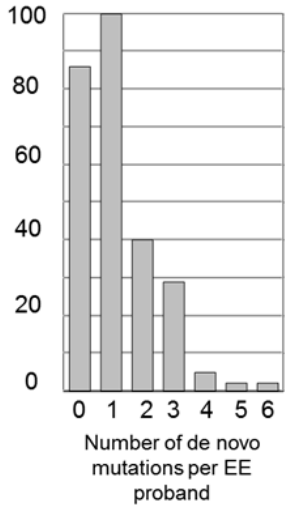
- Our understanding of the genetic basis of disease advancing at enormous pace
- Demand for modelling of genetic mutations is increasing placing an ever increasing need for generation of more mouse models bearing genetic mutations
  - Balance of ethical, societal and patient benefits
- Are there viable alternatives to mouse models that not only address the ethical concerns but also of real advantages
  - This will be the ultimate driver for reduction

LETTER

doi:10.1038/nature12439

De novo mutations in epileptic encephalopathies

Epi4K Consortium* & Epilepsy Phenome/Genome Project*



- Every offspring has a chance of a random genetic mutation occurring that are not in parents
- Most have 0-1, few have 2-3 and 4 or more occur very rarely
- Depending where the mutations are in our genome they can be *benign* or cause *severe disease*
- The majority of severe childhood epilepsy are caused by such mutations
- But with this genetic knowledge comes great hope that we can find treatments

Mutations in the human *KCNT1* gene give rise to neurodevelopmental disorders

Epilepsy of infancy with migrating focal seizure (EIMFS)

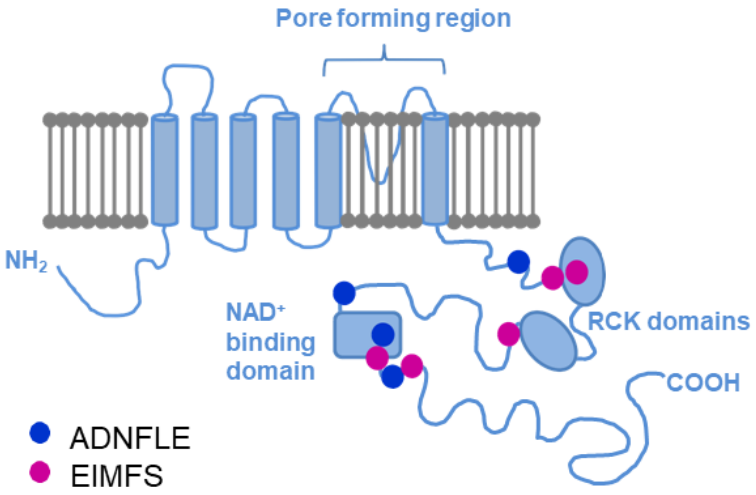
Barcia *et al.*, 2012, Nature Genetics

- Multiple cluster or continuous seizure
- Focal ictal activity which then migrate randomly to other brain regions

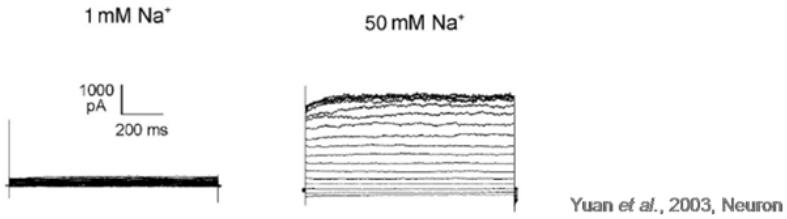
Mutations in the human *KCNT1* gene give rise to neurodevelopmental disorders

	ADNFLE	EIMFS
Age of onset	~7 years old	3-6 months of age
Cognitive dysfunction	Mild	Severe
Motor function	Normal	Developmental arrest
Response to AED	50%	0%

Sequence like a calcium-activated K^+ channel (Slack)



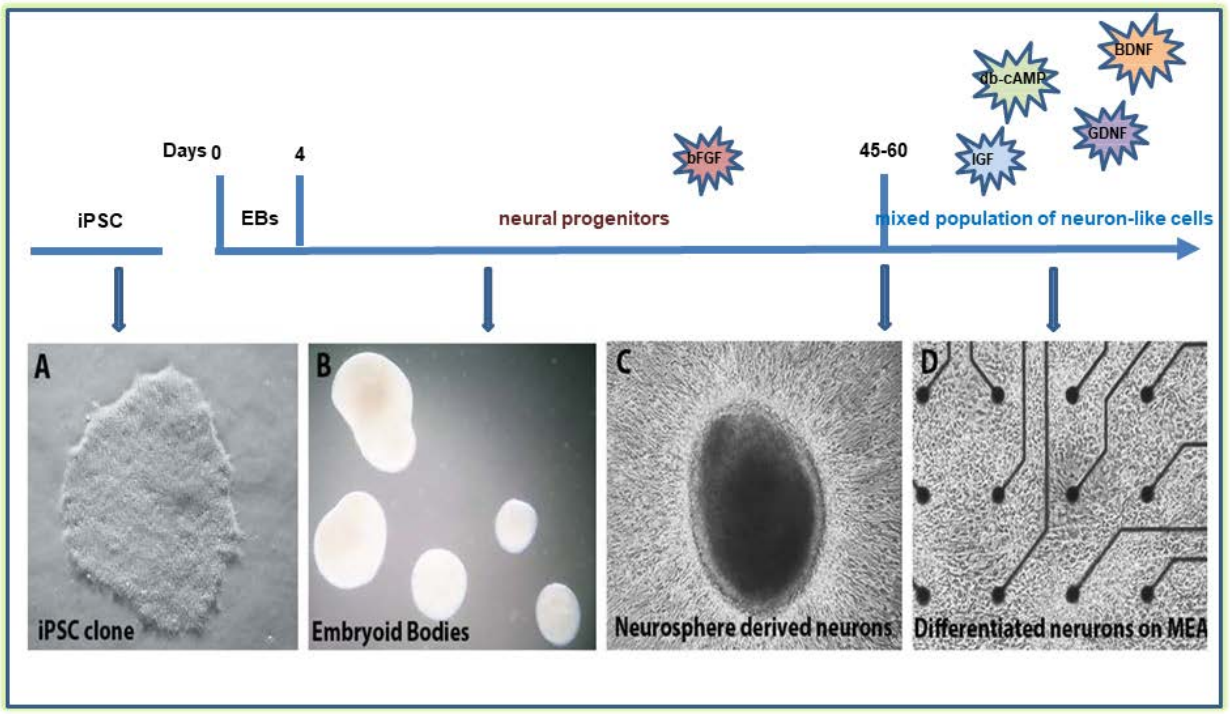
Enriched in electric organs that show high firing rates



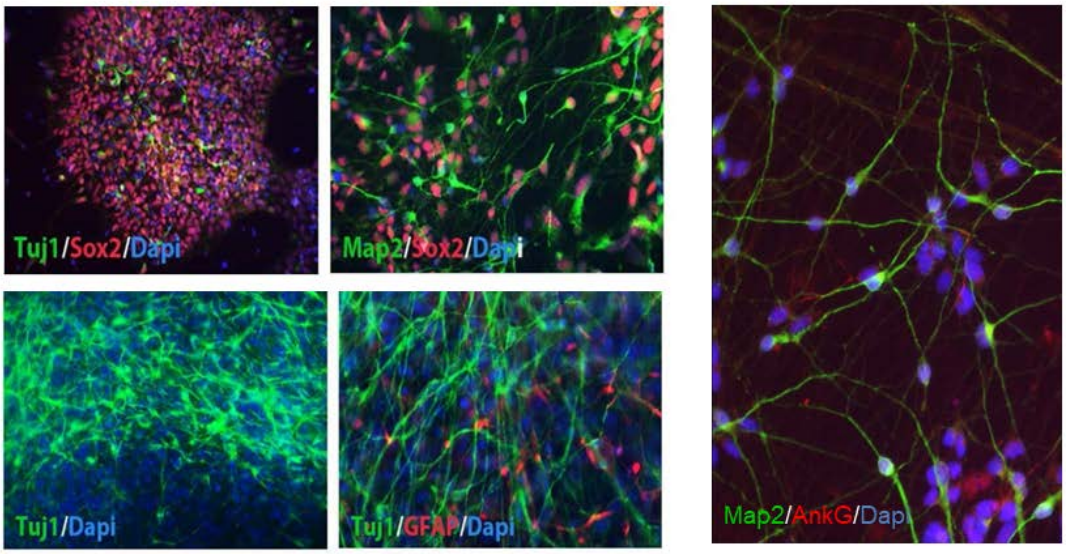
Yuan *et al.*, 2003, Neuron

Can we use human stem cell based models to understand the disease and develop therapy?

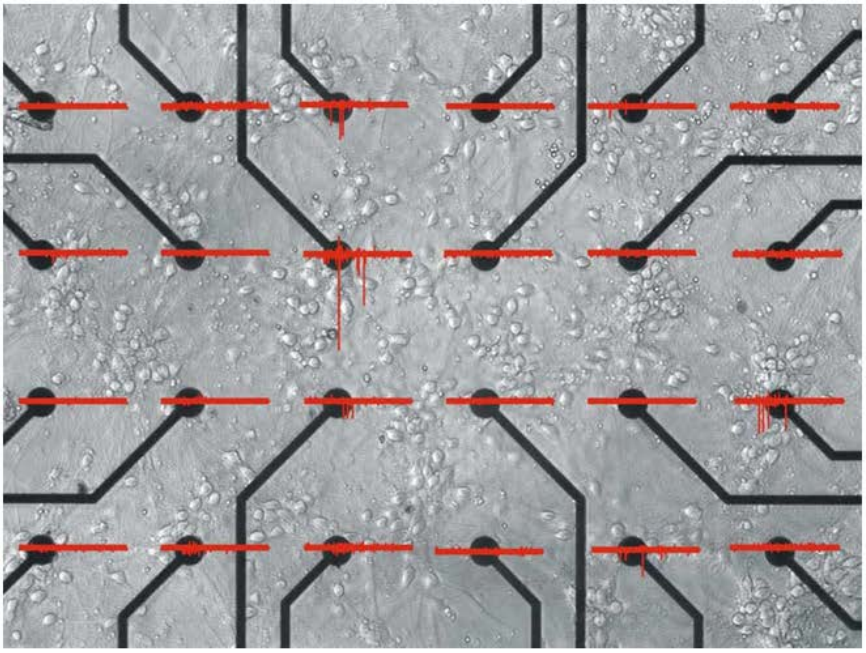
Turning stem cells into neurons



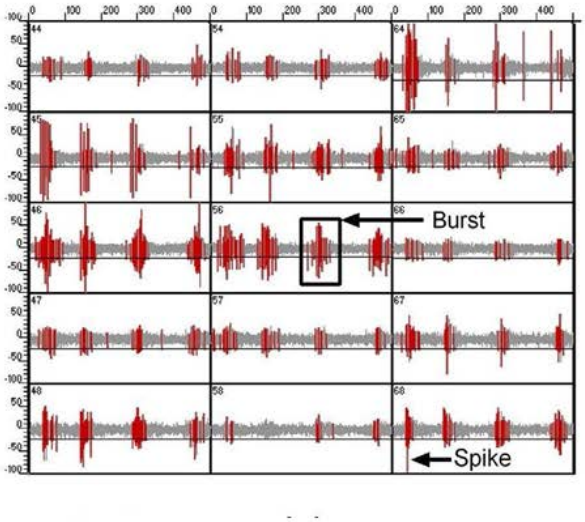
Markers of neuronal development

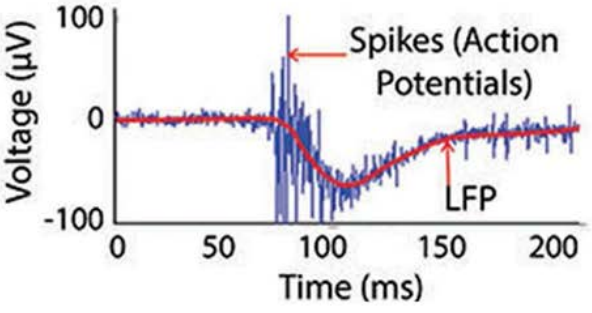


How to study:
Microelectrode arrays



MEA modelling





MEA modelling

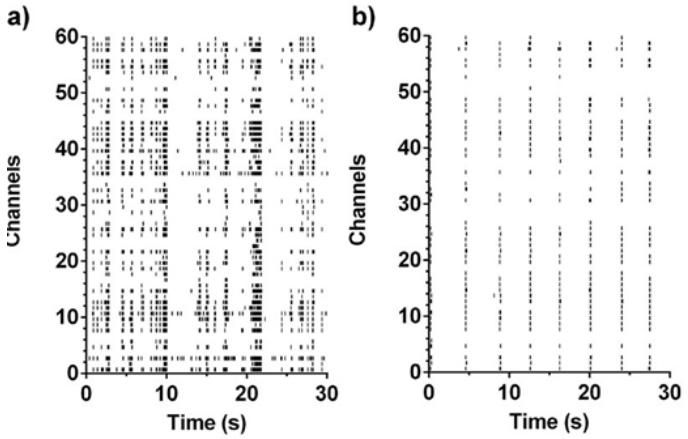


Fig 8: Raster plots showing a) baseline network activity b) Activity after the application of Conolidine 30uM

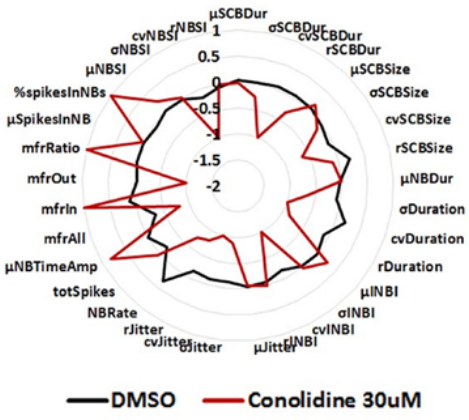
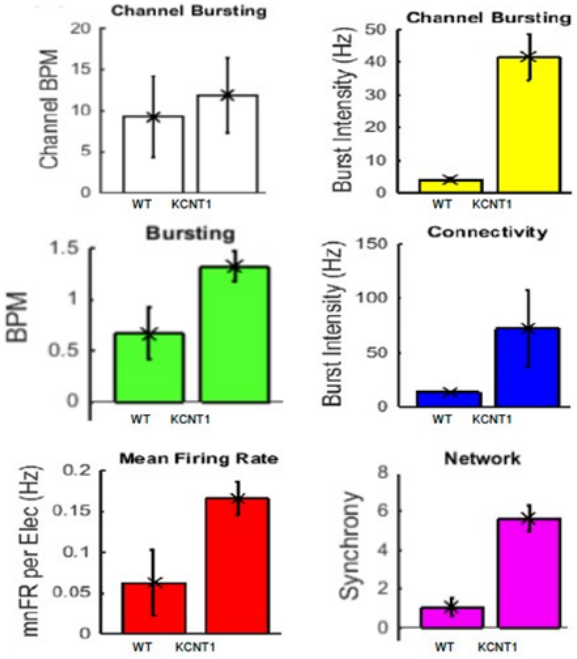
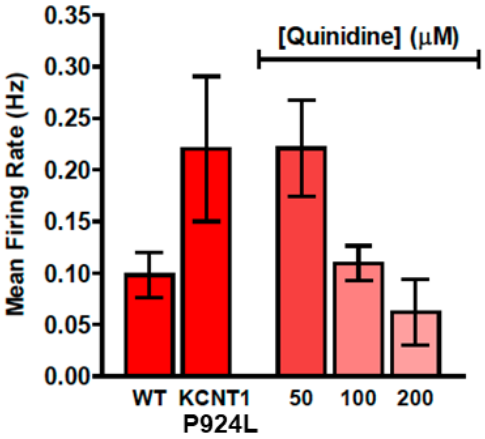


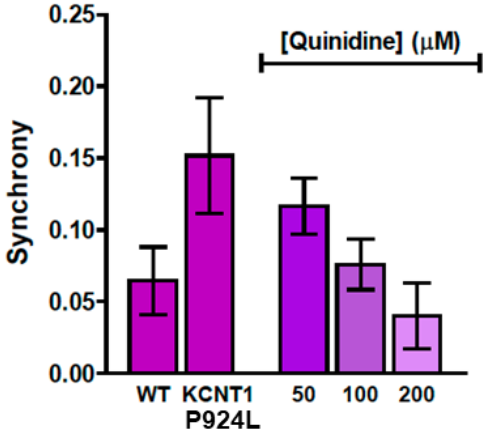
Fig 9: Radar plot showing the normalized change in features for Conolidine and its solvent DMSO

KCNT1(P924L) mutation and markers of enhanced excitability in MEAs



Quinidine reduces excitability in MEA model using human stem cell neurons





Clinical trial based on repurposing of quinidine

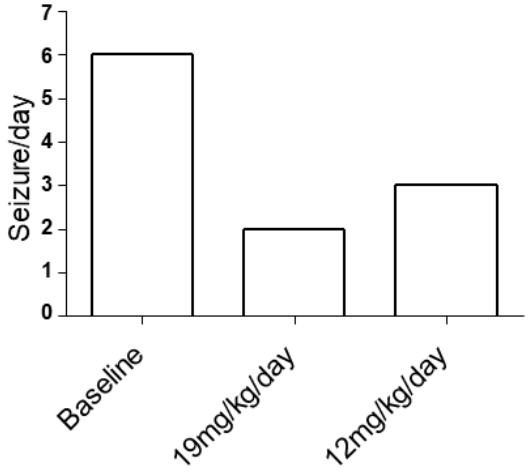
Quinidine improves EIMFS in very severe case

Female 11 years old

At 2.5 years age presented with 40 seizures per day and regressed to non-ambulatory non-verbal state

After one day of quinidine 19 mg/kg/d, seizures dropped to 2/d at 12 mg/kg/day seizures were 3/d

Improved bladder control, alertness, head control, sitting for first time



Quinidine in The Treatment of KCNT1 Positive Epilepsies. M Mikati, YH Jiang, M Carboni, V Shashi, S Petrovski, Slave, R Crimian, CJ Milligan, MY Li, A Grefe, A McConkie, SF Berkovic, IE Scheffer, S Mullen, M Bonner, S Petrou, D Goldstein*. Annals of Neurology Accepted*

Summary

- Demonstrates potential for animal free development pipeline to deliver repurposed drugs
- Massive potential for delivery of “precision medicine” in neurogenetic disease

How can we use computation to improve the quality of our brain stem cell models?

- In silico analysis typically performed after data is acquired which makes predictions about biology which are then tested and new data incorporated into model
- This cycle is slow and separates modelling from real biology
- Can we do modelling in real time by linking biology to computation?
- "Wetware in the loop" is our development that is a major step towards real time modelling for brain research

Information flow through neurons

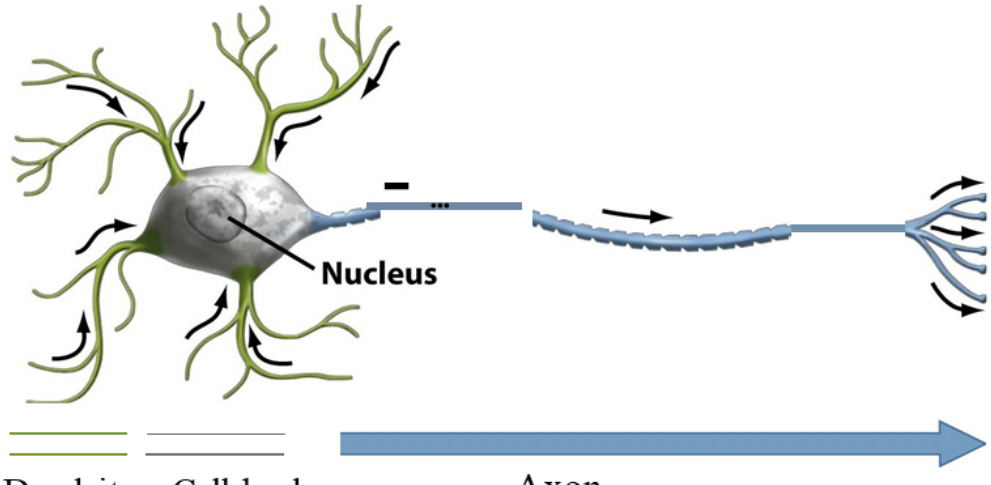
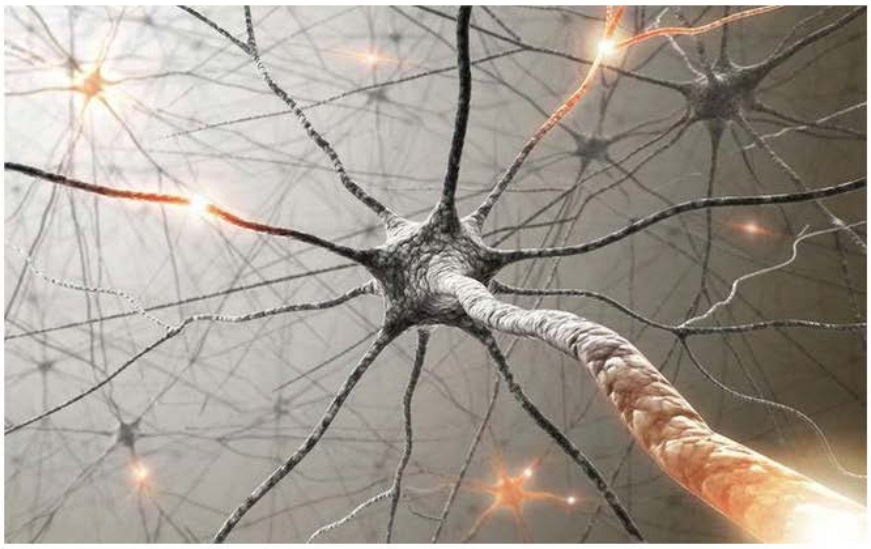
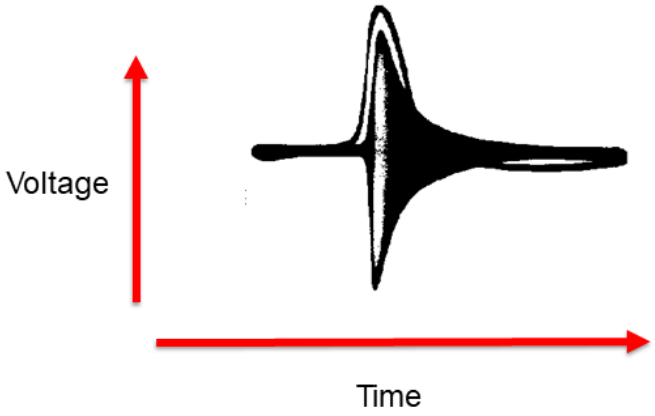


Figure 48-2b Biological Science, 2/e

Brain uses electrical signalling on massive scale for all real time processing of thoughts and actions



The “action potential” is the fundamental electrical signal in the brain



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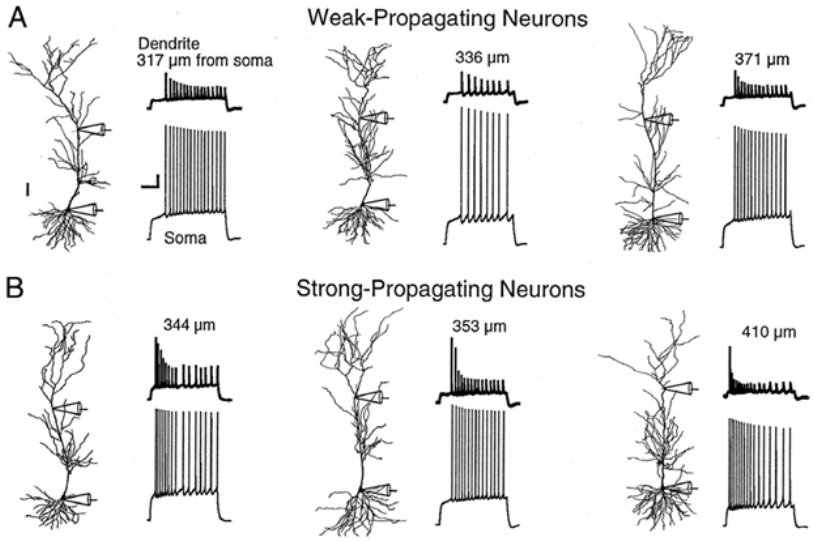
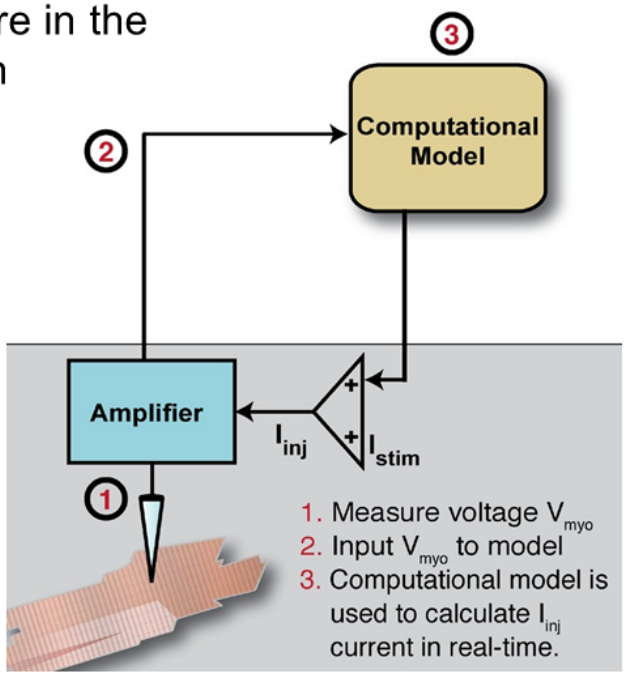


For brain and heart studies action potential properties are key

- However, in human stem cell based models of cardiac myocytes and neurons the action potentials are very often “immature” and do not reliably function

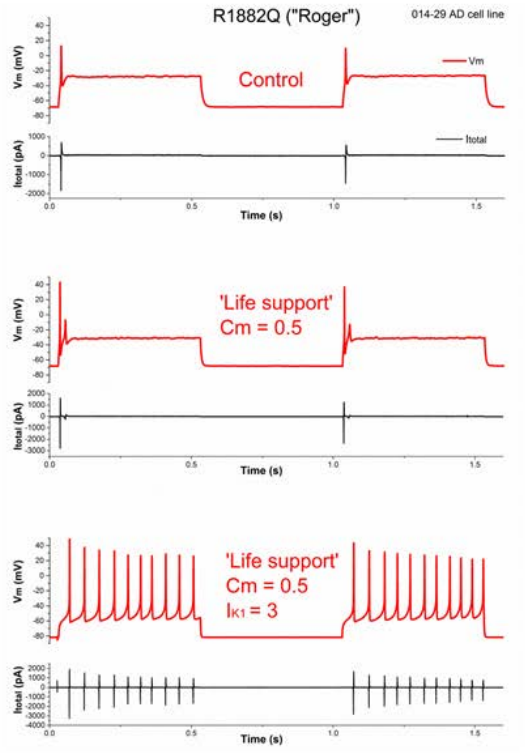
- Can we use real time computation approaches to provide “stem cell life support” to improve the quality of the action potential firing and subsequently the reliability and predictability of the models

The dynamic clamp as a basis of our real time wetware in the loop modelling solution

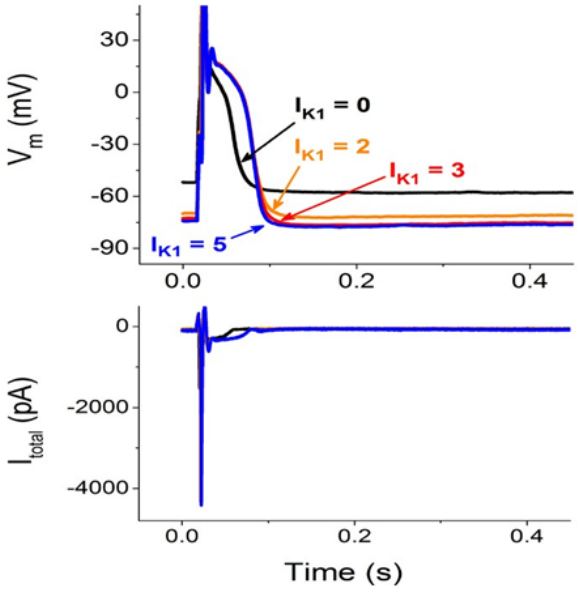


Stem cell or HEK cell expressing ion channel of interest

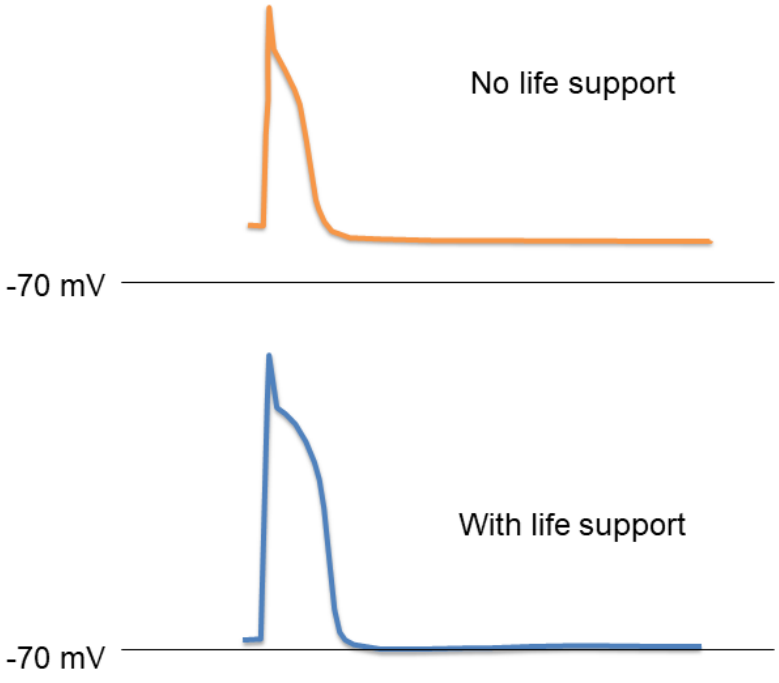
Life support with severe epilepsy patient-derived stem cell neurons



Life support in human stem cell derived cardiomyocyte



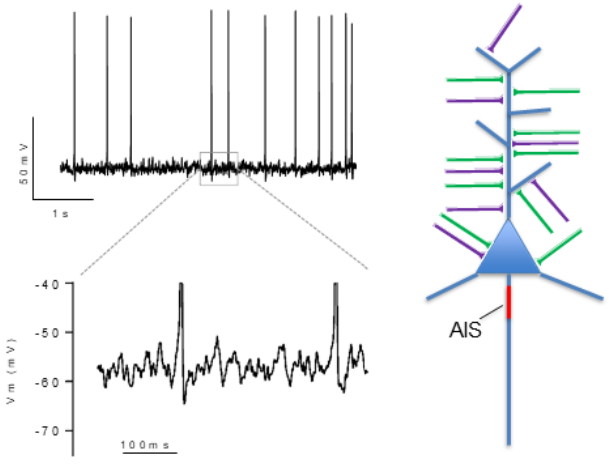
Life support in human stem cell derived cardiomyocyte

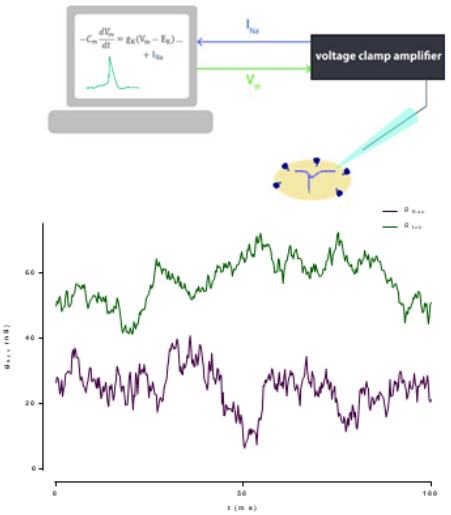


Using HEK cell lines alone can we use our real time computational system to with a cell line to recreate *in vivo* like neuronal recording

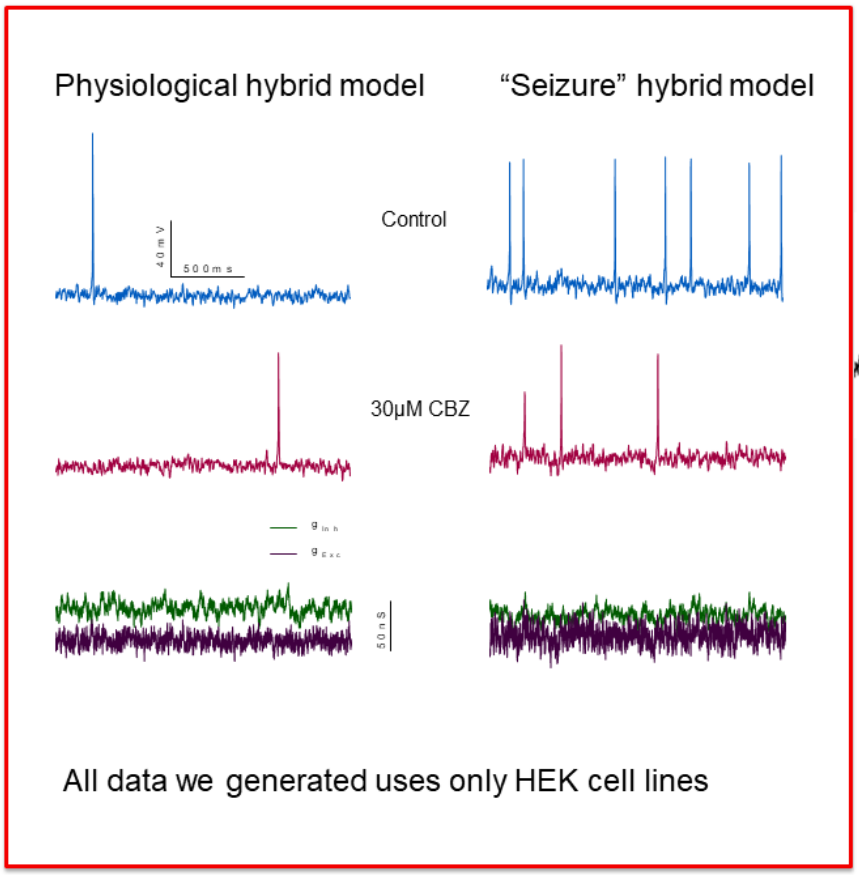
Pharmacology and mutant analysis with no patient or animal models needed

Modelling *in vivo*-like behaviour





Synaptic background noise generated with stochastic models (*Destexhe et al*)



Summary

- Provided examples of how stem cell and HEK cell modelling can couple with real time computation to provide high quality clinically predictive models of neurogenetic disorders
- Incorporation of human and even patient pharmacology, genetic and epigenetic environments offers tangible advantages over mouse models
- Viable alternatives now emerging for disease research and development of therapies in severe neurological disorders

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- Ingrid Scheffer
- David Goldstein
- Saul Mullen

- NHMRC
- DHB Foundation
- ARC CIBF

Gatekeeper benchmarking for AEC animal advocacy

John C Schofield
J&L Consulting, Dunedin New Zealand

The main function of Animal Ethics Committees (AEC) is to protect animals from harm; an advocacy role, whereas, scientists working with insentient bacteria or plants can essentially do as they please without any legislative oversight.

Use of animals in research and teaching has public approval and is ethically acceptable, when conducted under well controlled and regulated conditions. The most recent survey of public opinion on this matter was published in NZ in 2007 and it confirmed general acceptance and approval, based on a belief that appropriate checks and balances were in place to ensure no unnecessary pain and suffering. The AEC underpins all research and teaching use. It functions as a gatekeeper, hence it must operate in a clear, transparent and internationally commensurate manner across all institutions. A uniform standard is essential to ensure that the public concerns are addressed. How do AECs ensure that they operate as one?

Benchmarking is one practical answer. This involves measurement of the quality of an organisation's policies and procedures, when compared with its peers. The objectives are to determine where improvements can be made and compare performance levels with other institutions. In New Zealand, independent reviews are normally conducted every 5 years, while in Australia external reviews must be conducted at least every 4 years. The institution's performance is compared against their Code of Ethical Conduct in NZ or the Australian Code of practice for the care and use of animals for scientific purposes. However, there appears no formal mechanism for AECs to compare notes, because these reviews are confidential*. I suggest that both countries would benefit from a system to share improvements and advances in AEC performance amongst institutions.

This paper addresses the fundamental role of AECs in monitoring animal use for research, testing and teaching in both countries. Clearly the basis of all AEC performance are the relevant standards, however, in my experience, at an operational level, many personnel serving on AECs lack a working knowledge of these standards and generally follow the institutional customs established with time. In particular, Category C and D, or external members may play a more passive role in AEC functions, through allowing scientific institutional members to lead the application review process.

A number of strategies will be presented which might empower these external C and D members to act more forthrightly when faced with challenging applications.

*The Code recommends that institutions consider publishing their review report or at least the Executive Summary of that report on their web site.

The title of this paper summarises the content. It is my view that the AEC (Animal Ethics Committee) functions as a gatekeeper and an example will be discussed below. Some current benchmarking practices will be reviewed along with their perceived deficiencies. Finally, some strategies for improvement at the institutional or ANZCCART level will be proposed, in combination with a number of "empowerment activities" for AEC members.

The Australian code for the care and use of animals for scientific purposes[1], defines four compulsory categories of membership which are summarised as follows: Category A: veterinarian, Category B: a scientist, Category C: animal welfare representative and Category D: an independent external member representing the general public. The New Zealand Animal Welfare Act 1999 has equivalent membership but does not use category terminology.

AEC benchmarking is a less than scintillating subject, so I want to share my ideas and experiences in a new way; which started with my playing the Irish whistle, at the podium, to the tune of the rhyme below. It is an old English dance melody, dating back to 1853.

Half a pound of tuppenny rice,
Half a pound of treacle.
That's the way the money goes,
Pop! goes the weasel.
Every night when I get home
The monkey's on the table,
Take a stick and knock it off,
Pop! goes the weasel.

And a novel strategy is to ask the following questions:

1. Do weasels pop at your institution?
2. Would you know if they failed to pop?
3. What does successful weasel popping look like?
4. Do your weasels pop in compliance with the Australian code of practice's recommendations for weasel popping- in its 8th edition?

The reason for this approach, which may not be immediately apparent, is as follows. When confronted with a new discipline and a new committee appointment, for example when Category C or D members first join an AEC, much of the material they are expected to read will appear as a jumble of jargon. The proactive new appointee will start asking questions on issues which they know nothing about. A failure to ask questions, (in the absence of any supportive clarification from other AEC members) often leads to long term confusion and ignorance of the key issues.

My personal position on AEC functions can be simply summarised as an enthusiastic, crusading animal advocate, who believes that the anthropomorphic approach is of great value. This term can be defined as: "the showing or treating of animals, gods, and objects as if they are human in appearance, character, or behaviour".

I have come to appreciate just how challenging it is for Category C & D members when they first join an AEC. These members have an important but difficult role on the AEC: to ask searching questions, which reflect public concerns. Despite the difficulty, it is helpful to remember that the AEC exists as an animal advocate, and I believe that its main function is to protect animals from harm. In stark contrast, are scientists who use plants, earthworms

or bacteria, as experimental subjects. These researchers do not need AEC approval for their studies, because there is no harm caused. Furthermore, the use of animals in research and teaching is ethically acceptable under well controlled and regulated conditions. In my view, our legislation assigns the AEC a gatekeeper role. The term "gatekeeper" was first coined by Kurt Lewin (1890- 1947) a German Psychologist. The gatekeeper decides what information should move forward or not. It is now an important concept in communication studies.

For example, suppose a researcher proposes to the AEC to perform some eye irritancy studies for a new drug to treat eye disease. He decides to use rabbits, held in restrainers, and the test compound is to be placed onto the corneal surface of the eyes. This methodology is known as the Draize test. If the AEC is competent and current with the animal welfare legislation and literature, it would certainly refuse the Draize test proposal. Ideally it would suggest alternatives, such as the Hen's egg Chorioallantoic Membrane (HET-CAM) test, or the neutral red uptake assay.

What can the AEC compare itself with? How does an AEC determine whether it is up with the play? That it is doing its job properly, and what does success look like for an AEC?

Benchmarking is a measurement of the quality of an organization's policies, products, and their comparison with standard measurements, or with peers. The objectives of benchmarking are to determine what and where improvements are called for, and to analyze how other organizations achieve their high performance levels. Such comparisons can then be used to improve the functioning of another AEC.

Benchmarking standards which I am familiar with in Australia include the Australian code for the care and use of animals for scientific purposes (8th Edition, 2013) and in New Zealand, there is the Animal Welfare Act 1999, the Good Practice Guide for the use of animals in research, testing and teaching[2], and the Guide to the preparation of Codes of Ethical Conduct[3].

Another benchmarking strategy could be the external or independent reviews. In Australia these are ideally conducted once every 3 years (but no more than four years apart) and in New Zealand every 5 years.

The purpose statement for the Australian review is:

> "To establish evidence that all scientific and teaching activities adequately justified, that the welfare of animals is given due consideration and the AEC is effective"

The equivalent statement for NZ is:

> "To review compliance by the Code Holder and the AEC with the requirements of the Act, regulations and the Code of Ethical Conduct"

However, I don't think these reviews work at all well as benchmarking exercises between AECs. Since these reviews are confidential, the sharing of review findings between institutions is highly unlikely. In addition, these reviews do not appear to involve any examination of the quality of the AEC decisions, for I could find no terms of reference which encouraged reviewers to do so. In fact, the Performance Standards for Independent Reviews of Code Holders and Animal Ethics Committees, published by the NZ Ministry for Primary Industries, makes no mention of this matter[4].

Consequently, this begs the question; who monitors the gatekeepers? One could argue that the system encourages a silo mentality. Institutions keep their problems to themselves.
In New Zealand there is an informal network of concerned individuals, veterinary colleagues confer from time to time regarding AEC issues of concern. They share and benchmark animal welfare practices; the good, bad and the ugly, in order to seek solutions to immediate problems.

In Australia, there is a more formal structure; the Sydney Animal Research Ethics Group (SAREG), established 3.5 years ago by Paul Sou at UNSW [5]. They hold quarterly meetings and currently have approximately 20 active members. The Mission Statement for SAREG is:

"The mission of Sydney Animal Research Ethics Group (SAREG) is to provide a mutually supportive and positive learning environment in which every individual member has the opportunity to share experiences, and discuss matters related to animal research administration and service provision, including the specific approach that is taken at each member's own institution, in a confidential manner. This collaboration allows members to optimise processes at their own institution, and thus promote ethical animal research and welfare."

Strategies for improvement at the institutional or national level

Given the central importance of AECs, this matter might usefully be a regular item at ANZCCART conferences. The National Animal Ethics Advisory Committee (NAEAC) in NZ holds workshops every two years and might be a useful forum for benchmarking exercises. However, to be successful, such benchmarking would require a new level of transparency by institutional officials, willing to share concerns and potential problem solving strategies. Most certainly the SAREG model is a great development.

To return to the challenge confronted by new AEC members. Let me share another 'different approach'. If I was asked join a committee, about which I knew very little, for example a Weasel Popping committee, I would insist on the following:

Access to the "Weasel Popping for Dummies" course

1. To be coached by an expert Weasel Popper, as to what to expect
2. Advised on typical problems created by Weasel Poppers
3. Receive the relevant literature about Weasel Popping
4. Receive a Weasel Popper's glossary

The challenges which new external members face are several in my experience. They join a committee, often with a majority of well qualified academic experts. Unlike these experts, external members generally have minimal formal training in the scientific method and probably no knowledge of the techniques used by scientists. Anecdotal evidence suggests that most external members are often unaccustomed to vigorous debate and argument, whereas, academics generally thrive on and relish a good argument. Hence, when opinions are strongly held, external members can often feel intimidated by the scientists on the committee. This can result in a reluctance to ask questions; in case the inquiry is perceived to be 'stupid'. Hence external members may rely on the other members to make the right decision.

Some progress has been made in New Zealand to assist external members. The NZ Veterinary Association now has an on-line course for veterinarians[6]. The SPCA in NZ also intends to create an equivalent on-line course for its category C nominees.

There are some key strategies, which I would like to share, which may prove to be of assistance, particularly for C and D members.

1. Understand how the literature is used by researchers

a) It's very important to understand the value placed on the literature by scientists. It is their currency, their life blood, their raison d'etre. Scientists who don't get published, don't survive long. So much effort goes into this activity that most scientists hold the literature as almost sacred text, in my experience. It may also be useful to understand that scientists will generally preferentially cite literature that supports their proposal.

b) The literature is used to define, justify and promote whatever research idea is proposed. Scientists will only cite the literature which supports their proposal and avoid references which would challenge their application to the AEC. Hence, a balanced literature review of their research proposal might not be provided to the committee.

c) The AEC can ask the scientist to provide other additional references (which may challenge their animal model), so as to gain a more balanced perspective. There are review papers published which summarise the benefits and pitfalls of most animal models, but scientists, quite understandably might overlook these, in their research application.

d) Finally, the AEC can contact authors of recent publications, to get the "real deal". In my experience, this approach can deliver some surprising results. For example, when it is discovered that a surgical technique could only be accomplished by one of the authors, a lack of reproducibility might suggest a lack of scientific rigor.

e) It is worth noting that publication does not always confer ethical acceptability. Different cultures have different standards. For example, in the literature, rodents used for burn studies do not regularly receive analgesics to manage post-burn pain[7]. Depending on the location (country) of the burn study, the lack of analgesics may be 'justified' with the explanation that third-degree burns are insensate because the pain receptors have been destroyed by the burn injury. This theory is flawed in my view, since the margins of the burn still have pain receptors and this is why human burn victims are generally given intravenous analgesics as soon as possible. Fortunately, some journals are now more rigorous in the application of ethical standards, but regrettably there is no common international standard that can be relied on.

2. Use "the village idiot" approach.

a. Clearly it's easier for extroverts to play the idiot. One doesn't need to overdo the routine- but I am sure that you will understand these kind of reasonable questions.

"Can anyone help me here; I can't understand the justification?"

"How does she get from step A to step D, it seems information is missing?"

"You call that lay language!"

b. But what constantly amazes me is how often others on the committee also have the same question, but lack the confidence to ask it.

3. Apply “the Rotary 4-way test” The Rotary Club uses this test to evaluate the merits of any particular proposal.
Is it the truth?
Is it fair to all concerned?
Will it build goodwill and better friendships?
Will it be beneficial to all concerned?
If we apply this thinking to the use of animals in research testing and teaching, and use an anthropomorphic approach, we can ask ourselves:
Will it hurt me?
Will it help me?
What if it goes wrong?
Isn't there a better way of doing this?
The question: Will it hurt me? raises further questions such as; Where’s the morphine? How painful? For how long? When the committee member discovers in the application that no analgesics are proposed, that member might decide that they would not chose to be part of the study - if they imagined themselves as the animal.
The question: Will it help me? raises further questions such as; What scientific advance will result? Will meaningful data be collected? Justify why I must be used in this way please and finally, give me the big picture.

Category C: Gatekeeping the “Will it hurt me?” question could reasonably ask the committee:
“So if I was that animal, I don’t think I would be able to tolerate that procedure!” “Anyone here with a higher pain threshold than me? Anyone else concerned about this?”
The question: What if it goes wrong? raises further questions such as: What mistakes could happen? Sources of error? Probability of failure? We’re sure the researcher can actually do this correctly? What’s the batting average of this scientist at this surgery? What is the expected mortality rate for this surgery?

Category D: Gatekeeping the “What if it goes wrong? question”, is about misadventure, and can be asked of most procedures. The general public want assurances that animals do not suffer. Surgical success depends on competent surgeons. So some relevant questions to ask include:
“Exactly who is training the student to perform this surgery?”
“What is the expected survival rate for this brain surgery?”
“Who checks that the trainee is now competent?”
The question: Isn’t there a better way of doing this? raises further questions which reflect the Three R’s approach such as: Surely there is an alternative? Who else has done something similar? Can less invasive methods be used instead?

4. Ask for Gantt charts
The American engineer Henry Gantt, circa 1917, invented the horizontal chart, which he used as a timeline to plot construction deadlines.
The principle of Gantt charts can be used to provide an illustration of events which happen to individuals or groups of animals.

There are several advantages of Gantt charts:
a) Often a Gantt chart can summarise a complicated series of events, which is more helpful than multiple pages of narrative.
b) These charts present "research at a glance". They enable the reader to rapidly appreciate "the big picture".
c) Multiple procedures on animals can be clearly identified in a time line. Consequently, the AEC committee members gain a better understanding of the whole study and this then enables them to formulate questions about how and when animals are used.
In my experience, Gantt charts are not used enough in AEC applications and I would encourage AEC committee members to regularly ask scientists to use these charts in their application protocols.

5. Ask for pilot studies

A pilot study is a version of the main study that is run in miniature to test whether the components of the main study can all work together.
It is focused on the processes of the main study. It will therefore resemble the main study in many respects.

An African proverb from Ghana is a nice succinct summary of pilot studies which is worth remembering: "Never test the depth of the water with both feet".
The main goal of pilot studies is to assess feasibility so as to avoid potentially disastrous consequences of embarking on a large study - which could potentially "drown" the whole research effort.
Category C & D: Gatekeeping the "first time invasive study", might wish to ask the following:
"Clearly this researcher has never attempted such a surgery? So why we are happy for him to jump into a main study? Surely a limited pilot trial in the first instance would be prudent?"
It is recommended that AECs develop policies on when pilot studies are to be requested. Clearly the example above suggests a typical situation.

6. Ask for site visits

Visits by members of the AEC are a valuable monitoring strategy. They can readily confirm that animal welfare is being promoted in the research laboratory and determine that that procedures used on animals have first been approved by the AEC. Finally, site visits can be a helpful way to educate the AEC regarding new technologies.

However, it is most important that the visit team is qualified. To apply the novel example used initially, the site visit team should include an experienced weasel popper, who can identify popping weasels when they see them.

An example to illustrate the importance of appropriate expertise. The AEC site visit team invite themselves to observe brain surgery in anaesthetised rats. The Category A veterinarian expresses her view: "I can immediately see a couple of concerns with this surgical situation". Her AEC site visit colleagues are most surprised to learn that the surgery is being performed without sterile surgical drapes, and in addition, there is no evidence of eye protection ointment to prevent corneal drying during the 3-hour operation. Both concerns would have been missed, or overlooked by these other visitors. Had the veterinarian not been present, the researcher might have been signed off by the AEC visit

team as competent. As is the nature of research, these omissions might have been repeated in perpetuity.

Given the general limitations on time and personnel, within most institutions, there is merit in making strategic visits to observe experiments of high ethical cost, for example those involving survival surgical procedures. Admittedly, even relatively simple manipulations, such as intra-muscular drug injections have the potential to cause animal suffering if incorrectly performed. So AECs need to conserve their limited resources and decide on which experimental studies deserve their time. Policies on which research activities should routinely be visited is a useful and helpful strategy to develop.

Some suggested criteria to assist an AEC decide on which research activities to visit are as follows:

a) A new researcher/student performing invasive surgeries
b) A new and invasive procedure not previously attempted at the institution
c) A pilot trial with potential morbidity/mortality concerns
d) A study where the analgesia regime is questionable
e) At the invitation of the researcher to explain their new model
f) In response to a 'whistle blower' complaint
g) When a newly appointed senior academic refuses to attend mandatory training

When confronted with the difficult situation described in example (g) above, a recommended solution is to indicate to the new academic:
"Not a problem Professor, but because you can't make our training, we will visit your very first rabbit surgery. Please note that we not allow you any preliminary trials before the main event- since you claim such surgical expertise. We do expect that you will follow all our institutional survival surgery guidelines".

What generally follows, in my experience, is a rethinking of the Professor's priorities and a reluctant agreement, under sufferance, to attend training.

Perpetual protocol approval

A common challenge for AECs is what I call "protocol approval in perpetuity". By this I mean that the researcher believes that their application is approved for the full three-year term (or whatever applies at the institution) regardless of personnel changes.
A typical example to illustrate this phenomenon:
A protocol is approved for Dr. Smith's graduate student, Li Chen, to perform liver surgery on mice and the AEC visits to observe. Her surgical competence is confirmed. When Ms. Chen completes her studies and departs Dr. Smith's research lab, having graduated with a PhD, her replacement is one Barry Brexit who takes over the liver surgery on mice. Dr. Smith, not being concerned with details, doesn't advise the AEC of a change in personnel, a change in surgeon, because the protocol is already approved. Of major concern is that Brexit's surgical skills are minimal. *(Noting that in Australia, such a failure would be a serious breach of the Code.)*

This fictional, but typical scenario raises important questions:

a) Are approvals person specific?
b) What system does your AEC use to manage student surgeon succession?
c) How is the AEC notified when new personnel join a research team?

Summary

AECs can add value to their animal advocacy role when they share ideas and learn from each other. I believe that ANZCCART is well placed to assist with such benchmarking exercises in the future. Asking the right questions is a key role for external AEC members and I would encourage them to regularly ask for Gantt charts, pilot studies, site visits and if not already in place, to ask for policies and procedures to be created so as to assist the AEC determine when pilot studies and visits should be required.

Addendum

To clarify the unconventional weasel popping approach, at the conclusion of my presentation, I showed images of weasels; both small cottage and large industrial- strength types. In the textile industry, a spinner's weasel was a mechanical thread-measuring device in the shape of a spoked wheel, that accurately measured out yarn by making a popping sound to indicate the correct length had been reached. So the children's nursey rhyme does have a basis in industrial commerce.

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An overview of Alzheimer's disease and model systems used in research

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There is currently no cure or effective treatment for Alzheimer's disease, which is the most prevalent form of dementia, currently affecting more than 300,000 Australians and more than 45 million individuals worldwide. By 2050, it is estimated that nearly one million Australians and more than 130 million individuals globally will be burdened with a diagnosis of dementia, with a cost to the community in the hundreds of billions of dollars annually. As such, there is an urgent need to find effective therapeutic approaches that can either prevent or slow the course of disease.

One of the most common approaches utilised in neurodegenerative disease research involves the use of disease models, in which specific pathways can be genetically, pharmacologically or otherwise modulated in order to gain a greater understanding of disease mechanisms. The insight gained then allows for the development of a rational drug screening program that will generate candidate compounds to specifically target a particular protein or cellular cascade to effect an improvement in a given feature of a disease model. The burden of proof that a compound is safe and effective in a range of disease models is critical for the translation of the basic "bench science" through to the clinic.

In this presentation I will discuss Alzheimer's disease, and a number of the different model systems that are used to study the disorder, highlighting a number of the important caveats around their use and the potential implications for humans. Finally, I will present a vignette on my own research from the last decade that has focused on the role of metal ions (particularly zinc) in the onset and progression of Alzheimer's disease and how our unique collaboration with a local company (Prana Biotechnology) may lead to significant improvements in human health.

What is Alzheimer's disease?

The term "Alzheimer's Disease" (AD) was coined in 1910 by Emil Kraeplin to describe a series of cases initially described by Alois Alzheimer. The first case reported by Alzheimer appeared in his 1907 paper titled "Uber eine eigenartige Erkankung der Hirnrinde" ("About a strange disease of the cerebral cortex") (Alzheimer, 1907; Stelzmann et al., 1995; Small and Cappai, 2006), where he described the case of a 51-year-old woman who began experiencing a range of symptoms including disorientation, rapid memory loss and a host of other neuropsychological abnormalities. Following a rapid progression of her illness through to death, a post-mortem was conducted in which Alzheimer described significant atrophy and focal degeneration. In addition, he described how "the nucleus and the cell itself disintegrate and only a tangle of fibrils indicates the place where a

neuron was previously located" and further, "distributed all over the cortex, but especially numerous in the upper layers, there are minute miliary foci which are caused by the deposition of a special substance in the cortex" (Alzheimer, 1907; Stelzmann et al., 1995). Within these original descriptions lies the primary basic diagnostic criteria for AD – that is, widespread degeneration/atrophy in the brain together with the presence of abnormal "neurofibrillary tangles" (NFTs), representing the intracellular accumulation of abnormal tau proteins, and the extracellular accumulation of beta-amyloid (Aß) into "amyloid plaques". These features, which have subsequently been characterized in the medical literature (Glenner and Wong, 1984; Grundke-Iqbal et al., 1986), together drive the symptomatic presentation of the disease. The primary features that contribute to impaired quality of life in AD are classically associated with disturbances in memory, decision making and planning, the ability to recognize faces and objects, personality and mood changes and language problems. There are many excellent references in the literature describing the phenotype of Alzheimer's disease, as well as other details around the epidemiology, genetic associations with disease, risk factors and other aspects of the disease spectrum. These are both directed more towards the lay (Alzheimer's Association, 2015) and the scientific audiences (Ballard et al., 2011; Nisbet et al., 2015; Korolev, 2014; Karch et al., 2014; Amtul, 2015; Kumar et al., 2015; Scheltens et al., 2016).

How common is Alzheimer's disease?

This progressive neurodegenerative disorder represents the most common form of dementia (accounting for ~70% of all dementias), with the recent World Alzheimer Report stating that there are currently 46 million individuals living with dementia worldwide, with an economic cost near a trillion dollars annually (Prince et al., 2015). In Australia, there are currently in excess of 340,000 individuals with dementia, with a predicted increase to close to a million people locally (Alzheimer's Australia, 2015) and more than 130 million globally by 2050 (Prince et al., 2015). The prevalence of AD/ dementia is also closely linked to age, the greatest risk factor for the development of the disease, with rates of ~3.4% for Australians aged 70-74, 22.7% for those aged 85-89 and 42.2% for those 95 years and older (Access Economics, 2011). Despite this association with the ageing population, there is an emerging group of young onset dementia patients (aged less than 65 years), numbered around 200,000 in the USA alone, that are distinct from individuals that experience subjective cognitive decline (~13% of Americans aged 45 years and older reported experiencing progressively worse memory loss or confusion) (Alzheimer's Association, 2015).

What is the problem and how do we move forward?

Despite decades of research, the field is no closer to a cure, let alone an effective therapeutic that will intervene in the devastating neurodegenerative cascade that is AD. There are currently only a small number of drugs approved by the Food and Drug Administration (FDA) in the USA for the treatment of AD. These drugs are generally considered to provide some short-term benefit in a subset of patients, and are often used in conjunction with other non-pharmacological approaches to improve the quality of life of the individual. Details of the various therapeutic approaches and their respective mechanisms of action have been extensively reviewed in the past (Kumar et al., 2015; Ballard et al., 2011). It is clear, therefore, that one of the primary goals of the research community is to elucidate novel mechanisms and therapeutic approaches to either treat or prevent the development of disease. It is currently considered that the "treatment" of AD may be too difficult a mountain to climb, as the brain is a complex and in many ways delicate organ which is refractory to any form of "regrowth" in the same way as a broken bone or torn muscle might heal. Whilst there is research underway to try and elucidate mechanisms to "regrow" the brain (through

the use of endogenous stem cells, transplanted brain material and other pharmacological approaches for example), and indeed to maintain or even improve the function of the remaining brain material, this will remain the most difficult of paths towards success. Thus, there is currently a significant focus on the need for the early detection of AD so as to allow for the true prevention of disease before the loss/degeneration of precious brain matter occurs – although clearly for the millions of individuals currently impacted by this disease then a treatment strategy is also highly sought after.

In any case – whether the approach is targeted towards a prevention or a treatment strategy – there needs to be a suitable platform on which to develop and test candidate therapeutics.

Modelling human disease

The modelling of human disease is often a difficult process, as humans themselves are a complex and evolved species that are impacted by emotion, learning, language, geographic location, diet, exercise level and any number of other variables. Such factors may also contribute to both the onset and progression of disease, and so this means that defining a single non-human model in which to accurately recapitulate the features of many diseases is by default almost an exercise in compromise. Furthermore, these same considerations come in to play when human clinical trials are conducted – as these factors, together with others such as ethnicity, genetic factors (variants in specific genes can greatly impact your risk for certain diseases such as AD, and also impact how you respond to a given medication) and current medications and other factors can significantly impact the outcome of a given trial/ efficacy of a given therapeutic approach (it is also important to note that AD can be negatively *and* positively modulated by such variables).
This is particularly true for Alzheimer's disease – where it is clear that the cellular processes that precipitate disease occur many years before the clinical manifestation and diagnosis of the disease, and then they vary throughout the time course of the illness. Recent medical imaging studies (examining the amount of abnormal "amyloid" present in the brain), for example, have shown that it takes more than a decade to reach a threshold used to delineate a healthy control from a patient population, and nearly a further 20 years to then reach the mean level of amyloid burden found in established AD cases (Villemagne et al., 2013). Thus, defining the "human model" itself is very difficult given the many and varied paths towards disease and the innumerable confounding factors that can occur across lifespan.

Given the difficulties associated with human clinical trials (eg. ethics, time, cost, sourcing appropriately controlled cohorts of patients etc), the majority of scientific research focuses on preclinical animal models that help define the path towards human clinical trials.

In work done in the 1960's (McKinney and Bunney, 1969), there were a set of criteria put forward for a model system, which were later generalized to include the following, (1) the symptoms present in the animal model should be a good approximation of that found in the human condition (2) there should be quantifiable behavioural changes across the human/animal model that can be assessed (3) independent observers should agree on the criteria for assessing the subjective state (4) the effect of therapeutic intervention should be consistent between the humans and the animal model and, (5) the model should be able to be independently reproduced (Overall, 2000). These criteria have been modified/simplified in recent times to require, (1) face validity – meaning that the model has some resemblance to the human condition (2) predictive validity – meaning that the animal model can be utilized to establish the success of a given therapeutic approach and, (3) construct validity – meaning that the model has an appropriate theoretical foundation.

Given these requirements for a "valid" model, then consideration must be given to what aspect of Alzheimer's disease is modelled and in what system.

What to model in Alzheimer's disease?

Alzheimer's disease is a multi-factorial condition that is characterized by an evolution of disease and associated symptoms. As such, there are many different stages of disease that can be modelled which might not necessarily represent the full spectrum of AD, but rather provide a platform on which to study specific aspects of the disease. For example, animal models might recapitulate brain atrophy, neuronal loss, glial activation, modulation in neurotransmitter systems or alterations in specific cellular pathways. The argument can be made that the study of such processes in isolation eliminates the confounds present in more complete/complex models and allows a more rigorous understanding of the basic biology (although one must always question what is the primary driver for the symptoms present in any given model, and whether this has any relevance to the disease under study). In AD research, one of the primary areas of focus has been to generate models that contain the primary neuropathological features of the human disease – specifically, deposits of the amyloid plaque and the neurofibrillary tangle (Gotz and Gotz, 2009). As the primary drivers of disease, then the theory is that if a model exists that is characterized by these primary features of the AD brain, then many if not all of the associated cellular changes and even clinical symptoms will follow (there remains controversy in the field as to what is *the* primary cause of AD – the amyloid or the tau pathology. The weight of evidence now supports the notion that the amyloid pathology occurs first and precipitates tau pathology, the latter which is more closely correlated to cognitive deficits. This is largely summarized in the current iteration of the "amyloid cascade hypothesis" originally put forward by Hardy and colleagues (1991, 1992)). This would then provide a model that closely mimicked the human situation and would provide an excellent platform for drug discovery.

Before considering the "induced" models of disease, it is important to recognize that there are a number of "natural" non-human models in existence.

Are there "natural" models of Alzheimer's disease?

As noted earlier, there are several defining features of the AD brain that are believed to be the primary drivers of disease pathogenesis. Humans, however, are not unique in the existence of these neuropathological lesions, with several other species also known to express them in their brain. There are many mammalian species characterized by Aß and tau pathology, including dogs, cats, rabbits, goats, bears, wolverines, marmosets, monkeys, baboons and more (Oikawa et al., 2010; Braidy et al., 2015). Such species, therefore, perhaps represent some of the best models for the human disease given the natural development of "AD-like" pathology (this pathology arises because of the presence of the relevant conserved protein sequences between the different species; as will be discussed later, rodents, for example, have a slightly modified version of these proteins and so do not naturally produce specific AD-related pathology). What is immediately apparent, however, is that most of these animal species are quite inaccessible to the majority of scientific researchers and present a unique set of complications when considered in the context of medical research (eg. finding appropriate housing and facilities, sourcing the animals, costs of upkeep, existence of methods used for behavioural testing etc). The most commonly studied of these "natural" models are non-human primates (Podlisny et al., 1991; Oikawa et al., 2010; Ichinohe et al., 2009) and canines (Cummings et al., 1996; Gonzalez-Martinez et al., 2011). The former representing the closest approximation to humans, and as such, potentially having the greatest

relevance (Capitanio and Emborg, 2008). Indeed, Roelfsema and Treue (2014) note that "research with non-human primates represents a small component of neuroscience with far-reaching relevance that is irreplaceable for essential insights into cognitive functions, brain disease, and therapy". Practically speaking, however, such models are not typical in the field, and instead there is a host of other "induced" models of AD that have flooded the market. Whilst such models also present a unique set of complications and caveats, their relative ease of use (compared to a bear for example!) has made them a mainstay of preclinical research and drug testing for a host of significant human medical conditions.

What "induced" models of Alzheimer's disease are available?

There are numerous "induced" models used in AD research, the majority of which involve the over/expression of the key AD-related proteins amyloid and tau. There are models in everything from yeast (Bharadwaj et al., 2010; Moosavi et al., 2015), zebrafish (Newman et al., 2014), flies (Bouleau and Tricoire, 2015; Fernandez-Funez et al., 2015) and worms (Link, 2005; Wentzell and Kretzschmar, 2010). Such model systems present many advantages in the laboratory setting, and indeed provide platforms on which to address very specific hypotheses around Alzheimer's disease. However, they also present a number of caveats that have implications for our capacity to translate the findings into humans. This is, in part, why the typical screening protocols in industry (and indeed academia), follow a hierarchical pathway from least complex to more complex models (for example, cell culture or fly/worm models, followed by rodent models, then through to monkeys and finally man (De Felice and Munoz, 2016)), as the simple models allow rapid hypothesis testing and data generation, and then each step closer to the human brings increasing confidence that a given hypothesis or drug approach will successfully translate into the clinic. It is also critical to note that in terms of progressing novel medicines through into the clinic, it is crucial that there has been an extensive preclinical workup performed to establish, amongst other things, the safety and tolerability of a compound, as well as the potential mechanisms of action that will give rise to a theoretical clinical benefit (and indeed, taking one step backwards, it is also critical for the basic scientist to have a suite of preclinical data if they are going to obtain industry investment to further progress their compounds into development for ultimate human trials). These requirements are all very strictly laid out by the regulatory authorities and are designed to, amongst other things, limit adverse effects in man. As such, in order to test any compound in a human clinical trial, there is by default a requirement for there to have been complex animal modelling performed to justify moving forward into humans. As such, rodents represent a good middle ground in the laboratory setting, with regards to the complexity, caveats, and potential implications of the model, as well as the "acceptance" of the data derived from these models by industry partners and regulatory bodies. Two of the key features of rodent models has been the development of methodology to genetically modify the animals (this allows the scientist to ask very specific questions around any number of different human diseases or biological processes) and the development of sophisticated approaches (particularly the evolving touch-screen technology) to assess behavioural endpoints in the animals that have relevance to humans (such as anxiety, learning and memory and many other behaviours – this allows the scientist to examine the functional consequences of a given therapy/intervention/manipulation in the animal).

Whilst there is an incredible number of different rodent models utilized in AD research, the field has narrowed to a handful of more routinely used mouse models (summarized in, Onos et al., 2016). These animals, with strain names such as Tg2576, APP/PS1, 3XTg and others, are defined both by what genetic mutation has been engineered into them (and of what relevance this is to AD), and what the subsequent phenotype of the animal is (again relative to AD). These animals typically overexpress one of the two primary neuropathological features of AD (amyloid or tau, to give rise

to abnormal brain accumulations of these proteins that then drive cognitive dysfunction and other features characteristic of the human disease), and in some cases have been developed to express both the abnormal amyloid and tau proteins/lesions in the one animal (as noted above, rodents do not naturally develop the abnormal amyloid and tau pathology found in AD, as the proteins that generate these lesions and the biology of the animal is subtly different enough to preclude this – hence, transgenic mice that overexpress human versions of these proteins are generated). The result is that there are many different transgenic mouse models for AD, and this requires a clear understanding of what the model actually represents and can tell the investigator, and may also direct the specific set of questions that can be addressed in the given model. As an example, there are "aggressive" mouse models of AD where the animal may develop extensive brain pathology within four months of age (such as the TgCRND8 mouse), then there are models that may develop the same level of pathology later in life at ten to twelve months (eg. the APP/PS1 model) and finally, there are models that only develop this pathology much later in life (eg. after 15 months of age, as found in the Tg2576 model). So there is a great diversity in the models available with respect to *how* AD is being represented in these animals (as not only is the timing of the evolution of the neuropathology different between models, but other features also vary). Given that AD is an age-related disorder that only emerges in the older population, then it could be argued that the more aggressive animal models may not reflect the human disease process – however, holding animals for two years or more is impractical for many because of the associated costs and the time involved (indeed, having to wait two years or more for a single experimental result would be career suicide in our environment in which scientists live and die by their research output). Thus, the individual scientist must establish the best paradigm for their specific needs and situation. This broad discussion also raises the highly relevant and critical aspect of animal welfare and its relevance to research outputs.

The scientific importance of animal welfare

Whilst the importance of animal welfare from a moral/ethical standpoint is clear, the underlying relevance to the science is sometimes less apparent, and a number of these issues will be discussed here. At the most basic level, if an animal is "sick" or in some other way unusually impaired, then this will confound any dataset if the animal is left in an experimental trial. This is true from the perspective that a sick animal may perform differently in a given behavioural task, may respond differently to a given therapeutic approach and may have different physiological processes occurring that may independently alter disease onset or progression. It is important to note that the term "sick" is used in a very broad context, and should be read to include any impairment that could be perceived to impact normal animal physiology. Stress, for example, is a classic confound that is not immediately apparent, but one which can have profound implications for experimental outcomes (it can potentiate all manner of neurological diseases, cause memory impairment and many other outcomes that may directly impact the phenotype of a given animal model, or affect the performance/response of the animal in a trial). Stress can arise because of a host of reasons such as dominance issues in a cage or inappropriate handling. In addition, one of the emerging concepts in the field is that even apparently minor modifications within the animals' cage environment can impact experimental outcomes, including things such as a lack of sufficient bedding or nesting material, lack of appropriate environmental stimulation/socialisation, abnormal lighting, the colour of the individual caging systems, the position of those cages on a rack, the ambient temperature and so on (Toth, 2015; Mo et al., 2016). If such confounds creep into a study, they may impact the animals and ultimately inadvertently validate or refute a given hypothesis, which in turn may then lead on to further unnecessary experiments that waste animal lives, researcher time, effort and funding support. Whilst we lack the sophistication to fully tailor an

animal's environment to one that would be considered "optimal" from the animal's perspective, it is clear that significant consideration must be given to experimental design and animal welfare in any given experiment or any given animal housing scenario in order to maximize valid experimental outcomes and the potential to subsequently translate the findings into humans.

That being said, it can be argued that no animal model will ever fully recapitulate neurodegenerative or psychiatric disorders simply because of basic species differences (Kreiner, 2015) (not to mention issues around potential confounds as described above). Indeed, many of the mouse models utilized in AD research are more than adequate at modeling specific aspects of the pathology of the disease, but they often fail to accurately model the functional decline that characterizes AD (although as noted above, the approaches utilized to assess functional decline are becoming increasingly sophisticated, and as discussed later, the field is always on the search for *better* models). This raises the question of where animal modelling for AD has led us?

Where has Alzheimer's disease modelling led the field?

As discussed earlier, there are very few drugs licensed for the treatment of AD. Specifically, these treat the cognitive deficits present in the disease, and include the acetylcholinesterase inhibitors rivastigmine (Exelon), galantamine (Razadyne, Reminyl), tacrine (Cognex), and donepezil (Aricept), as well as the NMDA receptor antagonist memantine (Namenda). As noted, however, these drugs have only a modest effect for a period of time in a subset of patients. As we are no closer to truly effective symptomatic treatments, let alone disease modifying strategies that can prevent the disease, then research has been going along at a staggering pace across the globe at both the academic and the industry level. Much of this has involved animal research, which has cumulatively led to more than 1600 human clinical trials (930 completed, 590 currently active and 120 terminated) (De Felice and Munoz, 2016). The question has to be asked, therefore, why we don't have any better, or even newer, therapies than those listed above. This represents a significant debate in the field, with people questioning the validity of the animal models or their use/interpretation, the validity of the basic hypotheses around the cause of the disease, the robustness of the human clinical trials and so on. All these questions are directed towards understanding why we are failing, and why we have so many potential treatments for AD that work wonders in an animal model but fail to translate into the human population.

Laurijssens and colleagues (2013) recently outlined a number of generic reasons why animal models fail. These include (1) the model does not accurately represent the human disease or is based off an incorrect hypothesis, thus any therapeutic approach is targeted to something that is not relevant to the human condition. (2) Failures in translation to the patient, perhaps because of an incorrect dosing regimen or some other species difference that means the compounds are metabolized differently in animals vs man (the biological target itself may also be different in animals vs man, again complicating our ability to successfully translate studies and to engage a specific target in a human population), and (3) inappropriate clinical trial design (eg. study is not long enough, the patient cohort was flawed in some way, the methods of analysis were inappropriate or not sufficiently sensitive etc).

Considering the animal aspect, then clearly the caveats highlighted throughout this article are also going to have a significant impact on the successful translation of basic research into the clinic setting. There are also other issues – such as the potential for compensatory mechanisms to be present in mice that are not present in humans. This was recently reviewed by Kreiner (2015), and highlights how carefully we must consider/design animal experiments and subsequently interpret the outcomes. These and other concepts related to the difficulties in translating outcomes from

animal studies into successful human trials have been reviewed previously (Banik et al., 2015; Zeiss, 2015; Zahs and Ashe, 2010; Franco and Cedazo-Minguez, 2014, Amtul, 2016; Onos et al., 2016). From the other perspective, the human clinical side, there was also a very nice critique recently published (Karran and Hardy, 2014) that highlights some of the problems in successfully translating studies from preclinical to clinical settings.

Many of the therapeutic approaches that have come to clinical trial have been heralded as *the* compound that will save the ageing population from the devastation of AD. These compounds have been rooted in hypotheses that are central to many and have carried a weight of expectation from the field. The results, however, have been little more than a spectacular failure. Whilst it might be easy to invoke a host of animal/human differences to account for this, careful examination of the stepwise trajectory of a given compound from the bench to the clinic reveals a number of gaps in knowledge that may underlie at least some of the failures observed. In this regard, there are certainly examples where data at a given stage of the drug development pipeline has not supported the progression of a compound through into human clinical trial (Karran and Hardy, 2014). This speaks volumes both to the desperation of the field to try and find a cure, but also to the need for a rationale appraisal of all levels of the drug development process.

Conclusion

The utility of rodent models has proven invaluable in providing insight into the pathogenesis of AD (not to mention our understanding of general brain function) as well as in providing direction for the development of novel therapeutic approaches for the disease.

Arguably, however, the field must increase its rigor and sophistication when modelling human disease in animals and it must also rigorously evaluate all steps and experimental data that are generated from both the preclinical and clinical pipeline. This must be done within the context of the highest degree of animal welfare, but also with a mind to the many caveats that animal modelling brings and the many unique attributes afforded by the different species. As the field moves forward, we continue to march towards an era of decreased reliance on animals for the generation and validation of hypotheses, with cell-based techniques (Yang et al., 2016; Mungenast et al., 2016; Yi et al., 2015; Zhang et al., 2016) paving the way for a new frontier in personalized medicine.

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The care and feeding of the animal ethics officer – Organization and management of ethics data

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Charles Darwin University (CDU) Animal Ethics Committee (AEC) has particular challenges as it is currently the only AEC in the Northern Territory. Consequently, CDU AEC reviews and approves animal ethics application from a number of organisations in addition to applications from CDU researchers. These include government organisations, commercial companies, non-profit organisations and independent researchers. With the broad scope of work reviewed out by the AEC, a robust system is required for the organisation and management of data associated with the wide range of projects. Additionally, a system was required to keep track of the training and reporting requirements for the various researchers and projects to ensure compliance to the *Australian code for the care and use of animals for scientific purpose* (henceforth referred to as the code).

An Animal Ethics Database (AED) has been developed by the Research Systems and Performance (RSP) team of the Office of Research and Innovation (ORI) at CDU. Based on Oracle® Database platform, the AED was initially developed for easy filing and retrieval of project-related data. Since its development in 2013, the AED has been fine-tuned by the RSP team in conjunction with the animal ethics office requirements into a user friendly and functional database that provides numerous functionality over and above data storage requirement. In an external independent review of the AEC carried out in 2015, the reviewers were highly impressed by the ease and functionality of the AED, and recommended that it should be presented to the wider animal ethics community.

In addition to storing data, the AED is now capable of capturing due dates for annual progress / final reports allowing generation of reminders to principal investigators, due dates of compulsory ethics training for investigators and data for annual report required by the code and state authorities. Being an online system, the AED is also capable of securely distributing applications and reports to the AEC members for review and comments prior to the AEC meetings and semi-automated generation of correspondence letters based on the outcome of the AEC decision. A similar database has been created for the human research ethics office and this database can be translated for use by the human research ethics committee with a few adjustments to fulfil jurisdictional and operational requirements.

The presentation provides a brief overview on the functionality of the animal ethics database, namely the process of creation and submission of a new animal ethics application for review, through to the generation of the correspondence decision letters addressed to the researcher. It will also show how data from new applications, amendments, unexpected adverse events, animal usage and non-compliance can be easily retrieved for the generation of reports to the relevant authorities.

In the future, the AED will be further developed to include the addition of Standard Operating Procedures (SOP) and tracking for subsequent review of SOPs as required by the code. An automated letter generation for progress and final reports will also be implemented which is currently available in the human research ethics version of the database.

The Care and Feeding of the Animal Ethics Officer: Managing and organising ethics data

The animal ethics application process generates a substantial amount of data which is not only limited to applications, amendments and various reports; but also communication between the animal ethics committee (AEC), animal welfare officer and researchers.

The Australian Code for the Care and Use of Animal for Scientific Purpose (the Code) states that institutions must ensure that records related to the AEC business are maintained and where appropriate, should ensure that animal carers have access to records of approved project and activities. As such this creates a need for a robust database system to archive and easily retrieve the information stored.

In the Northern Territory, Charles Darwin University (CDU) AEC is responsible and authorised by the NT Animal Welfare Act for issuance of permits to individuals for conducting teaching or research involving animals. This adds an additional role of the AEC to safely store personal information of these personnel, and also the tracking of the status for the permits of these individuals, and their training requirements.

As with many other institutions, the ethics office is often responsible for the administration and support of the AEC in accordance to the Code. In light of these requirements by the Code and legislation, it would be useful for an animal ethics database to be able to manage these documents and processes on a single platform. This proceeding paper briefly describes the characteristic of an animal ethics database that will be useful for ethics office in managing and organising their ethics data.

Background

CDU AEC is currently the only AEC located in the Northern Territory. Consequently, CDU AEC handles a large number of applications within and external to the university, involving a wide range of projects. This results in a huge amount of ethics data that the AEC has to report back to the institution; and also to the various animal welfare branches in the States where the CDU AEC is licensed.

As with many other institutions, the CDU ethics office is responsible for the administration and support of the AEC in accordance to the code. One problem faced by many an ethics office is tracking of the submission of annual progress reports, and also the training of individuals working on each project. Another area of difficulty is the generation of the animal numbers according to various classifications such as type of activity and species for reporting of animal usage.

In view of these considerations, the Research system and performance team created an Animal Ethics Database to help overcome these issues. A demonstration database was presented to attendees at the 2016 ANZCCART conference and the factors that the CDU animal ethics office consider useful in an animal ethics database system.

What are the qualities of a good animal ethics database?

A good animal ethics database system should focus on 3 aspects: namely its accessibility, functionality and the operating environment and platform. This will allow users to manage various aspects relating to animals' ethics monitoring and applications on the same platform without the need for separate databases or spreadsheet.

Accessibility

An ideal animal ethics database should be an online based platform that is accessible over the inter- and intranet, and on various devices. As such, no additional specialised software or applications should be required for installation by users. The ability to be able to access the database from various devices is useful for animal carers who often utilise mobile type devices in animal facilities. In addition, the level of permission should be easily modified to allow different users to access different functions of the database. Examples include only permitting researchers to submit a new application, amendments and unexpected adverse event reports to existing projects, viewing existing files without allowing modification; to access by the AEC to view documents for the upcoming AEC meeting.

Functionality

The functions of a good animal ethics database should not only be limited to archival and retrieval of files and documents, but also include process management for the day to day activity of the ethics office and the AEC. We have identified 4 key functions that our ethics office administers, and a good animal ethics database system should be able to capture and assist in the management of these processes.

Management of research project

Management of research projects should be the primary function of an efficient animal ethics database and an ethics database should be developed around this capability. The users should be able to utilise the database to upload of project applications and any other associated documents with the project such as amendments, submission of reports, *ad hoc* reports by animal carers / welfare officer, as well as any correspondence between the researchers and the AEC. Research project management should also include the profiles and animal ethics training of investigators on the project, the animal usage, research sites / facilities where the project is taking place and inspection of these facilities, as well as the name of the organisation / research group carrying out the project.

Management of the AEC meetings

It will be useful if the animal ethics office is able to manage the animal ethics committee and the AEC meetings through the AE database. A good AE database should permit the transfer of project files to a scheduled meeting, so that AEC members can access these files from the meeting function, which is only made accessible to members. Furthermore, the ethics office should be able to manage committee members' details and their attendance at each meeting through the database.

Report generation

Reporting is a significant role of the animal ethics office and the database should be able to automatically generate reports based on parameters input into the reporting function. At Charles Darwin University, we are able to customise reports such as animal usage numbers, and projects according to various inputs such as type of animal, project status, principal investigators etc. In addition, we are able to generate automated reminders to researchers for submission of progress / final report, as well as generate a list of people who are due for animal ethics training.

Standard operating procedures (SOP) management

As part of the Code requirements, SOPs are required to be reviewed every three years by the AEC. A good animal ethics database should have the capability to store SOPs for document management and allow for a reminder when it is due for a review.

Operating environment and platform

The final aspect that is crucial to a quality animal ethics data base is the operating environment and platform that it is based on. It is essential to have a stable program that works on different operating systems and has a user friendly interface. In addition to this, it should provide a strict level of security especially if there are commercial in confidence projects that the AEC oversees.

Finally, given that the needs of the institution are an ever changing process, an ideal database should allow constant refinement, through the help of the institution's IT department to meet the need of the ethics office and the AEC.

The role in Zoos in Compassionate Conservation.

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Every year, millions of visitors across the globe step through the gates of zoos, immersing themselves in wildlife. Some come for a love of animals, some for a day out, but as soon as they step through those gates each one has the potential to be shaped and changed in the way they think about conservation.

While the science of animal welfare continually develops and evolves, we strive not only to meet minimum welfare standards, but to achieve positive welfare states and a life worth living for all animals in our care. This in turn becomes a double edged sword in the field of conservation within the zoo. With a visitor-ship that has become more socially conscious, displaying animals in positive welfare states provides the opportunity to create deeper understanding of animals, correlating to a more discernible action from visitors to conserve species. This drives conservation outcomes, but also allows the zoo to continue the work it is doing in husbandry and breeding of conservation species.

A new way of thinking, Compassionate Conservation, is being applied across the zoo system and is driving this conservation success. By looking at the interests and welfare of individual animals whilst aiming to improve conservation outcomes, greater conservation success can be seen on all fronts. This is a timely evolution of the way zoos operate, with Zoos Victoria's *Fighting Extinction* programs underway, both locally and globally.

No manuscript was submitted for this presentation