Choosing the right species in research

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When designing animal studies, investigators must choose a species that is appropriate for the research. In this paper, the author examines various criteria that can be used to guide this selection. He discusses the concepts of phylogenetic group and sentience and finds them not to be useful in the selection of appropriate species in biomedical research. He identifies other criteria that are more useful as justifications for species selection, including susceptibility to a targeted disease process, tendency to engage in a targeted behavior, suitable size for the experimental techniques to be used, presence of a large body of data relevant to the study, species specificity (the species itself is the target of the research), intergenerational interval, similarity to humans, contractual specification and existing guidelines. He proposes that investigators should use these justifications, and perhaps others, to choose the most scientifically appropriate species for animal studies.

In the course of animal research, investigators must choose for their studies animal species that are scientifically appropriate and most likely to produce valid results. Justification for species selection should be included in the animal research protocols reviewed by IACUCs. In some cases, investigators might be asked to include the consideration of phylogenetic group or sentience. But how are these characteristics applicable to species selection? Are they reasonable? And are there more useful criteria to guide this process?

PHYLOGENETIC GROUP

The use of phylogeny as a basis for species selection has been suggested in various sources. In 2008, on behalf of the US Department of Agriculture (USDA), the US Food and Drug Administration (FDA) and the Office of Laboratory Animal Welfare, Gipson *et al.*[1] wrote, "[When using] research procedures that may cause more than slight or momentary pain or distress to the animals involved…the principal investigator must consider alternatives (e.g., replacement with a species of a lower phylogenetic order or using alternative methods…)" and "When determining the appropriateness of a non-rodent species, consideration should be given to selecting a species of the lowest phylogenetic order that will yield the most informative data." No definition for 'lower' or 'lowest' is offered.

More frequently, this idea is stated in terms of a phylogenetic scale. In 1990, Prentice *et al.*[2] wrote, "Whenever it is compatible with the research objectives, animals that occupy the lowest possible position on the phylogenetic scale should be chosen." The most recent version of the *Guide for the Care and Use of Laboratory Animals* (eighth edition, 2011)[3] says, "Replacement refers to methods that avoid using animals. The term includes absolute replacements (i.e., replacing animals with inanimate systems such as computer programs), as well as relative replacements (i.e., replacing animals such as vertebrates with animals that are lower on the phylogenetic scale)." Here, 'scale' refers to an archaic term, scala naturae, meaning the great chain of beings[4]. Prior to the work of Charles Darwin, existing species were thought to sit on a direct line leading ultimately to humans. Each species in this upwardly linear arrangement added something to the contributions to brain, body and behavior made by species that preceded it. These preceding species were therefore lower[5]. In this way, 'lower' is used to mean 'less like a human'.

But Darwin[6] argued (and most scholars claim to accept the argument) that most existing species are arranged not on a direct line from one to another but rather in a tree-like array in which each existing species is located at the end of its own branch, with its ancestors lined up along the branch reaching back to

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the trunk. In a sense, it is possible to 'rank' species as higher or lower on a phylogenetic tree. On any given branch of a tree, some leaves are closer to the trunk of the tree and others are farther away. Leaves or species located closer to the trunk might be considered 'lower' than those located at the end of the same branch. But species closer to the trunk are, in general, extinct and do not participate in modern biomedical research. For example, early *Feliformia* (cat-like animals) could be considered lower on the felid branch than existing cats. This is meaningless in the context of scientific species selection, however, because early *Feliformia* cannot be used as research subjects as alternatives to modern cats. Existing cats are positioned at the end of the *Feliformia* branch, whereas existing dogs are positioned at the end of the *Caniformia* (dog-like animals) branch. Ranking one as higher or lower than the other is a bit like ranking an orange as higher or lower than an apple (to use a hackneyed analogy). It makes no sense, therefore, to rank phylogenetic groups as higher or lower relative to one another or to consider phylogenetic group when choosing a species for research.

SENTIENCE

What is sentience?

There does not seem to be a generally accepted definition of sentience in research animals. Boyle[7] defined sentience as "the capacity for emotion, pleasure and pain." For Broom[8], sentience was the "ability to experience pleasurable states, such as happiness, and aversive states, such as pain, fear and grief." Similarly, D'Silva[9] defined sentient creatures as "those who have feeling—both physical and emotional—and whose feelings matter to them." Silverman[10] defined a sentient animal as "one that has the ability to obtain and interpret stimuli from its internal and external environment and has at least a basic ability for memory, judgment and emotions."

Boyle[7] wrote that "[c]onsciousness may not be necessary for sentience", but she also wrote, "Roughly defined as the capacity for emotion, pleasure and pain, sentience is related to other brain capabilities of intelligence and consciousness." Duncan[11] noted that some invertebrates appear to be capable of learning and memory (e.g., *Aplysia*, fruit flies) but concluded that "whether or not this implies sentience is still open to debate." Wasserman[12] pointed out that "it is equally possible to imagine those behaviors to be performed without conscious accompaniment."

Collectively, these definitions imply that sentience involves happiness, pain, fear, grief, pleasure, emotion, ability to sense the internal and external environments and perhaps memory and judgment.

Which species are sentient?

The question of which species are sentient is open to debate. Many people might say that all mammals are sentient, and at least some might say that all animals are. According to Proctor[13], "Today it is generally accepted that at least the vertebrate species are sentient." Furthermore, she claimed, research has shown that "chimpanzees can be generous", "mice, rats and chickens demonstrate empathy" and several other species (starlings, dogs and honeybees) show optimism and pessimism. It is difficult to reconcile this statement with another observation in her paper: "The problem is, however, we cannot know exactly what, or how another is feeling…. This applies to both humans and animals, and means that it can be difficult to ultimately prove the capacity for sentience. This is particularly difficult for animals as they lack the power of speech to convey their feelings." Duncan[11] observed that "we can never prove conclusively that any organism is sentient… it is impossible to measure feelings directly."

But is there general agreement on which species are likely to be more or less sentient? Silverman[10] assumed that all mammals are equally sentient. He concluded, "Therefore, with regard to experiencing pain, there are no unequivocally 'higher' or 'lower' sentient species among the mammals." In terms of pain-related reactions, there are situations (ages or physiological and emotional states) when pain is experienced less or more. This is certainly true for humans; probably it is also true of other animals. A single animal can apparently be more or less sentient at different times; perhaps it is also possible for one species to be more or less sentient. We will not know this with certainty until we settle on a single, measurable definition of sentience and find a useful way of measuring it across species.

How has sentience been measured?

One of the major limiting factors in measuring sentience is that animals do not react to painful or other aversive stimuli in the same way. For example, dogs and cats respond to painful stimulation with increased vocalization, whereas sheep do not. It might be of great survival value, especially for prey species, to avoid responding outwardly to painful or aversive stimuli. This means that responses to pain might be difficult to detect in these species. Animal-specific training courses (e.g., The CITI Program, https://www.citiprogram.org/) point out that caretakers must know the species used in research well in order to detect their pain responses.

Here is an example from Danbury *et al.*[14]. Lame and normal chickens were offered feed with and without added carprofen (a non-steroidal anti-inflammatory drug used as an analgesic in veterinary medicine). Lame chickens consumed more drugged feed than did normal birds, but the difference was not significant. Both lame and normal chickens preferred drugged feed to undrugged feed. Notably, the walking ability of lame birds improved significantly with carprofen consumption in a dose-dependent manner, and birds with more severe lameness consumed significantly larger proportions of drugged feed. Danbury *et al.* concluded, "These findings may indicate that broilers are able to balance their intake of analgesic drug to match their level of pain." The authors are assuming that lameness is painful to chickens and that lame chickens' carprofen consumption represents self-administration of an analgesic to relieve the pain. But why, then, did normal chickens also prefer feed with added carprofen?

This example highlights the potential dangers of making assumptions about animal behavior that are based on our own experience. Such anthropomorphization, as Dewey[15] pointed out, "can be misleading even when it seems to be the simplest explanation for a behavior." Dewey cited, as an example, the ultrasonic vocalizations emitted by rat pups when they are separated from their mothers and exposed to cold temperatures. Observers have assumed these vocalizations to be cries of distress intended to gain the mother's attention and encourage her to retrieve the pup. But later research showed that this assumption was not entirely correct. Blumberg *et al.*[16] experimentally reduced venous blood pressure in the pups and found that vocalizations occurred whenever blood pressure dropped below a certain threshold. The vocalizations increased pressure in the abdomen, stimulating blood flow that helped to warm the pups. Blumberg *et al.* noted that their findings "underscore the potential pitfalls of anthropomorphic interpretations." Dewey observed that "the cries might serve a dual function, pumping blood and alerting the mother to a baby outside the nest."

People familiar with comparative psychology might experience a feeling of déjà-vu here. Myriad studies indicate that non-human species are intelligent in different ways and that our appreciation of their intelligence is limited by a lack of "species-appropriate tests of cognition"[4]. This means we cannot know whether one species is more or less intelligent than another[17]. The same can be said for sentience.

Given that we do not know with certainty which animals are sentient, it might be best from an animal welfare perspective to treat all animals as if they are sentient. As suggested in the International Guiding Principles for Biomedical Research Involving Animals[18], "[i]nvestigators and other personnel should never fail to treat animals as sentient, and should regard their proper care and use and the avoidance or minimization of discomfort, distress, or pain as ethical imperatives." Although the word 'sentience' was omitted in the 2012 revision of this document[19], the sense of the guidelines is the same.

WHAT TO DO?

If phylogenetic group and sentience are not useful criteria for guiding the selection of species in research, then how can investigators select animals to use? First, the IACUC and the investigator must justify the use of animals by weighing the potential harm to animals participating in the study against the potential benefits of that study. As Prentice *et al.*[2] stated, "According to a reading of the combined PHS policy [Public Health Service *Policy on Humane Care and Use of Laboratory Animals*] and the USDA rules, each IACUC must make a thoughtful and principled judgment as to whether or not the potential value of the research outweighs its ethical costs in terms of animal pain, distress and mortality." Likewise, Prentice *et al.*[20] observed that "[b]ecause a living creature serves as the experimental model, it is necessary ultimately to justify the use of animals in terms of an ethical cost-benefit assessment." There is no easy way to achieve this risk-benefit analysis; "it requires the thoughtful judgement of men and women who, using individual values, must strike the right balance between the needs of science and fulfillment of a moral obligation to treat laboratory animals humanely"[20].

Which species?

Principle III of the US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training[21] states that "[t]he animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results." In other words, investigators should choose the most suitable, most scientifically appropriate species, most likely to produce valid results.

What makes a species the most suitable scientifically?

There are a number of reasonable justifications for using a particular species in a research project.

Susceptibility to a disease of interest. For a given disease, some species might develop it more readily than others. For example, hamsters are particularly susceptible to pancreatic cancer, making them a favorite model for studying this disorder. On the other hand, the blind mole rat (*Spalax* spp.) is also used in cancer studies because it is resistant to many forms of cancer.

Tendency to express a behavior of interest. Some behaviors are unique to particular species, and scientists interested in those behaviors must therefore study the species that express them. The kelp gull (*Larus dominicanus*), for example, has some interesting behaviors that involve the unique, conspicuous red spot on the beak. Chicks peck at the spot to induce the mother to regurgitate food.

Size. Some species are too small for some studies. For example, many studies of bone healing are done in rabbits because they have bigger bones than mice or rats. It is much easier to manipulate and observe changes in the larger bones.

Presence of an existing body of work. There may have been many previous studies (by both the proposing investigator and others) of a particular phenomenon in a particular species. There would, therefore, be a large amount of data on that phenomenon in that species. This opens the possibility of using historical controls, and it may reduce the number of animals needed because procedures and handling methods for that species are established. If an investigator has been studying intestinal absorption in rats for years, it might be possible to reduce the number of rats needed for a new study by using data from previous studies on rats.

Species specificity. Investigators studying the characteristics of a particular species must use that species. If an investigator wants to study the hippocampus in cats, then cats are the only animals that are appropriate.

Gestational period or intergeneration interval. The short gestational period of rodents and flies makes them particularly suitable to genetic studies that require a number of generations of animals.

Similarity to humans. Some animals are more like humans than others. For example, the cardiovascular system of pigs is much like that of humans, making pigs a good choice for cardiovascular studies.

Contractual obligation. For some animal research contracts, the species to be used is specified by the contractor. To receive the contract, the investigator must agree to use that species. The IACUC must then decide whether the species justification in the protocol is adequate to allow approval.

Existing guidelines. For pre-clinical screens (safety evaluation programs) during drug development, for example, the FDA says, "Some factors that should be considered when determining the relevant species include: a) comparability of physiology and anatomy to that of humans; b) permissiveness/susceptibility to infection by, and replication of, viral vectors or microbial vectors for gene therapy; c) immune tolerance to a human CT [cellular therapy] product or human transgene expressed by a GT [gene therapy] product; and d) feasibility of using the planned clinical delivery system/procedure"[22]. Elsewhere, the FDA says, "Safety evaluation programs should include the use of relevant species. A relevant species is one in which the test material is pharmacologically active due to the expression of the receptor or an epitope… and demonstrate(s) a similar tissue cross-reactivity profile as for human tissues…. Safety evaluation programs should normally include two relevant species. However, in certain justified cases one relevant species may suffice…"[23].

There may be other good reasons for choosing one species over another in addition to these frequently encountered justifications. It is the investigator's responsibility to provide the IACUC with a complete discussion of the benefits of the study and, using points like those listed above, justification for the species selection. Using that information along with other required information regarding animal welfare in the proposed studies, the IACUC should be able to assess the merits of the study adequately.

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