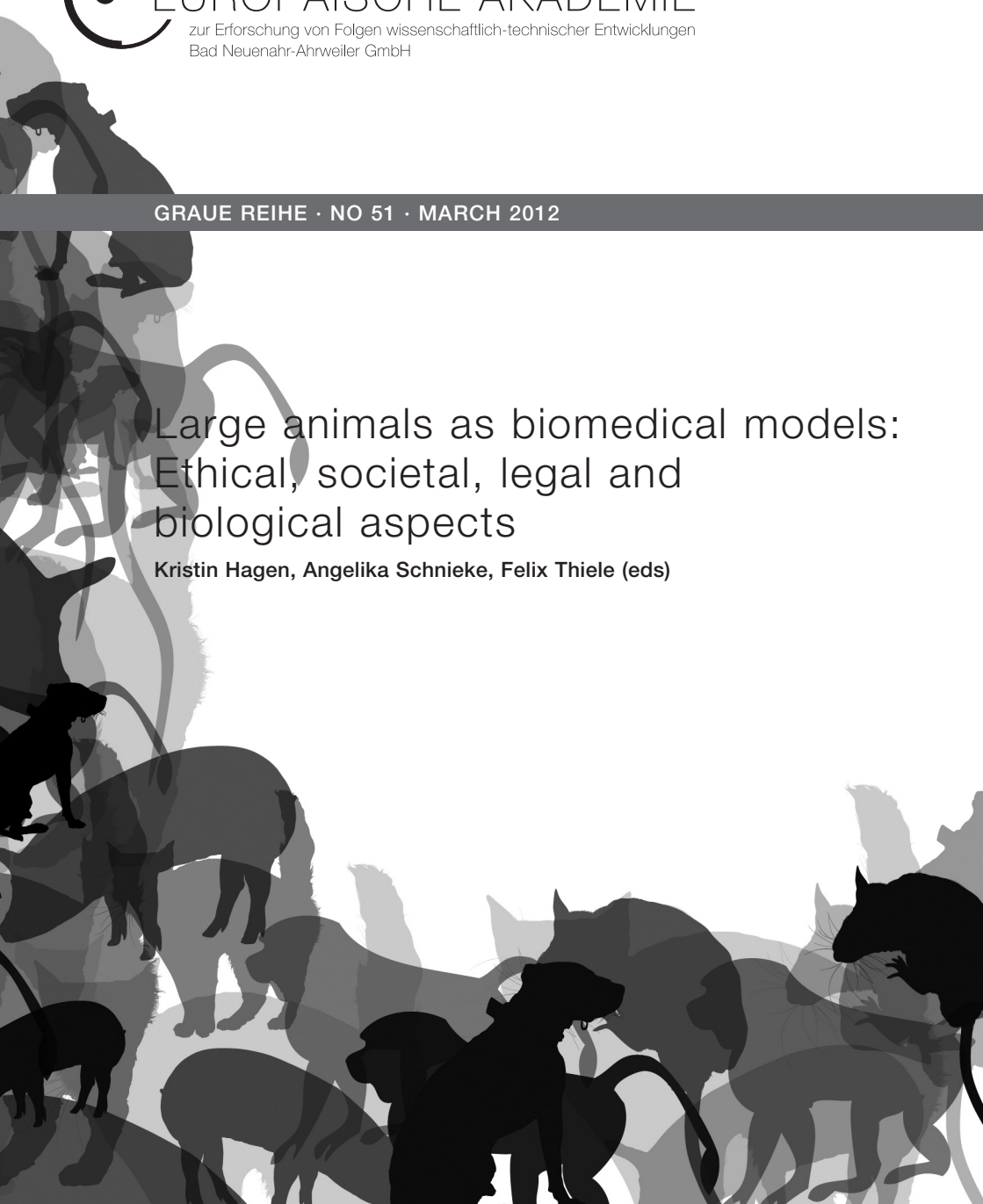
EUROPÄISCHE AKADEMIE
zur Erforschung von Folgen wissenschaftlich-technischer Entwicklungen
Bad Neuenahr-Ahrweiler GmbH

GRAUE REIHE · NO 51 · MARCH 2012

Large animals as biomedical models: Ethical, societal, legal and biological aspects

Kristin Hagen, Angelika Schnieke, Felix Thiele (eds)



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The texts of the "Graue Reihe" contain current editions and documentations which are developed by scientists of the Europäische Akademie zur Erforschung von Folgen wissenschaftlich-technischer Entwicklungen Bad Neuenahr-Ahrweiler GmbH. The academy is concerned with the scientific study of the consequences of scientific and technological advance for individual and social life and for the natural environment. The publications of the "Graue Reihe" are printed in the form of manuscripts and are published in loose succession edited by the **Europäische Akademie.** The volumes can be ordered free of charge or downloaded from the website of the Europäische Akademie GmbH (www.ea-aw.org/publications/graue-reihe).

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ISSN
1435-487 X

Editing
Katharina Mader

Layout • Printing
Heim für angewandte Grafik • Johannes Wütscher

Preface

This issue of the Graue Reihe is based on the international summer school "Does size matter? Ethical, societal, legal and biological aspects of large animals as biomedical models", which took place in Freising and Munich from October 10–14, 2011. The summer school was a co-operation of the Europäische Akademie GmbH with the Chair of Livestock Biotechnology at the Technische Universität München. The 15 junior participants representing various relevant disciplines at PhD and Postdoc level came from seven European countries and Canada to present and discuss their work. Leading researchers in veterinary science, biomedical research, philosophy and law brought diverse perspectives to a complex topic. Here, we present papers by eight junior and three senior participants.

We want to thank all lecturers and participants of the summer school for their insightful contributions. We explicitly extend our acknowledgement to those participants who have for various reasons (including copyright issues within their theses) not been able to contribute to this publication.

The assistance of staff at the TUM, in particular Barbara Bauer, and at the Europäische Akademie (Katharina Mader, Margret Pauels, Margret Heyen and Anja Schlochtermeier) was indispensable for the smooth organisation of the summer school. We are also greatful to Klaus Mainzer and Jörg Wernecke at the Carl von Linde-Akademie in Munich, where we presented the summer school to the public for discussion. This project was made possible by generous funding by the German Federal Ministry of Education and Research (BMBF).

Köln and Freising, March 2012
Kristin Hagen, Angelika Schnieke and Felix Thiele

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Editorial

Kristin Hagen, Angelika Schnieke and Felix Thiele

1. "Does size matter?"

Most of our knowledge of gene function in health and disease has been obtained by intensive study of certain key species, notably the nematode *C.elegans*, the fruitfly *D.melanogaster,* the frog *X.laevis,* zebrafish and the domestic mouse. Among vertebrates, mice are by far the best studied, because for decades these were the only mammals where methods of precise genetic modification were possible. For example, mice represented 59,3% of the approximately 12 million individual vertebrate animals registered in research within the EU in 2008 (European Commission 2010:7, 10). The same report listed the following figures for other mammals: rats 17,6%; other rodents including guinea pigs and rabbits 5,2%; ungulates 1,4%; cats, dogs and other carnivores 0,3%; and non-human primates 0,08%. All species of birds, reptiles, amphibians and fish taken together represented 15,9%.

"Large animal models" has become a common expression in biomedicine. It typically refers to any animal larger than mouse or rat, such as livestock (pigs, goats, sheep, cattle, horses, donkeys), cats, dogs and non-human primates[1]. This is, however, not a formal definition, but has rather developed within biomedical discourse, to distinguish such species from mice, rats, frogs, flies and worms[2]. Large animals often require different research infrastructure than small rodents, which has led to distinct research groups and distinct research facilities.

Against this background it should be clear that we are not interested in body size as a tool for the biological classification of animals. Rather, we take the common usage of the expression as a starting point. We observe that the use of large animal models is considered increasingly valuable for biomedical research while it may at the same time be subject to stronger regulation and ethical controversy than experiments with more common small animals. Can these observations be gener-

1 Carnivores and primates are occasionally excluded because they get special attention in some legal and ethical contexts and are granted special housing and management conditions (see Varga, 113ff, and Brukamp, 57ff. All page references in this Editorial refer to the Graue Reihe.). There is also a tradition from agricultural research to separate out "livestock biotechnology". Rabbits are often classified as small animal models. However, they are large and uncommon compared with mice, so they are sometimes also classified as large animal models (as by Antunes and Silva, 150ff), and relatively often found in large animal research facilities.

2 A similar line is drawn in the USA even on the legal level, where rats and mice bred for scientific purposes are explicitly excluded from laboratory animal protection legislation and statistics.

alized to all species considered large animal models, or do we need to look more closely at each species or particular taxa? What are the crucial attributes regarding the choice of species in biomedical research? What could be the criteria for differential ethical treatment of species, and why do some of the large animal species receive greater moral consideration?

In his contribution to this volume Robert Heeger addresses this latter question (13ff). He concludes that sentient animals have similar interests (e.g., in avoiding pain), but that other morally relevant aspects, notably cognitive capacity, capability to flourish, and sociability (which may, incidentally, be correlated with size) may give rise to species differentiation with regard to our moral duties. Capability to flourish and sociability are aspects that are not yet strongly represented in animal ethics, which has been focused on pain perception and cognitive capacity, but Heeger's stance that all sentient animals have similar interests with regard to pain, and that the extent of an animal's mental complexity beyond sentience is nevertheless morally relevant, is more generally accepted.

Does an animal's cognitive ability determine its capacity to suffer? In testing this connection, James Yeates focuses on pain (24ff). His analysis of available empirical evidence leads him to conclude that there are numerous connections between cognitive processes, and thus probable capacity, and experience of pain, but that these connections are diverse and depend on context, and that there is insufficient evidence to conclude that the cognitive abilities of an animal makes it experience more or less pain *overall*. As Yeates points out, the preferential use of "lower" animals (but above the threshold of experiencing pain at all) would need other logical defence – maybe linking cognition to other forms of suffering than pain, or arguing that cognitive abilities somehow make animals more worthy of moral consideration (or worthy of moral consideration in other respects) irrespective of suffering. This would correspond to Heeger's argument that the more capacities an animal possesses the more forms of potential mistreatment it can experience, and that we need to consider ethical models that go beyond suffering.

As Ralf Müller-Terpitz writes in his contribution about the legal framework of large animal experimentation (47ff), if comparable results can be obtained with "less developed animals", these should be preferred. However, this provision is made in the context of non-human primates. The only other species specifically mentioned in European legislation are cats and dogs, who are required to receive special treatment, but not to be replaced by other species. The reason for special

treatment of cats and dogs is "a high level of public concern as to the fate of such animals" (Article 26 Directive 2010/63/EU, European Parliament 2010).

The Directive does not pay much more attention to the justification of the differential treatment of primates: It simply states that "use of non-human primates is of the greatest concern to the public" (Article 17) and that the use of great apes is restricted because they are "the closest species to human beings with the most advanced social and behavioural skills" (Article 18). In practice, when preferential use of less cognitively developed animals for scientific purposes is advocated, the comparison refers exclusively to primates. In this context, it is therefore difficult to distinguish between concern for primates on the grounds of cognitive capacity, or other grounds, such as relatedness to humans[3].

Kirsten Brukamp, in her contribution on treatment guidelines and ethical frameworks for research involving non-human primates (57ff), points out how particular cognitive abilities make suffering beyond pain more likely: e.g., when an individual of a social species is deprived of social contact. However, such distinction alone would not make a case for the differential treatment of primates, as evidence is accumulating in many taxa of cognitive abilities. This may be one reason why Brukamp does not rely on potential suffering alone, but calls for a bioethical position to go beyond pathocentrism and take into account primates' similarities and close-relatedness with humans, valuing complex (human-like) cognitive capacities as such. At the same time, she pinpoints a dilemma: "The vast similarities to humans are the reason why primates are examined in research, and exactly those similarities warrant special treatments that primates deserve [...]" (64).

The weight of this dilemma of epistemic versus ethical concerns is questioned by Lara Kutschenko in her analysis of relevant similarity in the light of biomedical experimentation (69ff). Kutschenko argues that from an epistemological point of view, the relatedness and similarity of humans and non-human primates are not sufficient reasons for using primates in research because humans and non-human primates may not be similar with regard to the specific research question. According to Kutschenko, different factors will be decisive depending on research question and context. In some cases, specific cognitive capacities or specific brain structures may be important factors.

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3 This phenomenon has sometimes been coined "primatocentrism". However, within other areas of animal protection, where for example the protection of wild animals is the issue, cetaceans will sometimes also be placed high on the moral hierarchy – probably due to our ample knowledge of their cognitive capacities and sociability.

Lene Vammen Søndergaard and Mette Herskin (84ff), whose focus is on Alzheimer's disease, take as a starting point the goal of replacing primates with other animals as models for neurodegenerative diseases, while going beyond the rodent model. They point out that the longevity, larger body size, and more complex brain of the pig have been crucial factors for choosing pigs rather than rodents. By focusing on physiological homology, Søndergaard and Herskin's approach illustrates Kutschenko's thesis regarding the difficulty of extrapolations: the early-stage practicalities of developing suitable animal models necessitates reference to homology in the hope that this will enhance the likelihood of relevant similarity.

Where Kutschenko argues from a philosophical point of view, Brigitte von Rechenberg (100ff) reaches very similar conclusions on the basis of her experience in musculoskeletal research. Here, body size is often a relevant biomechanical factor. However, von Rechenberg's more general conclusion is that close attention to the research question and the experiments designed to address it are crucial for the optimal choice of model species, and striving for optimal research quality is a moral requirement in animal research.

The notion of relevant similarity is convergent with analysis of animal models in terms of their validity. Orsolya Varga (113ff) introduces the concept of validity and points out that the predictive validity of an animal model can only be known with hindsight. In attempts to maximise predictive validity it can make sense to consider aspects of face validity (including physiological homology and evolutionary similarity) and construct validity (tailoring the animal's attributes to the research question). However, according to Varga, there is no evidence that choosing large animals because of size-related homologies increases a model's validity, unless there is a direct biomechanical or physiological connection as exemplified in von Rechenberg's work.

The work by Barbara Rütgen and her colleagues (126ff) describes another process of model development. Factors that support the use of the dog as an alternative model for haematopoietic malignancies include similarity of molecular changes in dogs and humans, and their shared environment. The latter is interesting because it shows that factors other than phylogenetic or physiological similarity, i.e., in this case the shared environment, can influence the validity of an animal model. While Rütgen and co-authors describe how the use of animals in which a disease occurs spontaneously can have a positive impact on validity (and avoids inducing disease in model animals) they do however note that "the value of the canine model also depends on the availability of rodent models that can reproduce the

disease as it occurs in dogs." (130) This approach of using different animal models, notably, one small rodent model and one large animal model, is very common. In the United States, current FDA (Food and Drug Administration) requirements are that pre-clinical trials of a biomedical drug or device should use at least two different animal models, rodent and non-rodent.

The example of canine models for haematopoietic malignancies highlights another aspect of inter-species value theory: animal models for veterinary rather than human medical progress. Katy Beck, as part of her work on transmissible spongiform encephalopathy (135ff) raises the question: If the research is to benefit, say, cattle, would that make it more justifiable to also use cattle in the experimental work? While at first sight it may seem intuitive that the pains of being an experimental animal for the benefit of one's own species are more worthwhile than for other species, this does not stand up to closer scrutiny. Admittedly, when people consent to be subjects in biomedical experiments they will probably be motivated more by benefits to human than to veterinary medicine. But an analogy with other species relies either on the assumption of altruistic feelings[4] and some form of consent, which is unlikely in most animals, or on a moral theory that promotes the sacrifice of individuals for a common good[5].

In choosing the model animal species for a particular research project, the predictive value and the quality of the research data gained are of central importance, but there is also a legal requirement to use the "species with the lowest capacity to experience pain, suffering, distress or lasting harm" (Article 13 Directive 2010/63/ EU, European Parliament 2010). This is context dependent; when it comes to minimizing negative impact the actual procedures are relevant because animal species may experience procedures differently. Aspects such as domestication, social structure and behavioural needs play an important role. This is also an area where the size question actually comes up: When tissue or blood sampling is required, small animals will be at a disadvantage. In some cases, several mice may be required to provide a sufficient mass of sample where a single larger animal would suffice. Insufficient attention is often paid to what the choice of species actually means to the animals involved. The work by Luis Antunes and Aura Silva (150ff) provides a

4 Some evidence for altruistic feelings is available in some taxa, including primates.

5 Even if the latter were supported, the matter would still be complicated in animals. For example, it is questionable whether cattle in European agriculture have a sufficiently good life that they could benefit from such sacrifice at all, and whether there would, in the particular case of prion diseases, not be far more just approaches that required changes in the farming systems rather than biomedical research.

counter-example example: The rabbit is chosen on the basis of thorough consideration of its species- (and breed-) specific characteristics and needs.

How are we supposed to know which species will be least harmed in a particular research situation? We do have some species-specific biological evidence to support such choice, but **we are very far from a deep understanding of the cognitive abilities** and needs of *any* animal species, and we do not have a reliable inter-species (non-anthropocentric, i.e., allowing comparison between non-human animal species or individuals) theory of cognition, let alone an inter-species value theory. Knowledge in comparative cognitive psychology and ethology is rapidly advancing. But what about an inter-species value theory as proposed by David DeGrazia (1996)? **How** can we separate out and weigh notions of ability to perceive pain and suffer, having specific cognitive capacities, and similarity/relatedness/relationships with humans? Much more work is needed, and it seems understandable if there is some confusion in animal ethics committees that have to consider such questions.

If there are traditions (or other mechanisms) to preferentially allow research with rodents, these may not serve research quality or animal protection. There is no reason to assume that large animals are generally more prone to suffering than small rodents. The initiative to use more large animals is occasionally constructed as a way to increase research quality and induce less animal suffering overall: Using a more suitable animal model to investigate a particular question may result in fewer animals being used in the process of achieving comparable scientific progress[6]. The aim should be to achieve highest quality of research, and this should include the correct choice of species. At the same time it has to be kept in mind that *all* species considered here are capable of suffering and have highly developed cognitive abilities.

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6 However, it should be kept in mind that large animal biomedical research – like all biomedical research – is not primarily an animal protection initiative, but has its main motivation in answering scientific questions.

References

DeGrazia D (1996) Taking Animals Seriously: Mental Life and Moral Status. Cambridge University Press, Cambridge

European Parliament (2010) Directive 2010/63/EU of the European Parliament and the Council on the protection of animals used for scientific purposes of 22 September 2010, Official Journal of the European Union 2010 No. L 276/33 of 20 October 2010

European Commission (2010) Report from the Commission to the Council and the European Parliament. Sixth Report on the Statistics on the Number of Animals used for Experimental and other Scientific Purposes in the Member States of the European Union, COM(2010) 511 final/2

Experimenting on animals: When does their size matter morally?

Robert Heeger

1 Introduction

The debate about research involving animals ranges broadly over two distinct questions. The first asks whether animal research yields useful knowledge that could not be gained from other sources. The second concerns whether it is morally acceptable for humans to use animals in ways that can cause them harm. A positive answer to the scientific question does not settle the moral question, for it may be the case that an experiment that yields useful and relevant information is not morally acceptable. Therefore, one needs to consider the question of whether experimenting on animals can be morally justified.

But how should one tackle this question? One seemingly simple and attractive strategy would be to start by considering the relative *moral status* or moral importance of human beings and animals. One could suppose that there are three general positions to choose from. The first is that human beings have a moral importance that animals lack and that for this reason there is a clear moral dividing line between human beings and animals. According to the second position, there is a sliding scale of moral importance which ranges from humans on the top to single-celled creatures at the bottom. The third position is that human beings and either all, or at least sentient, animals are moral equals, which could imply that it is morally wrong to subject an animal to treatment that would be unacceptable in the case of humans. The question of whether animal experiments are morally justifiable could be regarded as merely a matter of deciding which of the three general positions is the most adequate – the position of a dividing line, that of a sliding scale, or that of moral equality.

However, this strategy appears not to settle the question of justification in a helpful manner. Two examples may illustrate this. First, suppose it was possible to establish that which the positions of a dividing line and of a sliding scale assume: that humans have a higher moral status than animals. Yet, this is not enough to show that animals can justifiably be sacrificed for human purposes, for it may be that the morally 'higher' humans have a moral duty of care and compassion for 'lesser' beings. Second, from the moral-equality position does not necessarily follow that harming animals in research should not be carried out, because the

moral-equality position could, in principle, allow for the conclusion that harmful experiments should be conducted both on animals and humans.

Since it appears that consideration of the relative moral status of human beings and animals does not settle the question of the justification of animal research, a different strategy has been brought forward. It has been proposed that a promising approach may be to ask about the *morally relevant features* of humans and animals. That means taking two steps. The first is to ask what features could make humans and animals subjects of moral concern, thus imposing constraints or limits on how they may be treated. The second step is to ask what weight such features should have in deciding the moral acceptability of research (Nuffield 2005:40–1, 48). The present paper is favourable to this proposal. It will focus on the first step just mentioned, the question of what features could be morally relevant. According to widespread moral convictions, there are mainly four features that have the potential to give rise to moral concern: sentience, higher cognitive capacities, capability for flourishing, and sociability. At least one or all of these features may be applicable to specific animals, albeit to differing degrees. The moral convictions here referred to should not be dismissed as manifestations of an irrational attitude, for they can in many cases stand the test of critical reflection. For example, they can be supported by moral principles and be compatible with empirical evidence.

The size of experimental animals is not one of the features mentioned. Size as such is no moral touchstone. But size can matter morally if it is related to one or more of those features that may give rise to moral concern. This I will try to put forward.

2 Sentience

One feature that can give rise to moral concern about humans and animals is their sentience. That humans and animals are sentient beings is morally important for instance for the following reasons. It matters to sentient beings how they are treated. They can be harmed and benefited. A basic moral principle says that it is wrong to cause them harm. If we share this view, we have reason to take a closer look at the sentience of animals.

How should we conceive of animal sentience? A sentient being is a being capable of having feelings, that is to say, mental states, such as sensations or emotional states that are typically pleasant or unpleasant. In the literature on the mental life of animals it is stated that many animals – most or all of the vertebrates and

possibly some others, such as the cephalopods – can experience a wide variety of feelings. This is asserted, not as absolutely certain but as very reasonable and presumptive given currently available evidence (DeGrazia 1996: ch 5).

Let us first look at the unpleasant feelings. The animals mentioned can experience pain and distress. And there is good reason to think that most, if not all, of them can experience fear and anxiety and, one may assert even more confidently, suffering.

Some clarification of these terms is appropriate. *Pain* is an unpleasant or aversive experience typically associated with actual or potential tissue damage. *Suffering* is a highly unpleasant emotional state associated with more-than-minimal pain or distress. It has an umbrella function to the states of distress, fear, and anxiety. *Distress* is a typically unpleasant emotional response to the perception of environmental challenges and to equilibrium-disrupting stimuli. *Fear* and *anxiety* appear to work hand in hand. *Fear* is an emotional response to what is taken to be danger – a response that is typically prompted by a known object in the immediate environment, and typically focuses attention to facilitate protective action. *Anxiety* is an emotional response to what is taken to be a threat to one's physical or psychological well-being. Typical behavioural and physiological details of anxiety found in animals are (1) motor tension, as seen in shakiness and jumpiness, (2) autonomic hyperactivity (sweating, pounding heart, increased pulse and respiration, diarrhoea), (3) inhibition of behavioural repertoire in novel situations, and (4) hyperattentiveness, as seen in vigilance and scanning. There are probably great qualitative differences among the anxious states of mind experienced by different species. Animals capable of having fear and anxiety are probably capable of suffering.

The literature discusses not only aversive states but also positive states. It says that the animals can have pleasures and enjoyments and, therefore, feel happy.

Also these terms need some clarification. *Pleasures* are not simply sensations but feelings of some sort. Pleasures are desired for their own felt qualities (the attitude model, as stated by Sidgwick 1907:131). There are good reasons to believe that many animals can have pleasure. (1) Like the aversive mental states pleasure has a function. The aversive states motivate doing things that tend to make the unpleasant experiences stop. Pleasure, too, is motivating. It attracts one to what is generally beneficial. (2) There are physiological indications: neural pathways apparently associated with pleasure have been located in the brains of mammals, birds, and fish. (3) There are common sense observations of many animals acting as if they experience pleasure. *Enjoyment* stands for preferring, liking or desiring a pleasurable experience. The dog who finds eating pleasurable enjoys the activity.

The horse who gets pleasure from scratching against a post enjoys doing so. *Feeling happy* is an occurring mental state of an individual over some stretch of time. Animals can feel happy because they can enjoy themselves. This is not the same as being happy in the long term. For example, in Aristotle and the classical utilitarians, the words 'being happy' can sum up a person's well-being over a lifetime. One who is happy in this sense is disposed to make an overall positive judgement about the way his or her life is going, and feels good about it. Animals cannot be happy in this sense, unless they can make evaluative judgements about their lives as wholes, something one might reasonably doubt. But they can be happy in the sense that they feel happy. We could speak of their having happy lives if they mostly feel happy during their lifetimes.

In conclusion, if (1) sentience is as morally important as stated in the beginning of this section, if (2) a sentient being is one capable of having pleasant or unpleasant feelings, and if (3) it is very reasonable to assert that many animals can have such feelings, then (4) there is reason to state that all of these animals possess the morally important feature of sentience.

3 Higher cognitive capacities

Sentience is not the only feature that can give rise to moral concern. More features are worth considering. One of these is that a being can have higher cognitive capacities. This means it can not only feel pain and pleasure, but can, for instance, also be conscious, have the powers of memory and anticipation, and the capability of learning. Human beings and many animals can be said to have these capacities in common. That they have them is relevant to our moral behaviour towards both human beings and animals: the capacities ought to have a determining influence on our moral duties towards them. In order to find out what this implies for the treatment of animals, we can use the more familiar human case as a point of reference. Using the human case as an aid, we can take a closer look at higher cognitive capacities and say that having them is being capable of self-consciousness, or the use of language, or moral agency, or autonomy. Their moral relevance is that we are morally obliged to show respect for others as beings who have these capacities. For example, we are not allowed to interfere with the moral agency of these beings by using them in potentially harmful experiments without their consent. This gives rise to the important question of whether animals too can have the just mentioned capacities. If they can have them, or can have them to a certain degree, then this is relevant to our moral behaviour towards them. For example, we should not ignore that an

animal may lack the capacity for full moral agency, but may nevertheless have other ways of expressing dissent to certain treatments, say by seeking to flee. Generally speaking, showing respect for animals that have higher cognitive capacities is taking into account that there are more morally questionable ways of mistreating them than there are of mistreating animals that do not exhibit such capacities. I will now focus on the four capacities mentioned: self-awareness, the use of language, moral agency, and autonomy (DeGrazia 1996:ch. 7; Bermudez 2007).

3.1 Self-awareness

Are any nonhuman animals self-aware? One can usefully distinguish different sorts of self-awareness. The first and perhaps most basic one is *bodily self-awareness:* awareness of one's own body as distinct from other things, the rest of the environment. An example is the rabbit's bodily self-awareness as she goes for a carrot. Another sort of self-awareness is *social self-awareness,* which involves understanding one's social relations to others in one's group, the expectations that follow from these, and how to work within these expectations towards desired goals. For instance, velvet monkeys have extensive knowledge about the particularities of social relationships within their group. This knowledge includes considerable understanding of how they themselves fit into the social structures of the group. Many other higher mammals are socially self-aware: other monkey species, Great Apes, the lesser apes, elephants, and dolphins. A third sort of self-awareness is *introspective self-awareness* of some of one's own mental states. Some observations suggest that certain apes may have this complex activity.

The three sorts of self-awareness may fall on a continuum of complexity – from simpler to more abstract mental capacities. So, one may say that self-awareness is not all-or-nothing but comes in different degrees.

3.2 Language

Language seems to enhance enormously the conceptual powers of a mind. Thus, an interesting part of understanding animals is understanding to what extent, if any, the mentality of animals is boosted by language. But the discussion about whether any animals have language is controversial. One reason is that scholars set differing standards for language possession.

An influential traditional standard says that language must meet two necessary conditions: an act of communication must have reference and syntax, that is to say, it must have content and it must follow some rules that determine a word's or sign's function by its position among other words or signs. According to this standard, beings either have language or don't have it.

There are researchers who find this all-or-nothing view debatable. They argue that it seems more profitable to understand language as both multidimensional and gradational. Two dimensions are language *comprehension* and language *production*. For instance, when children learn their first language and when adults learn foreign languages, their comprehension of others' language exceeds productive capabilities. Both communicative feats – the receptive and the productive - come in degrees. Two other dimensions of language are the *reference* or content of words or signs and the understanding of *syntactic rules*. Also these achievements – referential and syntactical achievements – come in degrees.

In the literature about animal minds, it has been brought forward that also some animals show such communicative feats. Let me just mention two examples given there. Dolphins and sea lions have shown to comprehend combinations of commands, demonstrating some capacity to master syntactical rules (Herman and Morrel-Samuels 1990:287, 296–7). Some apes have been taught to ask for things that interest them. These communications were first made nonverbally and later verbally (by pressing keys on a keyboard). They included, for instance, to ask for a tool to solve a problem, or to announce an intention (to go 'outdoors') (Savage-Rumbaugh and Brakke 1990:325f).

If one does not keep to the all-or-nothing view of language but allows that there are different kinds and degrees of linguistic achievement, then it seems reasonable to say that some apes and cetaceans have used, and many of their con-specifics can learn, certain forms of language.

3.3 Moral agency

There are several defensible ways of understanding moral agency. One way is to take actions of animals as expressions of traits or *dispositions* that, in humans, are considered virtues. Such actions are, according to some authors, forms of moral agency. Many animals reveal dispositions to respond to natural goods and evils in socially useful ways. Mammals provide many examples. Mothers care tenderly for their babies. Orphans are adopted by other members of a group. Some-

times animals care for old or feeble companions. Apparently, compassionate acts towards con-specifics are frequently observed.

Another way of understanding moral agency is to require virtuous action that is *independent* of conditioning and instinct. The literature supplies many examples of apparently virtuous behaviours that may be inexplicable in terms of instincts of conditioning. One such example is the case of porpoises helping drowning sailors (Sapontzis 1987:34); another example is altruistic or compassionate behaviour of chimpanzees (Goodall 1993:34).

A third way of understanding moral agency includes three requirements. A moral agent must be capable of (1) deliberating on the basis of what he/she takes to be moral reasons, (2) acting on the basis of such deliberation, and (3) justifying his/her decisions with an explicit argument appealing to moral reasons. It is possible that no animal fully meets this standard. Perhaps animals who make certain tough choices (as in Goodall's example) actually deliberate and act for moral reasons. The same may be true of dolphins who rescue humans (even if no tough choice is involved, Sapontzis' example). But the requirement of moral justification, which involves giving an explicit argument, seems not to be within animals' repertoire.

Perhaps this third way manifests the highest degree of moral agency known to terrestrial beings. However there are different kinds and degrees of moral agency.

3.4 Autonomy

Autonomy is not simply liberty – or freedom – of action. Liberty or freedom of action is the absence of external constraints that impede one from doing what one wants. Autonomy is more involved. Freedom of action implies governing one's actions by one's desires (doing what one wants to do). Autonomy implies governing these first-order desires by second-order desires (so that one wants what one wants to want, Frankfurt 1971:829–39). Autonomy is a second-order capacity to reflect critically upon one's first-order desires and the ability either to identify with these or to change them in light of higher-order preferences or values (Dworkin 1988:108). My higher-order preferences must stop somewhere with preferences or values that are 'given' to me, that come from 'outside' me. What non-chosen influences would be autonomy-subverting? Clear examples are obsessions, compulsions, coercive threats, hypnosis, and the onset of dementia. But autonomy admits of degrees. There is more-or-less autonomous action.

Do animals possess the capacity for critically evaluating the desires that move them to act, and sometimes modifying them on the basis of higher-order valuations? We do not know. The requisite mental complexity is very high. Abstraction is required, for the individual must be able to step back from her motivations and evaluate them. Perhaps we should be open to the possibility of some instances of autonomy of animals. But it would seem that the mental complexities involved are so high that probably very few – if any – nonhuman animals are autonomous.

Concluding, the higher cognitive capacities of a being are morally relevant. They ought to have a determining influence on our moral duties towards this being, as is obvious from the human case. Different animals can have one or more of the four mentioned capacities to a smaller or greater extent. The degree of an animal's mental complexity beyond sentience is significant for our moral duties towards this animal.

4 Capability to flourish

A further basis of moral concern is the idea of animals having a *telos,* a good, or species-specific needs. One might say that the animals *flourish* if they are able to satisfy their species-specific needs and to develop and use those capacities that animals of their species as a rule display. The concept of flourishing is morally important because it expresses a more comprehensive idea of animal well-being than just freedom from pain and suffering. It enables one to say that things may go well or badly for an animal depending on how specific environmental conditions relate to its usual species-specific development. The concept seems to have clear force in relation to identifying circumstances that fundamentally violate the expression of significant biologically determined features of a species. For example, experimental animals spend most of their lives in cages or pens, not actually undergoing procedures. They need adequate space for a range of natural behaviours: appropriate social behaviour, exercise, foraging and play and solid floors of appropriate material. Where they are housed in small and barren cages, they cannot perform their full range of species-specific behaviours. Inadequate environments have been the direct cause of a range of adverse physiological and psychological effects. This is not to say that animals can flourish only in their natural environments. If they are provided with a sufficiently complex environment, they may in principle be able to develop their potential in similar ways to animals living in the wild. The important question to ask is whether their – more or less – artificial environment is appropriate with regard to their species-specifics capacities and needs.

5 Sociability

Many authors in philosophy and animal ethics see sociability as creating a level of moral concern. According to these authors, being a member of some form of complex community creates moral relations of rights and duties. In our context, this idea can be developed in mainly two ways.

First, there are animals which have established links with humans and come to share their lives and fate in complex ways – particularly dog, cat and horse. Humans have special responsibilities to these beings who form part of a community with them. One may say that humans can have moral duties to animals due to their connectedness with them, and that the strength of these duties is (also) a function of the human-animal relationship.

Secondly, not only the relationship to humans establishes certain responsibilities, but also relationships that animals have among themselves. The Nuffield Council on Bioethics writes that this becomes perhaps most clear in considering animals such as primates. The species-specific capacities that these animals normally develop also include complex social interactions with other animals. Many argue that expression of this behaviour is usually severely restricted in research. It is feared that such infringements cannot be alleviated in the same way as physiological pain and suffering (by pain relieving medicines). The cage sizes that can be provided in conventional laboratories will always be inadequate. There are also concerns about how these social animals might experience the death of other research animals with which they have established relationships. Similar arguments could be made with regard to other social animals, such as dogs. It seems plausible that sociability may interact with other features: if social dislocation causes distress or suffering or interferes with flourishing to a significant degree, the overall effect on the animal could be potentially serious.

6 Moral concern and ethical theory

Four features have been presented which, according to widespread moral convictions, can give rise to moral concern. Moreover, it has been stated in the introduction that the size of experimental animals can matter morally if it is related to one or more of those four features. In order to reach a conclusion, two questions need briefly be taken up. First, why do the four features rightly give rise to moral concern? Secondly, why can size matter if it is related to these features? Looking for

answers, it seems useful also to turn to ethical theory because ethical theory can serve critical reflection on moral convictions.

Several authors in ethical theory, especially in animal ethics, have this answer to the question of why the four features rightly give rise to moral concern. The features regard the interests of animals, and ethics is centrally concerned with interests. Interests or, collectively, welfare or well-being, form a large part of the subject matter of ethics. Interests include both 'preference-interests' and 'welfare-interests' (Regan 1983), that is to say, the term 'interest' covers both *having* an interest in something or taking an interest in that thing, and the fact that something *is in one's interest,* that it has a positive effect on one's good, welfare, or well-being.

The second question was: why can size matter if it is related to the four features? An important, but possibly controversial answer one can get from ethical theory is some principle of equal consideration saying that equal moral weight should be given to the relevantly similar interests of different individuals. The crucial concept here is that of relevantly similar interests. If equal consideration is extended to animals, then one must pay heed to their relevantly similar interests and to their relevantly different interests. Sentient animals, including humans, have relevantly similar interests, for example, in avoiding pain. Pain is pain, no matter who has it. We could say that at the level of sentience, size does not matter morally. But regarding higher cognitive capacities, capability to flourish, and sociability we can note differences between animals, and these differences can be related to size. Then size does matter morally.

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References

Bermudez JL (2007) Thinking Without Words: An Overview For Animal Ethics. The Journal of Ethics 11:319–335

DeGrazia D (1996) Taking Animals Seriously: Mental Life and Moral Status. Cambridge University Press, Cambridge

Dworkin G (1988) The Theory and Practice of Autonomy. Cambridge University Press, Cambridge

Frankfurt H (1971) Freedom of the Will and the Concept of a Person. J Philos 68:829–39

Goodall J (1993) Chimpanzees – Bridging the Gap. In: Cavalieri P, Singer P (eds) The Great Ape Project: Equality Beyond Humanity. St. Martin's Press, New York

Herman L, Morrel-Samuels P (1990) Knowledge, Acquisition and Asymmetry Between Language Comprehension and Production. In: Bekoff M, Jamieson D (eds) Interpretation and Explanation in the Study of Animal Behavior, vol. I., Westview, Boulder, CO

Nuffield Council of Bioethics (2005) The Ethics of Research Involving Animals. London, www.nuffieldbioethics.org (22 February 2012)

Regan T (1983) The Case for Animal Rights. University of California Press, Berkeley

Sapontzis SF (1987) Morals, Reason, and Animals. Temple University Press, Philadelphia

Savage-Rumbaugh S, Brakke KE (1990) Animal Language: Methodological and Interpretive Issues. In: Bekoff M, Jamieson D (eds) Interpretation and Explanation in the Study of Animal Behavior, vol. I., Westview, Boulder, CO

Sidgwick H (1907) Methods of Ethics, 7th ed., Macmillan, London

Brain-pain: Do animals with higher cognitive capacities feel more pain? Insights for species selection in scientific experiments

James W. Yeates

Abstract

Recent legislative moves, such as the new EU Directive, encourage scientists to reduce suffering, by minimising pain and by using animals of lower cognitive abilities. This underlies the use of invertebrates instead of vertebrates, non-mammals instead of mammals, and restrictions on the use of certain species, such as primates, especially great apes.

If the basis for using "lower" animals is to avoid suffering, then its rationale depends on whether an animal's cognitive abilities alter its capacity to suffer. This paper explores and tests this connection, focusing on the relationship between an animal's cognitive capacity and its ability to experience pain.

However, empirically testing this hypothesis is philosophically problematic. Instead, a theoretical approach is needed to identify ways in which cognition capacities may prevent, cause, alleviate, attenuate or augment pain. These cognitive capacities can then be empirically investigated.

Pain involves both sensory and affective cortical areas such as the limbic and paralimbic systems and somatosensory cortex, which interact with other areas of cortical processing. Based on these neurological pathways, this paper looks at three (overlapping) aspects of pain processing: (1) sensory processing such as gate control theory, attention and distraction effects, episodic memory; (2) affective processing such as stimulus appraisal, biopsychosocial and diathesis-stress models; and (3) doxastic processes such as pain beliefs and problem solving.

The paper concludes that the capacity to suffer pain requires a base-line cognitive ability ("sentience"), which includes both sensory and affective components. Mammalian species, such as pigs and rodents, appear to be above this threshold. The possession of higher cognitive abilities is not a necessary condition of sentience. For sentient animals, different animals may have varying propensities to experience pain in different contexts. For example, it may be tentatively suggested that animals with lower cognitive abilities may be more prone to some forms

of acute pain, whereas those with higher capacities may be predisposed to chronic pain syndromes.

However, there is insufficient evidence to conclude that "higher" animals experience more or less pain overall. As such, the basis for the use of lower animals needs more defence. This defence might link cognition to other forms of animal suffering.

1 Introduction

An animal's pain experiences may be determined by many different factors. They depend on the stimuli received. But, in addition, empirical evidence suggests that pain experiences are also affected by other factors. For humans, pain reportage and physical impairments are related only modestly and not all of the variation is necessarily due to differences in the stimuli involved (Waddell and Main 1984; Flor and Turk 1988). In mice, inflammation may not always be accompanied by sensitisation (Larsson et al. 2006). Some of these factors may have a genetic basis, insofar as genetic factors appear to partly determine pain perception, for example in mice (Belknap et al. 1983; Panocka et al. 1986; Mogil 1999; Mogil et al. 1999a, b, 2000; Shir et al. 2001) and humans (Norbury et al. 2007; Nielsen et al. 2008; Fillingim et al. 2009). The apparent occurrence of intra-specific variation in individuals' pain experiences raises the hypothesis that there might be inter-specific variation in individuals' pain experiences, i.e., individuals of different species may have different capacities for pain experiences.

One determinant of variation in pain experiences may be differences in psychological capacities/cognitive ability (Price 2000; Woolf and Slater 2000). Despite a long tradition that the cerebral cortex was taken to be uninvolved in pain processing (mainly based on observations on humans following cerebral lesions; Head and Holmes 1911), studies are increasingly demonstrating the involvement of cortical areas such as the limbic and paralimbic systems and somatosensory cortex. There is a parallel increase in evidence that pain signalling and experience can depend on cognitive state (Price 2000). Contemporary theories and paradigms to pain and its control, such as gate control theory, biopsychosocial and diathesis-stress models, have stressed the importance of psychological, conative, affective and attitudinal elements (amongst others). For example, on diathesis-stress models, cognitive ability may be a significant predisposition, which can interact with later injurious stresses.

Such relationships suggest there may be a more general relationship between capacities for cognition and pain. If an animal's cognitive processes can affect its experience of pain, then it seems plausible to consider whether an animal's *capacity* for cognitive processes can affect its *capacity* to experience pain.

It has been frequently observed that there is inter-species variation in cognitive capacities. We might consider this in terms of species with capacities for more or less complex cognitive processes – i.e. what is often termed "higher" and "lower" cognitive capacities. This is undoubtedly an over-simplification. Yet it is an inherent and popular simplification, with adherents from Aristotle, through Morgan, to contemporary folk psychology views of humans as more cognitively complex than other species. This issue has substantial practical significance. Beliefs about animals' cognitive abilities appear to affect how people treat them (Davis and Cheeke 1998) and many philosophers defend differential respect for humans versus other animals against accusations of speciesism by pointing to humans' higher cognitive functions (e.g. Ben-Zeev 1982; Donagan1982; Gewirth1982). Similar arguments appear to underlie the greater protection afforded to primates, and to the placement of species on a *"scala naturae"* of neurophysiological sensitivity. Such a scale is then used to select experimental non-human animal models: less cognitively-developed animals should be, *ceteris paribus,* used in place of higher ones in experiments that may cause pain or other suffering. Taking this view as a starting hypothesis, we can ask the question of whether there might be relationships between animals' cognitive capacities and their capacities to experience pain. More specifically, do "higher" cognitive capacities make an animal likely to experience "increased" pain experiences? Whether these *scala naturae* assumptions are empirically well-grounded has received little attention yet.

This paper aims to begin an exploration of whether the *scala naturae* of neurophysiological sensitivity is justified by evaluating whether an animal's cognitive capacities increase the likelihood or intensity of its pain experiences. This discussion focuses on the axiological aspects of pain experiences, the things that make it unpleasant and worth avoiding (see Yeates 2011). This aspect of pain experiences will depend upon modifiers such as the intensity, duration, frequency of pain experiences, alongside the animal's propensity or likelihood of experiencing pain. It can also depend on the animal's response to the pain, insofar as how they may cope or process the experience. This may lead to other affective responses (e.g. fear or anxiety), although this paper focuses on initial and directly associated feelings (e.g. feelings about the pain experience), rather than such wider or longer-term effects. We are primarily concerned with whether higher cognitive function

leads to pain of higher intensity, duration or propensity. For shorthand, I shall use the terms "more" or "less" pain to denote any such variations.

2 Pain sensations

Pain is, in one sense, information. It "encodes" information about noxious states, such as their intensity, location and duration. This section considers the processing of information about intensity, location and duration, which we shall describe as the *sensation* of pain.

The basic perception of such dimensions in mammalian brains appears to be in the somatosensory cortices (Kenshalo and Isensee 1983; Kenshalo et al. 1988; Chudler et al. 1990; Bushnell et al. 1999), with anatomical, EEG, source-analysis and intracranial recording suggesting that the earliest pain-induced brain activity is in the secondary somatosensory cortex (Kunde and Treede 1993; Tarkka and Treede 1993; Ploner et al. 1999b; Craig 2002). Lesions in the somatosensory cortices have led to deficits in pain sensation (Ploner et al. 1999b; Bowsher et al. 2004) and neurones correlating to skin pain have been identified in primary and secondary somatosensory cortices of monkeys (Robinson and Burton 1980; Kenshalo et al. 1988; Dong et al. 1989) and rats (Lamour et al. 1983; Guilbaud et al. 1992; Follett and Dirks 1994) and the frontal cortex of rats (Mantz et al. 1988, 1990). Specifically, the primary somatosensory cortex appears to localise cutaneous pain in primates (Kenshalo and Isensee 1983) and rats (Morrow et al. 1998; Paulson et al. 2000).

However, the somatosensory cortices (SSC) are not uniquely important in pain experiences. For example, a human subject who suffered a lesion in both primary and secondary somatosensory cortices appeared unable to localise or precisely describe a pain stimulus, but reported a poorly-defined unpleasant feeling (Ploner et al. 1999a). In monkeys, somatosensory cortices appear to be equivalently stimulated in both awake and asleep subjects (Kenshalo and Isensee 1983; Kenshalo et al. 1988; Chudler et al. 1990). So, while the SSC may be important in the temporo-spatial *location* of painful stimuli, it is not clear what function they serve in the intensity of frequency of pain experiences. This would suggest that the higher cognitive abilities associated with greater SCC function do not necessarily lead to greater pain experiences.

Nevertheless, they may have less direct effects. The capacity to process information about spatial and temporal location and intensity may have some axiological

effects on the pain experience. The spatial location of pain is important in central (but not peripheral) allodynic effects whereby pain experiences sensitise the animal to experiencing more pain or increase the intensity of pain felt after subsequent painful stimuli in the same location. Where such responses are adaptive, animals with the cognitive complexity for allodynia may avoid greater future pain from worsening injuries. However, where such responses are maladaptive they may increase pain.

The capacity to determine the timing of pain might affect pain experiences in several ways. The ability to temporally locate pain allows animals to know *when* pain will begin or end and *that* pain will begin, end or continue. The latter knowledge may help animals to cope, and higher animals that are able to anticipate pain's end may therefore find pain experiences less unpleasant. Conversely, higher animals' knowledge that pain is likely to continue may exacerbate their negative feelings. Whether temporal location is beneficial may depend on what pain the animal experiences, insofar as these will affect the animal's beliefs about them. For example, higher animals may be better able to cope with repeated acute pain (Duncan and Petherick 1991) because the animal learns that the stimuli are of short duration and thus anticipates the end of the feeling (although higher animals may also anticipate future painful experiences in the interim). In contrast, higher animals may be less able to cope with long-term pain because they know that the pain will continue.

3 Attention

The perception of painful stimuli may also depend on the animal's attention (Steinmetz et al. 2000). This observation is based on electrophysiological and behavioural studies in humans (Seminowicz and Davis 2007), monkeys (Dubner et al. 1981; Bushnell et al. 1984; Hsiao et al. 1993; Meftah et al. 2002), cats (Casey and Morrow 1983) and chicken (Gentle 2001) and the increasing number of established human psychiatric analgesic therapies based on altering patients' attention (Eccleston 1995). Evidence from human studies suggests two ways attention can affect pain experiences: depending on *distraction* and *focusing*.

3.1 Distraction

Distraction appears to reduce pain experiences in humans and monkeys (McCaul and Haugtvedt 1982; Bushnell et al. 1985; Miron et al. 1989; Petrovic et al. 2000;

de Wied and Verbaten 2001; Dowman 2004; Terkelsen et al. 2004; Veldhuijzen et al. 2006). Utilising cognitive resources can reduce pain-related brain activity in humans, corresponding to neurobiological changes in primary and secondary somatosensory, insula, and cingulated cortices (Bushnell et al. 1999; Peyron et al. 1999; Longe et al. 2001; Frankenstein et al. 2001; Bantick et al. 2002; Tracey at al. 2002; Petrovic et al. 2004; Seminowicz et al. 2004; Valet et al. 2004, Wiech et al. 2005), trigeminal nucleus caudalis and medial thalamus (Bushnell et al. 1984; Bushnell and Duncan 1989). Non-human animals with the cognitive capacity to be distracted may thus also experience less pain.

More precisely, the effect of increased ability to be distracted would depend on the underlying mechanism of that distraction. It may occur through the animal's cognitive resources being completely sidetracked towards processing another stimulus at the expense of pain experiences in an all-or-nothing fashion. Alternatively, it could be that the animal's attention is partially divided between painful and "other" stimuli (Eccleston and Crombez 1999), in an incremental reduction depending on the strength of the distraction. *Ceteris paribus,* animals more able to be sidetracked would experience pain less often (because they are sidetracked); animals more able to divide attention would experience pain of lower intensity (because the attention is divided). If both mechanisms obtain, higher animals more able to divide attention might be expected to experience pain more frequently but of lower intensity.

3.2 Focusing

Cognitive flexibility in attention allows animals not only to be distracted, but also to focus on painful stimuli. In general, focusing on pain appears to increase the intensity of the experience in humans (Absi and Rokke 1991), which would suggest an increased capacity to focus may be associated with increased capacity to experience pain.

More precisely, focusing may be based on two mechanisms analogous to those for distraction. It could be that animals are sidetracked towards pain, or that animals can alter the division of attention incrementally. The sidetracking account would suggest that (lower) animals that are more likely to be sidetracked are also more likely to focus: since they have limited resources they can be expected to focus on biologically salient stimuli (Crick and Koch 2006) such as pain (Downar et al. 2003). The dividing attention account would suggest that (higher) ani-

mals able to divide attention are also less likely to focus because they can spare resources across a less focused range of processes. If both mechanisms obtain, then lower animals may experience pain more often *and* of higher intensity. Thus, the capacity to modulate attention can increase or decrease overall pain experiences (Table 1). The effect of increased capacity for focusing may outweigh the effect of increased capacity for distraction – indeed focusing may have a more significant effect.

Another complication to the above account is the evidence that at least some of the alterations in pain experiences, due to attention, may be because attention alters whether pain is processed by sensory or affective pathways. Which pathway is activated may alter the experience of pain (Malow et al. 1987). This may vary for acute versus chronic pain. For acute pain, focusing on sensory elements appears to decrease the severity of pain experiences and focusing on affective elements seems to increase the severity of experiences (Leventhal et al. 1979; Ahles et al. 1983; Dar and Leventhal 1993), whereas the relationship may be reversed for chronic pain (Rosenstiel and Keefe 1983; Phillips 1987; Vlaeyen and Linton 2000).

4 Affective processing and emotional coping

The limbic and paralimbic, i.e. anterior cingulate cortex and insular cortex, also appear to be involved in pain processing (Craig 2002, 2003; Bushnell and Apkarian 2006), with the insular cortex recruited immediately following the somatosensory activation (Frot and Mauguiere 2003). The limbic system appears to be especially important for emotional and motivational aspects of pain sensation (Bushnell and Apkarian 2006), with a significant correlation between anterior cingulate cortex (ACC) activity and a stimulus' reported unpleasantness (Tölle et al. 1999) and pain intensity being attenuated by ACC lesions (Hassenbusch et al. 1990; Wilkinson et al. 1999) and cingulotomies (Foltz and Lowell 1962; Foltz and White 1968).

Limbic involvement is evinced in many mammals, including humans (Lentz et al. 1998; Hutchinson et al. 1999; Frot and Mauguiere 2003), non-human primates (Friedmann and Murray 1986; Rausell and Jones 1991; Shi and Apkarian 1995; Koyama et al. 1998; Dostrovsky and Craig 1996), cats (Craig and Dostrovsky 2001) and rabbits (Sikes and Vogt 1992). This suggests that many animals of various neurophysiological sensitivities may experience affective elements of pain (e.g. its unpleasantness). Indeed, since the function, ontogeny and evolution of limbic

systems may be somewhat independent from prefrontal cortex development, prefrontal activity and capacities are not necessary for pain experience. The quality of the pain experience may be modulated by them, in particular the dorsal frontal cortical region may act as a top-down modulator of pain. This would suggest that animals with greater cortical activity may actually experience *less* painful experiences (Lorenz et al. 2003).

However, neurophysiological sensitivity may also vary how well an animal can cope using affective processing, insofar as the capacity for other emotions may modulate the intensity of pain experiences. On the one hand, pain may be reduced by the presence of other feelings such as pleasure (Yeates and Main 2008) and anger (Janssen et al. 2001; Burns et al. 2003), although affective coping may depend on pain severity (Nicassio et al. 1995; Robinson et al. 1997; Riley et al. 1999). Learning may also reduce pain experiences, for example conditioning may promote stress-induced analgesia (Flor et al. 2002b) and operant conditioning programmes may lead to lower pain-ratings (Flor et al. 2002a). On the other hand, other emotional states may increase pain experiences. They might delay recovery from painful pathology (Salovey et al. 2000). They might increase pain-sensitivity or cause pain, or vice-versa, or co-dependence (Robinson and Riley 1999). Anger may be associated with higher levels of pain (Fernandez and Turk 1995; Greenwood et al. 2003), although this may depend on gender (Burns et al. 1996) and location of pain-source (Bruehl et al. 2003) and cause of pain (Materazzo et al. 2000). Stress can also increase pain, as suggested by increased activation of the nucleus of the solitary tract and amygdale (De Lange et al. 2005) and the association between stress and Complex Regional Pain Syndrome in humans.

Cognitive biases can also affect pain experiences in humans (McCracken et al. 1999, 2002). For example, anxiety can be associated with increased pain experiences (Graffenried et al. 1978; Rhudy and Meagher 2000; Keogh and Cochrane 2002), analgesia consumption (Nelson et al. 1998; Kain et al. 2000; Caumo et al. 2002) and chronic pain prevalence (McWilliams 2003). Indeed, anxiety sensitivity (anxiety about likely anxiety symptoms) also appears related to chronic pain (Asmundson et al. 1999a, b; Norton et al. 1999) and lower acute pain thresholds (Keogh and Mansoor 2001; Keogh and Cochrane 2002). Depression seems to be associated with chronic pain (Banks and Kerns 1996; Briley 2003; Greenberg et al. 2003), and antidepressants can be effective in controlling pain. The capacity for such biases may therefore lead to increased pain experiences. The reciprocal biases, such as optimism, may decrease pain.

More generally, the effect of animal's cognitive processing may depend on the animal's context. In particular, emotional processing may decrease pain where it is controllable, but increase uncontrollable pain where responses are maladaptive. The effect of emotional processing may also differ for chronic versus acute pain, insofar as the limbic system appears to be more important in chronic pain than acute pain (Apkarian et al. 2001). What kind of emotional processing occurs is likely to depend on the animal's previous experiences and underlying cognitive state (especially on diathesis-stress models). For example, it seems likely that animals subject to laboratory husbandry conditions and painful interventions will more commonly suffer negative biases than positive biases. If so, then the net effect of the capacity for emotional biases may be to increase pain.

5 Doxastic processing, pain beliefs and control

Insofar as pain is "information", an animal's doxastic reasoning abilities can affect how it processes painful stimuli. Higher animals may entertain beliefs about a pain's cause, duration, permanence and the animal's ability to control it (e.g. as assessed in Pain Beliefs and Perceptions Inventory (Williams and Thorn 1989; Williams and Keefe 1991) and Survey of Pain Attitudes (Jensen et al. 1994) tools), which may affect pain experiences and treatment response (Williams and Keefe 1991; Williams et al. 1994; Tait and Chibnall 1998). Such beliefs require varying neurophysiological sensitivity. For example, beliefs about one's coping ability, so-called "self-efficacy"(Bandura 1977; Bandura et al. 1987, 88; Weidenfeld et al. 1990; Lester et al. 1996; Villemure and Bushnell 2002) may require abilities to conceptualise pain, control and possibly self; the ability to receive beliefs through communication may be significantly enhanced by verbal abilities (as in cognitive therapy and other training).

These beliefs may reduce pain and there is some evidence from human studies that knowledge by itself reduces unpleasantness associated with pain (mainly anxiety; Johnson 1973), especially if it makes animals "accept" pain (McCracken 1998; McCracken et al. 1999, 2003; Ridson et al. 2003; Viane et al. 2003). However, such beliefs may also increase pain, depending on the beliefs and their accuracy. Animals may also "catastrophise" about pain, and thereby worsen pain experiences, as has been demonstrated in humans (Zautra and Manne 1992; Jacobsen and Butler 1996; France et al. 2002; Tripp et al. 2003). So whether such capacities make pain worse or better is probably highly contextual. For example, being able to anticipate control may lead to less pain in the placebo effect (Benedetti et al.

2005; Haour 2005), but may also lead to a nocebo effect, where negative expectations increase reported pain (Benedetti et al. 1997). Higher doxastic capacities may be beneficial for animals who experience predictable, controllable and impermanent pain, whereas animals experiencing pain that appears unpredictable, uncontrollable and permanent may suffer more from knowing this.

The ability to entertain pain beliefs might allow higher animals' pain to be reduced by cognitive therapy. Whether this means higher animals will experience less pain depends on two things. Firstly, it depends on whether cognitive therapy is thought to act on the "lower" pain processing or on "higher processing". If the former, then this would suggest that higher animals' amenability to cognitive therapy affords them the potential to experience less pain.[1] If the latter, then it could be argued that cognitive therapy only "undoes" the harm of having higher cognitive capacities. This latter model is supported by the apparent usefulness of strategies in cognitive therapy such as teaching patients to recognise irrational or harmful beliefs that distort experiences (Meichenbaum 1985). Animals that cannot distort beliefs would not need this therapy, and so would feel less pain. This would suggest that animals with lower neurophysiological sensitivity would experience less pain. Secondly, whether higher animals will experience less pain will depend on whether they actually, in fact, receive cognitive therapy.

6 Discussion

Based on this conceptual analysis and review of empirical evidence, there are reasons to consider that the capacity to experience pain may be increased *or* decreased by increased cognitive capacities. Cognition appears to increase pain in some cases but decrease it in others, and the multiple relationships cannot be generalised into a single overall statement that different animals experience more or less pain *per se* as an overall net effect (which fits with our popular belief that less intelligent humans do not thereby experience less pain). Even when there is an effect of cognition on pain processing, it may be that this has no qualitative effect on an animal's actual subjective pain experiences. It is commonly considered that pain must be reduced by at least 30% for the reduction to be meaningful to patients (Farrar et al. 2000, 2001, 2003; Cepeda et al. 2003; Salaffi et al.

1 Cognitive therapy may also reduce the stress, anxiety etc of pain, including by altering the assessment and processing of painful experiences, without altering the pain itself, and may involve teaching problem solving, thereby decreasing the likelihood of future pain experiences although this is arguably not a defining aspect of cognitive therapy (D'Zurilla and Goldfried 1971; Meichenbaum 1985).

2004; Hanley et al. 2006). Thus, only if human/non-human differences reduce/increase pain by this amount will there be a meaningful difference in its experience. There is insufficient evidence that variations in the effect of mammals' differing cognitive capacities reach this threshold (and they appear to be equally likely to reach it on either side).

These considerations suggest that we should reconsider the received wisdom of using animals of "lower" cognitive complexity in preference to "higher" ones. The fact that we cannot identify a net relationship from this analysis does not of course mean that there is *no* relationship between cognitive capacities and the capacity to experience pain. However, the absence of grounds for a belief either way suggests that there are no grounds for an ethical (or legal) stance that we should use less neurophysiologically complex animals in painful experiments. There is insufficient evidence or reason to support the defence of routine differential treatment of species based on species-relative variations in capacity to experience pain. For example, there is no defence that painful experiments should use mice in preference to chimpanzees (or humans) as a refinement of that experiment.

As a corollary, this paper does suggest that species selection be defended for specific experiments. For example, evidence described above suggests that higher capacities for doxastic processing may decrease pain when the sources of the pain are controllable (e.g., in promoting healing), but may increase pain when it is not controllable (e.g., in knowing pain is unpredictable). This would imply that animals with higher doxastic reasoning abilities might be better used in experiments where they are given the opportunity to use those abilities to cope. Similarly, higher cognitive capacities for emotional processing may decrease pain when pain sources cannot be eliminated (e.g., in emotional coping) but increase pain when pain sources can be eliminated (e.g., in depression). Animals experiencing pain that appears predictable, controllable and impermanent may benefit from being able to have corresponding beliefs; animals experiencing pain that appears unpredictable, uncontrollable and permanent may suffer more from knowing this. This suggestion is corroborated by (limited) evidence that problem-focused coping was found to be better than emotion-focused coping in situations perceived as controllable; whereas emotion-focused coping was found to be better than problem-focused coping in situations perceived as uncontrollable (Forsythe and Compas 1987). This would suggest that animals with more sophisticated or potent emotional coping abilities may be better used in experiments where pain cannot be evaded, and their use should be avoided where pain can be evaded. As another example, there may be reason to expect that higher animals will experience more

chronic pain, because of the effects of "malfunctioning" cognitive processes, but less acute pain, due to the effects of coping abilities. Chronic pain is common in humans, for example, with a reported prevalence of 20% (Gallagher 1997), although there is no comparable figure for non-human animals. If higher cognitive capacity does predispose to more chronic pain but less acute pain, then this suggests that animals of higher neurophysiological sensitivity should be used for studies involving acute pain; whereas animals with lower neurophysiological sensitivity should be used for studies involving chronic conditions.

This discussion also suggests a number of other possible refinements. It is clear that individual's cognition can alter their pain experiences, and knowledge of the specific relationships can be used to refine experiments. For example, experiments can try to alter animal's processing to improve their problem-solving, coping or processing to avoid allodynic, sensitisation or chronic pain syndromes. Scientists may also consider using attentional effects within refinement strategies. In addition, attention to variations between individuals, even within the same species, may help to minimise pain experiences in practice.

This paper is only a start, and further work may elucidate relationships in more detail. For example, many of the effects of cognitive complexity on pain experiences may depend on interactions and relations between different elements discussed above. Already mentioned is the effect of attentional changes on whether pain is processed by sensory or affective pathways. This may alter the experience of pain (Malow et al. 1987). As another example, the anterior cingulated cortex not only has a role in emotion, but also a well-recognised role in attention (Davis et al. 1997; Derbyshire et al. 1998), so there may be interactions between attentional and affective processing. Such ideas would need further consideration, as evidence comes to light. It is important that such further work avoids focusing only on specific elements of relationships between cognition and pain experiences, insofar as a holistic approach is needed to answer the question of species selection for experiments.

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Table 1: Speculate effects of higher distraction and focusing capacities on frequency and intensity of pain experiences

	Distraction			*Focusing*			*Overall*
Mechanism	Sidetracking	Divided attention	Both	Sidetracking	Divided attention	Both	(cancel-ling out and addition)
Definition of "higher"	Ability to be sidetracked away from pain	Ability to divide atten-tion to pain onto other stimuli	Ability to divide attention instead of being side-tracked away from pain	Ability to be sidetracked towards pain	Ability to divide attention to other stimuli onto pain	Ability to be more sidetracked towards pain instead of dividing attention	Ability to divide atten-tion more than be side-tracked to or from pain
Higher animals	Lower frequency	Lower intensity	Higher fre-quency but lower intensity	Higher frequency	Higher frequency	Higher intensity	Higher frequency
Lower animals	Higher frequency	Higher intensity	Lower fre-quency but greater intensity	Lower frequency	Lower frequency	Lower intensity	Lower frequency

References

Absi M, Rokke PD (1991) Can anxiety help us tolerate pain? Pain 46:43–51

Ahles TA, Blanchard EB, Leventhal H (1983) Cognitive control of pain: attention to the sensory aspects of the cold-pressor stimulus. Cognitive Ther Res 7:159–178

Apkarian AV, Thomas PS, Krauss BR, Szeverenyi NM (2001) Prefrontal cortical hyperactivity in patients with sympathetically mediated chronic pain. Neurosci Lett 311:193–197

Asmundson GJ, Norton PJ, Norton GR (1999a) Beyond pain: The role of fear and avoidance in chronicity. Clin Psychol Rev 19:97–119

Asmundson GJ, Norton PJ, Veloso F (1999b) Anxiety sensitivity and fear of pain in patients with recurring headaches. Behav Res Ther 37:703–713

Bandura A (1977) Self-efficacy: toward a unifying theory of behavioral change. Psychol Rev 84:191–215

Bandura A, O'Leary A, Taylor CB, Gauthier J, Gossard D (1987) Perceived self-efficacy and pain control: opioid and nonopioid mechanisms. J Pers Soc Psychol 53:563–571

Bandura A, Cioffi D, Taylor CB, Brouillard ME (1988) Perceived self-efficacy in coping with cognitive stressors and opioid activation. J Pers Soc Psychol 55:479–488

Banks SM, Kerns RD (1996) Explaining high rates of depression in chronic pain: A diathesis-stress framework. Psychol Bull 119:95–110

Bantick SJ, Wise RG, Ploghaus A, Clare S, Smith SM, Tracey I (2002) Imaging how attention modulates pain in humans using functional MRI. Brain 125:310–319

Benedetti F, Amanzio M, Casadio C, Oliaro A, Maggi G (1997) Blockade of nocebo hyperalgesia by the cholecystokinin antagonist proglumide. Pain 71:135–40

Benedetti F, Mayberg HS, Wager TD, Stohler CS, Zubieta JK (2005) Neurobiological mechanisms of the placebo effect. J Neurosci 25:10390–10402

Ben-Zeev A (1982) Who is a rational agent? Can J Philos 12:647–661

Bowsher D, Brooks J and Enevoldson P (2004) Central representation of somatic sensations in the parietal operculum (SII) and insula. Eur Neurol 52:211–25

Briley M (2003) New hope in the treatment of painful symptoms in depression. Curr Opin Invest Dr 4:42–45

Bruehl S, Chung OY, Burns JW (2003) Differential effects of expressive anger regulation on chronic pain intensity in CRPS and non-CRPS limb pain patients. Pain 104:647–654

Burns JW, Johnson BJ, Mahoney N, Devine J, Pawl R (1996) Anger management style, hostility and spouse responses: Gender differences in predictors of adjustment among chronic pain patients. Pain 64:445–453

Burns JW, Kubilus A, Bruehl S (2003) Emotion induction moderates effects of anger management style on acute pain sensitivity. Pain 61:165–175

Bushnell MC, Duncan GH, Dubner R, He LF (1984) Activity of trigeminothalamic neurons in medullary dorsal horn of awake monkeys trained in a thermal discrimination task. J Neurophysiol 52:170–187

Bushnell M, Duncan G, Dubner R, Jones R, Maixner W (1985) Attentional influences on noxious and innocuous cutaneous heat detection in humans and monkeys. J Neurosci 5:1103–1110

Bushnell MC, Duncan GH (1989) Sensory and affective aspects of pain perception: Is medial thalamus restricted to emotional issues? Exp Brain Res 78:415–418

Bushnell MC, Duncan GH, Hofbauer RK, Ha B, Chen JI, Carrier B (1999) Pain perception: is there a role for the primary somatosensory cortex? Proc Nat Acad Sci USA 96: 7705–7709

Bushnell MC, Apkarian AV (2006) Representation of pain in the brain. In: McMahon SB, Koltzenburg M (eds) Wall and Melzack's Textbook of Pain. Elsevier, Philadelphia, pp 107–124

Casey KL, Morrow TJ (1983) Nocifensive responses to cutaneous thermal stimuli in the cat: stimulus–response profiles, latencies, and afferent activity. J Neurophysiol 50:1497–515

Cepeda M, Africano J, Polo R, Alcala R, Carr D (2003) What decline in pain intensity is meaningful to patients with acute pain? Pain 105:151–157

Chudler EH, Anton F, Dubner R, Kenshalo DR Jr (1990) J Neurophysiol 63: 559-569

Caumo W, Schmidt AP, Schneider CN, Bergmann J, Iwamoto CW, Adamatti LC, Bandeira D, Ferreira MB (2002) Preoperative predictors of moderate to intense acute postoperative pain in patients undergoing abdominal surgery. Acta Anaesth Scand 46: 1265–1271

Craig AD (2002) How do you feel? Interoception: the sense of the physical condition of the body. Nat Rev Neurosci 3:655–666

Craig AD (2003) A new view of pain as a homeostatic emotion. Trends Neurosci 26:303–307

Craig AD, Dostrovsky JO (2001) Differential projections of thermoreceptive and nociceptive lamina I trigeminothalamic and spinothalamic neurons in the cat. J Neurophysiol 86:856–870

Crick FC, Koch C (2006) What are the neuronal correlates of consciousness? In: Hemmen JL, Sejnowski TJ (eds) 23 problems in systems neuroscience. Oxford University Press, Oxford, pp 474–90

Dar R, Leventhal H (1993) Schematic processes in pain perception. Cogn Ther Res 17:341–57

Davis KD, Taylor SJ, Crawley AP, Wood MI, Mikulis DJ (1997) Functional MRI of pain- and attention-related activations in the human cingulate cortex. J Neurophysiol 77:3370–3380

Davis SL, Cheeke, PR (1998) Do domestic animals have minds and the ability to think? A provisional sample of opinions on the question. J Anim Sci 76:2072–2079

De Lange RP, Geerse GJ, Dahlhaus M (2005) Altered brain stem responsitivity to duodenal pain after a single stressful experience. Neurosci Lett 381:144–148

De Wied M, Verbaten M (2001) Affective pictures processing, attention and pain tolerance. Pain 90:163–172

Derbyshire SW, Vogt BA, Jones AK (1998) Pain and Stroop interference tasks activate separate processing modules in anterior cingulated cortex. Exp Brain Res 11:52–60

Donagan A (1982) Comments on Aaron Ben-Zeev, 'Who is a Rational Agent?' Can J Philos 12:663–666

Dong WK, Salonen LD, Kawakami Y, Shiwaku T, Kaukoranta EM, Martin RF (1989) Nociceptive responses of trigeminal neurons in SII-7b cortex of the awake monkey. Brain Res 484:314–324

Dostrovsky JO, Craig AD (1996) Nocicpetive neurons in primate insular cortex. Society for Neuroscience Abstracts 22:111

Dowman R (2004) Distraction produces an increase in pain-evoked anterior cingulate activity. Psychophysiology 41:613–624

Downar J, Mikulis DJ, Davis KD (2003) Neural correlates of the prolonged salience of painful stimulation. Neuroimage 20:1540–1551

Dubner R, Hoffman DS, Hayes RL (1981) Neuronal activity in medullary dorsal horn of awake monkeys trained in a thermal discrimination task. III. Task-related responses and their functional role. J Neurophysiol 46:444–464

Duncan IJ, Petherick JC (1991) The implications of cognitive processes for animal welfare. J Anim Sci 69: 5017–5022

D'Zurilla TJ, Goldfried MR (1971) Problem-solving an behaviour modification. J Abnorm Psychol 78:107–126

Eccleston C (1995) The attentional control of pain: methodological and theoretical concerns. Pain 63:3–10

Eccleston C, Crombez G (1999) Pain demands attention: a cognitive-affective model of the interruptive function of pain. Psychol Bull 125:356–366

Farrar J, Portenoy R, Berlin J, Kinman J, Strom B (2000) Defining the clinically important difference in pain outcome measures. Pain 88:287–294

Farrar J, Young Jr J, LaMoreaux L, Werth J, Poole R (2001) Clinical importance of changes in chronic pain intensity measured on an 11-point numerical pain rating scale. Pain 94:149–158

Farrar J, Berlin J, Strom B (2003) Clinically important changes in acute pain outcome measures: a validation study. J Pain Symptom Manag 25:406–411

Fernandez E, Turk DC (1995) The scope and significance of anger in the experience of chronic pain. Pain 61:165–175

Flor H, Turk DC (1988) Chronic back pain and rheumatoid arthritis: predicting pain and disability from cognitive variables. J Behav Med 11:251–265

Flor H, Knost B, Birmbaumer N (2002a) The role of operant conditioning in chronic pain: An experimental investigation. Pain 95:111–118

Flor H, Birmbaumer N, Schultz R, Grusser SM, Mucha RF (2002b) Pavlovian conditioning of opiod and nonopiod pain inhibitory mechanisms in humans. Eur J Pain 6:395–402

Follett KA and Dirks B (1994) Characterisatoin of responses of primary somatosensory cerebral cortex neurons to noxious visceral stimulation in the rat. Brain Res 656:27–32

Folkman S, Lazarus RS, Dunkel-Schetter C, DeLongis A, Gruen RJ (1986) Dynamics of a stressful encounter: cognitive appraisal, coping and encounter outcomes. J Pers Soc Psychol 50:992–1003

Foltz EL, Lowell EW (1962) Pain 'relief' by frontal cingulumotomy. J Neurosurg 19:89–100

Foltz EL, White LE (1968) The role of rostral cingulumotomy in 'pain' relief. Int J Neurol 6:353–373

Forsythe C, Compas B (1987) Interaction of cognitive appraisals of stressful events and coping: Testing the goodness of fit hypothesis. Cognitive Ther Res 11:473–485

France CR, France JL, al'Absi M, Ring C, McIntyre D (2002) Catastrophzing is related to pain ratings, but not nociceptive flexion reflex threshold. Pain 99:459–463

Frankenstein UN, Richter W, McIntyre MC, Remy F (2001) Distraction modulates anterior cingulate gyrus activations during the cold pressor test. Neuroimage 14:827–836

Friedman DP, Murray EA (1986) Thalamic connectivity of the second somatosensory area and neighbouring somatosensory fields of the lateral sulcus of the macaque. J Comp Neurol 252:348–374

Frot M, Mauguiere F (2003) Dual representation of pain in the operculo-insular cortex in humans. Brain 126:438–450

Gallagher RM (1997) Primary care and pain medicine: A community solution to the public health problem of chronic pain? Med Clin N Am 83:555–583

Gentle MJ (2001) Attentional shifts alter pain perception in the chicken. Anim Welfare 10: S187–194

Gewirth A (1982) On Rational Agency as the Basis of Moral Equity: Reply to Ben-Zeev, Can J Philos 12:667–671

Graffenried BV, Adler R, Abt K, Nuesch E, Spiegel R (1978) The influence of anxiety and pain sensitivity on experimental pain in man. Pain 4:253–263

Greenberg PE, Leong SA, Birnbaum HG, Robinson RL (2003) The economic burden of depression with painful symptoms. J Clin Psychiat 64(S7):17–23

Greenwood KA, Thurston R, Rumble M, Waters SJ, Keefe FJ (2003) Anger and persistent pain: Current status and future directions. Pain 103:1–5

Guilbaud G, Benoist JM, Levante A, Gautron M, Willer JC (1992) Primary somatosensory cortex in rats with pain-related behaviours due to a peripheral mononeuropathy after moderate ligation of one scietic nerve: neuronal responsitivity to somatic stimulation. Exp Brain Res 92:227–45

Haour F (2005) Mechanisms of the placebo effect and of conditioning. Neuroimmunomodulat 12:195–200

Hassenbusch SJ, Pillay PK, Barnett GH (1990) Radiofrequency cingulotomy for intractable cancer pain using stereotaxis guided by magnetic resonance imaging. Neurosurgery 27:220–223

Head H, Holmes G (1911) Sensory disturbances from cerebral lesions. Brain 34:102–254

Hsiao S, O'Shaughnessy D, Johnson K (1993) Effects of selective attention on spatial form processing in monkey primary and secondary somatosensory cortex. J Neurophysiol 70:444–447

Hutchinson WD, Davis KD, Lozano AM, Tasker RR, Dostrovsky JO (1999) Pain-related neurons in the human cingulate cortex. Nat Neurosci 2:403–405

Jacobsen PB, Butler RW (1996) Relation of cognitive coping and catastrophizing to acute pain and analgesic use following breast cancer surgery. J Behav Med 19:17–26

Janssen SA, Spinhoven P, Brosschot JF (2001) Experimentally-induced anger, cardiovascular reactivity and pain sensitivity. J Psychosom Res 51:479–485

Jensen MP, Turner JA, Romano JM, Lawler BK (1994) The relationship of pain-specific beliefs to chronic pain adjustment. Pain 57:301–9

Johnson JE (1973) Effects of accurate expectations about sensations on the sensory and distress components of pain. J Pers Soc Psychol 27:261–275

Kain ZN, Severino F, Alexander GM, Pincus S, Mayes LC (2000) Preoperative anxiety and postoperative pain in women undergoing hysterectomy. A repeated-measures design. J Psychosom Res 49:417–422

Kenshalo DR Jr, Isensee O (1983) Responses of primate SI cortical neurons to noxious stimuli. J Neurophysiol 50:1479–1496

Kenshalo DR Jr, Chudler EH, Anton F, Dubner R (1988) SI nociceptive neurons participate in the encoding process by which monkeys perceive the intensity of noxious thermal stimulation. Brain Res 454:378–382

Keogh E, Cochrane M (2002) Anxiety sensitivity, cognitive biases and the experience of pain. J Pain 3:320–329

Keogh E, Mansoor L (2001) Investigating the effects of anxiety sensitivity and coping on the perception of cold pressor pain in healthy women. Eur J Pain 5:11–22

Koyama T, Tanaka YZ, Mikami A (1998) Nociceptive neurons in the macaque anterior cingulated activate durig anticipation of pain. Neuroreport 9:2662–2667

Kunde V, Treede RD (1993) Topography of middle-latency somatosensory evoked potentials following painful laser stimuli and non-painful electrical stimuli. Electroen Clin Neuro 88:280–289

Lamour Y, Willer JC, Guilbaud G (1983) Rat somatosensory (Sml) cortex 1: Characteristcs of neuronal responses to noxious stimulation and comparison with responses to non-noxious stimulation. Exp Brain Res 49:35–45

Larsson MH, Rapp I, Lindström E (2006) Effect of DSS-induced colitis on visceral sensitivity to colorectal distension in mice. Neurogastroent Motil 18(2):144–152

Leventhal H, Brown D, Shacham S, Engquist G (1979) Effects of preparatory information about sensations, threat of pain and attention on coldpressor distress. J Pers Soc Psychol 37:688–714

Lenz FA, Rios M, Zirh TA, Chau D, Krauss G, Lesser RP (1998) Painful stimuli evoke potentials recorded over the human anterior cingulate gyrus. J Neurophysiol 79:2231–2234

Lester N, LeFebvre JC, Keefe FJ (1996) Pain in young adults – III: Relationships of three pain-coping measures to pain and activity inference. Clin J Pain 12:291–300

Longe SE, Wise R, Bantick S, Lloyd D, Johansen-Berg H, McGlone F, Tracey I (2001) Counter-stimulatory effects on pain perception are significantly altered by attention: An fMRI study. Neuroreport 12:2021–2025

Lorenz J, Minoshima S, Casey KL (2003) Keeping pain out of mind: the role of the dorsolateral prefrontal cortex in pain modulation. Brain 126:1079–1091

Malow RM, West JA, Sutker PB (1987) A sensory decision theory analysis of anxiety and pain responses in chronic drug abusers. J Abnorm Psychol 96:184–189

Mantz J, Milla C, Glowinski J, Thierry AM (1988) Differential effects of ascending neurons containing dopamine and noradrenaline in the control of spontaneous activity and of evoked responses in the rat prefrontal cortex. Neuroscience 27:517–526

Mantz J, Godbout R, Tassin JP, Glowinsky J, Thierry AM (1990) Inhibition of spontaneous and evoked unit activation in the rat medial prefrontal cortex by mesencephalic raphe nuclei. Brain Res 524:22–30

Materazzo F, Cathcart S, Pritchard D (2000) Anger, depression and coping interactions in headache activity and adjustment: A controlled study. J Psychosom Res 49:69–75

McCaul K, Haugtvedt C (1982) Attention, distraction, and cold-pressor pain. J Pers Soc Psychol 43:154–162

McCracken LM (1998) Learning to live with the pain: Acceptance of pain predicts adjustment in persons with chronic pain. Pain 74:21–27

McCracken LM, Spertus IL, Janeck AS, Sinclair D, Wetzel FT (1999) Behavioural dimensions of adjustment in persons with chronic pain: Pain-related anxiety and acceptance. Pain 80:283–289

McCracken LM, Gross RT, Eccleston C (2002) Multimethod assessment of treatment process in chronic low back pain: comparison of reported pain-related anxiety with directly measured physical capacity. Behav Res Ther 40:585–594

McCracken LM, Eccleston C (2003) Coping or acceptance: What to do about chronic pain? Pain 105:197–204

McWilliams LA, Cox BJ, Ens MW (2003) Mood and anxiety disorders associated with chronic pain: An examination in a nationally representative sample. Pain 106:127–133

Meftah E, Shenasa J, Chapman C (2002) Effects of a cross-modal manipulation of attention on somatosensory cortical neuronal responses to tactile stimuli in the monkey. J Neurophysiol 88:3133–3149

Meichenbaum D (1985) Stress Innoculation training. Pergamon press: NY

Miron D, Duncan G, Bushnell M (1989) Effects of attention on the intensity and unpleasantness of thermal pain. Pain 39:345–352

Morrow TJ, Paulson PE, Danneman PJ, Casey KL (1998) Regional changes in forebrain activation during the early and late phase of formalin nociception: Analysis using cerebral blood flow in the rat. Pain 75:355–365

Nelson FV, Zimmerman L, Barnason S, Nieveen J, Schmaderer M (1998) The relationship and influence of anxiety on postoperative pain in the coronary artery bypass graft patient. J Pain Symptom Manag 15:102–109

Nicassio PM, Schoenfeld-Smith K, Radojevic V, Schuman C (1995) Pain coping mechanisms in fibromyalgia: relationship to pain and functional outcomes. J Rheumatol 22:1552–1558

Norton GR, Norton PJ, Asmundson GJ, Thompson LA, Larsen DK (1999) Neurotic butterflies in my stomach: The role of anxiety, anxiety sensitivity and depression in functional gastrointestinal disorders. J Psychosom Res 47:233–240

Paulson PE, Morrow TJ and Casey KL (2000) Bilateral behavioural and regional cerebral blood flow changes during painful peripheral mononeuropathy in the rat. Pain 84:233–245

Petrovic P, Petersson K, Ghatan P, Stone-Elander S, Ingvar M (2000) Pain-related cerebral activation is altered by a distracting cognitive task. Pain 85:19–30

Petrovic P, Petersson K, Hansson P, Ingvar M (2004) Brainstem involvement in the initial response to pain. Neuroimage 22:995–1005

Peyron R, García-Larrea L, Grégoire MC, Costes N, Convers P, Lavenne F, Mauguière F, Michel D, Laurent B (1999) Haemodynamic brain responses to acute pain in humans: sensory and attentional networks. Brain 122:1765–80

Phillips HC (1987) Avoidance behaviour and its role in sustaining chronic pain. Behav Res Ther 25:273–279

Ploner M, Freundt HJ and Schnitzler A (1999a) Pain affect without pain sensation in a patient with a postcentral lesion. Pain 81:211–214

Ploner M, Schmitz F, Freund HJ, Schnitzler A (1999b) Parallel activation of primary and secondary somatosensory cortices in human pain processing. J Neurophysiol 81:3100–3104

Price DD (2000) Psychological and neural mechanisms of the affective dimension of pain. Science 288:1769–72

Rausell E, Jones EG (1991) Chemically distinct compartments of the thalamic VPM nucleus in monkeys relay principal and spinal trigeminal pathways to different layers of the somatosensory cortex. J Neurosci 11:226–237

Rhudy JL, Meagher MW (2000) Fear and anxiety: Divergent effects on human pain thresholds. Pain 84:65–75

Ridson A, Eccleston C, Crombez G, McCracken L (2003) How can we learn to live with pain? A Q-methodological analysis of the diverse understandings of acceptance of chronic pain. Soc Sci Med 56:375–386

Riley JL 3rd, Robinson ME, Geisser ME (1999) Empirical subgroups of the Coping Strategies Questionnaire-Revised: a multisample study. Clin J Pain 15:111–116

Robinson CJ, Burton H (1980) Somatic submodality distribution within the second somatosensory (SII), 7b, retroinsular, postauditory and granular insular cortical areas of M. fascicularis. J Comp Neurol 192:93–108

Robinson ME, Riley JL 3rd, Myers CD, Sadler IJ, Kvaal SA, Geisser ME Keefe FJ (1997) The Coping Strategies Questionnaire: a large sample, item level factor analysis. Clin J Pain 13:43–49

Robinson ME, Riley JL (1999) The role of emotion in pain. In: Gatchel RJ, Turk DC (eds) Psychological factors in pain. Guilford press, NY, pp 74–88

Rosenstiel AK, Keefe FJ (1983) The use of coping strategies in chronic low back pain patients: relationship to pain characteristics and current adjustment. Pain 17:33–44

Salaffi F, Stancati A, Silvestri C, Ciapetti A, Grassi W (2004) Minimal clinically important changes in chronic musculoskeletal pain intensity measured on a numerical rating scale. Eur J Pain 8:283–291

Salovey P, Detweiler JB, Steward WT, Rothman AJ (2000) Emotional states and physical health. Am Psychol 55:110–121

Seminowicz D, Mikulis D, Davis K (2004) Cognitive modulation of pain-related brain responses depends on behavioral strategy. Pain 112:48–58

Seminowicz DA, Davis KD (2007). Interactions of pain intensity and cognitive load: the brain stays on task. Cereb Cortex 17:1412–1422

Shi T, Apkarian AV (1995) Morphology of thalamocortical neurons projecting to the primate somatosensoty cortex and their relationship to spinothalamic terminals in the squirrel monkey. J Comp Neurol 361:1–24

Sikes RW, Vogt BA (1992) Nociceptive neurons in area 24 of rabbit cingulated cortex J Neurophysiol 68:1720–1732

perception and heart rate variability but not a nociceptive withdrawal reflex. Acta Physiol Scand 180:405–414

Tölle TR, Kaufmann T, Siessmeier T, Lautenbacher S, Berthele A, Munz F, Zieglgänsberger W, Willoch F, Schwaiger M, Conrad B, Bartenstein P (1999) Region-specific encoding of sensory and affective components of pain in the human brain: a positron emission tomography correlation analysis. Ann Neurol 45:40–47

Tracey I, Ploghaus A, Gati JS, Clare S, Smith S, Menon RS, Matthews PM (2002) Imaging attentional modulation of pain in the periaqueductal gray in humans. J Neurosci 22:2748–52

Tripp DA, Stanish WD, Reardon G, Coady C, Sullivan MJ (2003) Comparing postoperative pain experiemces of the adolescent and adult athlete after anterior cruciate ligament surgery. J Athl Training 38:154–157

Valet M, Sprenger T, Boecker H, Willoch F, Rummeny E, Conrad B, Erhard P, Tolle TR (2004) Distraction modulates connectivity of the cingulo-frontal cortex and the midbrain during pain-an fMRI analysis. Pain 109:399–408

Veldhuijzen D, Kenemans J, de Bruin C, Olivier B, Volkerts E (2006) Pain and attention: attentional disruption or distraction? J Pain 7:11–20

Vlaeyen JWS, Linton SJ (2000) Fear-avoidance and its consequences in chronic musculoskeletal pain: a state of the art. Pain 85:317–332

Viane I, Crombez G, Eccleston C, Poppe C, Devulder J, van Houdenhove B, DeCorte A (2003) Acceptance of pain is an independent predictor or mental well-being in patients with chronic pain: Empirical evidence and appraisal. Pain 106:65–72

Villemure C, Bushnell MC (2002) Cognitive modulation of pain: How do attention and emotion influence pain processing? Pain 95:195–199

Waddell G, Main CJ (1984) Assessment of severity in low back disorders. Spine 9:204–208

Weidenfeld SA, O'Leary A, Bandura A, Brown S , Levine S, Raska K (1990) Impact of perceived self-efficacy in coping with stressors on components of the immune system. J Pers Soc Psychol 59:1082–1094

Wiech K, Seymour B, Kalisch R, Enno SK, Koltzenburg M, Driver J, Dolan RJ (2005) Modulation of pain processing in hyperalgesia by cognitive demand. Neuroimage 27:59–69

Wilkinson HA, Davidson KM, Davidson RI (1999) Bilateral Anterior Cingulotomy for Chronic Noncancer Pain. Neurosurgery 45:1129–1136

Williams DA, Thorn BE (1989) An empirical assessment of pain beliefs. Pain 36:351–8

Williams DA, Keefe FJ (1991) Pain beliefs and the use of cognitive-behavioural coping strategies. Pain 46:185–190

Williams DA, Robinson ME, Geisser ME (1994) Pain beliefs: Assessment and utility. Pain 59:72–78

Woolf CJ, Slater MW (2000) Neuronal plasticity: increasing the gain in pain. Science 288:1765–1768

Yeates J (2009) Breeding for pleasure: the value of pleasure and pain in evolution and welfare ethics. Speaker abstracts of the UFAW International Symposium 2009:7 (http://www.ufaw.org.uk/documents/SPEAKERABSTRACTS2009_000.pdf, 24 February 2012)

Zautra AJ, Manne SL (1992) Coping with rheumatoid arthritis: a review of a decade of research. Ann Behav Med 14:31–39

Large animals as biomedical models – a legal perspective

Ralf Müller-Terpitz

1 Subject

Due to a need for better portability of research results to human beings an increasing recourse to "large animal models" for biomedical research has been observed in recent years. In such models, animals like, for instance, rabbits, dogs, cats, pigs, horses and in particular primates are being used for scientific experimentation purposes.

From a legal point of view, this development *inter alia* leads to the question under which conditions scientific experiments with larger animals are compatible with animal welfare law. Possible legal obstacles with regard to such animal experiments include the biological proximity of some large animal species to humans, some species' possibly higher cognitive abilities, and their potentially higher ability to perceive suffering and pain. Furthermore, it is alleged that the public perception and the moral evaluation of animal experimentation in Europe, especially when affecting larger animals, have significantly changed (see **authorisation** authority of Bremen for animal experiments, referred to by the Administrative Court of Bremen 2010).

The following essay will analyze whether these potential obstacles are of legal relevance. For this purpose, the legal framework of animal experimentation in Europe and particularly in Germany will be described briefly (2) before focusing on specific legal issues on the level of the national German (3) and European Union law (4). The essay will end with some general conclusions regarding the subject (5).

2 Legal framework

2.1 European level

At European level, the legal question regarding the subject of animal experimentation is stipulated by the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes of 18 March 1986 (European Treaty Series No. 123), as amended according to the provisions of the Protocol of 22 June 1998 (European Treaty Series No. 170). This Convention was drafted and adopted under the auspices of the Council of Europe (Strasbourg) as an international treaty between the Member States of this international organization. It therefore represents a classic instrument of public international law to harmonize differing national legislation in the respective Member States. Nevertheless, the Convention has only been ratified by 22 of 47 members, including the European Union and the Federal Republic of Germany.

The Convention stipulates minimum standards only (Article 4 of the Convention). Amongst others, its main subjects of regulation are the conduct of scientific experiments with vertebrate animals following the "3R-approach" (replace – reduce – refine), the authorisation procedure for such experiments (Article 6 – 13 of the Convention), the care and accommodation of animals used for scientific purposes, its killing, the control of breeding and supplying, the education and training of scientific staff and statistical information. The Convention applies to any vertebrate animal used or intended for use in any experimental or other scientific procedure where that procedure may cause pain, suffering, distress or lasting harm (Article 1 paragraph 1 sentence 1 of the Convention). The term "procedure" is defined as any experimental or other scientific use of an animal which may cause it pain, suffering, distress or lasting harm, including any course of action intended to, or liable to, result in the birth of an animal in any such conditions. However, the elimination of these conditions by a successful use of anaesthesia, analgesia or other methods does not place the use of an animal outside the scope of this definition (Article 1 paragraph 2 letter c of the Convention). *Inter alia,* such experimental procedures may be performed for the avoidance and prevention of (human or animal) disease, including the production of drugs, substances or products, and the diagnosis or treatment of such disease (Article 2 letter a of the Convention). Where it is planned to subject an animal to a procedure in which it will or may experience *severe and enduring* pain that procedure must be specifically declared

and justified to, or specifically authorised by, the responsible authority (Article 9 paragraph 1 of the Convention). Furthermore, the states being party to the Convention are obliged to take appropriate measures in order to ensure that no such procedure is carried out *unnecessarily*. From the aforementioned provision it can be concluded that the necessity of such experimental procedures depends amongst others on a satisfactory declaration to the responsible authority that the intended scientific experiment is of *sufficient importance* for meeting essential needs of man or animal. However, the size of an animal is without any legal relevance for the authorisation of such experiments.

As the Convention represents a "classic" instrument of international law, its implementation by national law (animal welfare legislation) is indispensable. Only these implementing provisions, which in Germany are laid down in the Animal Welfare Act (see below 2.3), are directly binding for researches planning to perform experiments with (large) animals.

2.2 European Union level

By contrast, the legal situation on the level of EU law is more differentiated. At this level, relevant legal documents can be found both on the so-called level of primary and of secondary EU law:

2.2.1 Primary EU law

With regard to primary EU law, Article 13 of the Treaty on the Functioning of the EU (TFEU) has to be mentioned in the first place. This provision has been inserted in EU treaty law by the Treaty of Lisbon which entered into force on 1 December 2009, replacing an almost identical Protocol on the protection and welfare of animals to the Amsterdam Treaty of 1997. This provision reads as follows: "In formulating and implementing the Union's agriculture, fisheries, transport, internal market, *research* […] development and […] policies, the Union and the Member States shall, since animals are sentient beings, pay full regard to the welfare requirements of animals, while respecting the legislative or administrative provisions and customs of the Member States relating in particular to religious rites, cultural traditions and regional heritage" (emphasis added).

Article 13 TFEU contains a so-called cross-sectional treaty provision, applying to all kind of EU measures (of in particular administrative or legislative nature)

while formulating and implementing the aforementioned EU policies. The question whether Article 13 TFEU is prevailed by an "anthropocentric", "ecocentric" or "pathocentric" understanding, i.e. grants protection to animals only for the benefit of human beings or also because of their *intrinsic* value, can neither be answered with the help of its wording nor by taking account of its genesis. However, from a legal point of view this question is rather of ethical than of juridical importance and can thus be left undecided. In contrast, it is of greater significance that the European Union taking actions in the political areas as stipulated by Article 13 TFEU is not only obliged to pay full regard to the welfare standards of animals as sentient beings but also has to respect the legislative or administrative provisions and customs (like for instance religious rites or regional heritage) of the Member States. Furthermore, the European Union has to consider that scientific research is protected by Article 13 of its **Charter of Fundamental Rights** ("The arts and science research shall be free of constraint."). As consequence, EU primary law stipulates a fundamental right which has to be respected by the EU institutions while formulating and implementing their political actions (see Article 6 paragraph 1 of the Treaty on European Union). In particular, it is a task of the European legislator to achieve a proportional balance between these conflicting aspects – animal welfare on the one side, national provisions and customs as well as the freedom of research on the other side.

2.2.2 Secondary EU law

With regard to animal experimentation, the EU legislator has fulfilled this task by stipulating the Directive 2010/63/EU of the European Parliament and the Council on the protection of animals used for scientific purposes of 22 September 2010 (Official Journal of the European Union 2010 No. L 276/33 of 20 October 2010). This relatively young directive, to be transposed into national law till 10 November 2012 (Article 61), will substitute the Council Directive 86/609/EEC of 24 November 1986 regarding the protection of animals used for experimental and other scientific purposes (Official Journal of the European Communities 1986 No. L 358/1) from 1 January 2013 on (Article 62). The latter directive merely transferred the above sketched guidelines of the European Convention (2.1) into EU law and therefore mainly maintains – even literally – their provisions, only setting out minimum standards that have to be observed when carrying out animal experiments for scientific purposes. In contrast, the new directive sets out a stricter protection of laboratory animals, comparable to current German legal stand-

ards which always exceeded the minimum standards as stipulated on European and EU level. This issue still will have to be touched on below (see *sub* 4).

EU law is considered to be "supranational law", which means that it is directly binding on national authorities and – in any case of conflict – superior to national law. Thus, national law being contrary to EU law may not be applied or has to be interpreted in a manner being compatible with superior supranational law. Although directives address the EU Member States themselves, they may be directly applicable to national individuals – in the present context: a researcher – when their provisions are not properly transferred into national law in time and its content is sufficiently precise, i.e. does not depend on any further legal concretization by the failing Member State.

2.3 National level

Finally, persons carrying out scientific animal experimentation in Germany are directly bound by national legal provisions underneath the constitutional level regarding animal welfare.

With respect to the constitutional level, the legal situation in Germany is comparable to the status in EU primary law as described above: Whereas Article 5 paragraph 3 of the Basic Law grants the freedom of research ("Art and scholarship, research and teaching shall be free."), Art. 20a Basic Law, containing a so-called state objective clause, stipulates: "Mindful also of the responsibility toward future generations, the state shall protect the natural bases of life *and the animals* by legislation and, in accordance with law and justice, by executive and judicial action, all within the framework of the constitutional order" (emphasis added). As animals are only protected "within the framework of the constitutional order", which comprises – as shown – the freedom of research (Article 5 paragraph 3 Basic Law), *and* "in accordance with law", it is – like on the European level – the (national) legislator´s task to proportionally determine the borderline between the fundamental right of research on the one side and the protection of animals on the other.

In Germany, this task of legislative concretization has been fulfilled by the Animal Welfare Act of 18 May 2006 (Federal Law Gazette 2006 I 1206), thereby transposing the minimum standards of the above mentioned European Convention and EU directive 86/609/EEC into national law. According to its section 1 sentence 1, it is the aim of this Act to protect the lives and well-being of animals, based on the

responsibility of man for their fellow creatures. Nevertheless, section 1 sentence 2 as well as the subsequent provisions regarding animal husbandry, killing, animal breeding and, in particular, experiments on animals reveal that this is not an absolute but only a relative protection as – generally speaking – "good (anthropocentric) reasons" may justify pain, suffering or harm inflicted upon animals (regarding the anthropocentric interpretation of this provision see Cornils 2011: 97; Müller-Terpitz 2005: 108).

3 Focus: National law

Thus, the admissibility of animal experiments is stipulated in sections 7 to 9a of the German Animal Welfare Act. According to its section 7 paragraph 1, the expression "experiments on animals" means any operation or treatment for experimental purposes which may cause the animals pain, suffering or harm or any experimental treatment on the animal genotype which may cause the genetically modified animals or their carrier animals pain, suffering or harm. Besides, scientific experiments may only be carried out on animals if they are indispensable (i) for the prevention, diagnosis or treatment of diseases, suffering, bodily defects or other abnormalities or the detection or exertion of influence of physiological conditions or functions in human beings or animals, (ii) for the detection of environmental hazards, (iii) for the testing of substances or products to ensure that they are safe in terms of human or animal health or that they are effective against animal pests, or (iv) for the important topic of basic research (section 7 paragraph 2 Animal Welfare Act). The decision whether experiments on animals are indispensable shall be based in particular on the scientific findings available at the time and on checks whether the same purpose can be achieved by other methods or procedures. In addition, experiments on vertebrates may only be carried out if the pain, suffering or harm is "ethically justifiable" in view of the experiment's purpose (section 7 paragraph 3 sentence 1 Animal Welfare Act). Experiments causing lasting or repeated severe pain or suffering to vertebrates may only be performed if the results are expected to be of "outstanding importance" for the fundamental needs of human beings or animals including the solution of scientific questions (section 7 paragraph 3 sentence 2 Animal Welfare Act). The alleged change of ethical values amongst the people of Europe regarding the admissibility and necessity of animal experimentation, especially if the latter affects larger animals, is not a topic of legal relevance and thus has to remain unconsidered.

Albeit such a harm-benefit-analysis can be performed with regard to a concrete experiment, i.e. on a case-by-case basis only, the provision nevertheless reveals that from a legal perspective the size of an animal *per se* is no relevant category with regard to the admissibility of scientific animal experiments. Therefore, even experiments with non-human primates, causing material distress or harm, may be admissible as long as results for major needs of human beings or animals are to be expected. These results may also concern questions of basic research (see above), e.g., questions regarding the construction and functioning of the human brain. Although non-human primates are from an evolutionary point of view very closely related to human beings and thus possibly dispose of comparable cognitive abilities to perceive suffering and pain, they may in principle be object of an (even lethal) animal experiment as long as there exists no other possibility to use less developed animal species or other scientific methods in order to obtain comparable results (cf. Administrative Court of Bremen 2010 with annotation by Gärditz 2010, Lorz and Metzger 2008: vor § 7 margin no. 7).

4 Outlook: European Union law

The above mentioned new Directive 2010/63/EU on the protection of animals used for scientific purposes (see Sect. 2.2.2) entered into force in November 2010. Nevertheless, the respective Member States still have time to adopt their national laws and regulations to this directive until 10 November 2012 (Article 61 Directive 2010/63/EU). Directive 86/609/EEC will then be repealed with effect from 1 January 2013 (Article 62 Directive 2010/63/EU).

This new directive aims at improving the welfare of animals used in scientific procedures by raising the minimum standards for their protection according to latest scientific developments. That is why the minimum standards of EU law approach the always stricter national standards in Germany now. Therefore, numerous provisions of the directive are already set out under German law and do not ask for further transposition into national law.

Nevertheless, with regard to some very important aspects of animal welfare the new directive exceeds or at least specifies existing national legal standards by concretizing specifications and restrictions with regard to non-human primates (like, e.g., macaques), used for scientific experiments. Albeit, these specifications are comparable to the current legal status or at least administrative practise under the German Animal Welfare Act: Article 8 paragraph 1 of the Directive stipulates

that specimens of non-human primates shall not be used in scientific procedures, with the exception of those procedures meeting the conditions of basic research or aiming at the preservation of the species being subject to animal experimentation, the avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality or their effects in human beings or the development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs and other substances or products with a view to human beings. Furthermore, the experiment has to be scientifically justified to the effect that the purpose of the procedure cannot be achieved by the use of species other than non-human primates. Notwithstanding the above sketched regulation, great apes (like, e.g., orang utan, gorilla and chimpanzee) shall not be used in such procedures. Nevertheless, where a Member State has justifiable grounds for assuming that action is essential for the preservation of the species or in relation to an unexpected outbreak of a life-threatening or debilitating clinical condition in human beings, it may adopt at least *provisional measures* allowing the use of great apes in procedures having the purposes referred to in Article 8 paragraph 2 of the Directive (Article 55 paragraph 2 Directive 2010/63/EU).

In principle, these specifications and restrictions seem to be congruent with the already existing national regulation. At this point again, European law stipulates restrictions with regard to highly developed animals such as non-human primates and great apes without absolutely excluding the possibility of using such animals for scientific purposes (however, critical and detailed with regard to the legislative competence of the European Union and the restrictions of the freedom of research Cornils 2011; Gärditz 2012). In other articles, the directive even explicitly stipulates provisions with regard to large animal models such as cats and dogs (cf. Article 31 and 32 of the directive). Notwithstanding the difficult legal questions regarding the EU directive's validity, one can therefore at least observe as a general rule that the size of the animal *per se* is also under the new directive without any relevance for the admissibility of animal experimentation.

5 Conclusion

In recent years, an approximation of the supranational and national law regarding the regulation of animal welfare in scientific contexts has been observed. In this process, the supranational as well as the national legislator had to find a fair balance between two conflicting principles: the freedom of research on the one side and the aspect of animal protection on the other.

Although the standards of protection under the German Animal Welfare Act have to be considered as relatively high, the size of an animal used for scientific purposes is in principle without relevance. What matters is the question whether the use of a certain animal model – notwithstanding the issue whether this model is based on a "small" or "large" animal – is necessary to achieve a specific scientific aim and can thus not be replaced by another model. Nevertheless, scientists using vertebrates are obliged to weigh up their scientific purposes against the pain and suffering caused to the animals. Only if scientists pursue scientific purposes of high value (such as basic research or testing of drugs for treatments on humans), the causing of severe pain and suffering to experimental animals is admissible from a legal point of view. Again, the size of an animal *per se* is not a relevant weighing factor but the question which aptitude an animal disposes of to perceive pain and suffering. Therefore, the use of non-human primates is admissible only under very restrictive legal conditions. The use of great apes is even generally forbidden. The approach of the strict German animal welfare law has now also been adopted by the law of the European Union which has to be transposed into national law till November 2012. Although justified doubts have been raised in particular with regard to the EU´s legislative competence for such an overall approach to animal experimentation, one can observe that the increase of protection on EU level, especially with regard to primates and great apes, does in principle not contradict our current national regulation.

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References

Administrative Court of Bremen (2010) Decision of 28 May 2010, no. 5 K 1274/09. Deutsches Verwaltungsblatt:1044–1048

Cornils M (201) Reform des europäischen Tierversuchsrechts, Lit Verlag, Münster

Gärditz K F (2010) Annotation to Administrative Court of Bremen, Decision of 28 May 2010, no. 5 K 1274/09. Deutsches Verwaltungsblatt:1048–1052

Gärditz K F (2012) Invasive Tierversuche zwischen Wissenschaftsethik und Wissenschaftsfreiheit. Wissenschaftsrecht Beiheft 21 (2012):97–128

Lorz A, Metzger E (2008) Tierschutzgesetz, Verlag CH Beck, München

Müller-Terpitz R (2005) The "Uniqueness" of Human Beings in Constitutional Law. In: Duncker HR, Prieß K (eds) On the Uniqueness of Humankind. Springer-Verlag, Berlin Heidelberg, pp 107–122

Research involving non-human primates: treatment guidelines and ethical frameworks

Kirsten Brukamp

1 Animal experimentation

Experimental research on non-human primates is a controversial topic in biomedical ethics. Proponents argue that this type of investigation is indispensable, whereas opponents claim that the animals are subjected to inappropriate suffering. Given these disagreements, specific guidelines for the treatment of captive non-human primates for laboratory research have already been developed and should be continuously refined. These recommendations need to be consistent with an ethical framework for animal research that pays tribute to the extensive similarities between non-human primates and humans.

Four fundamental perspectives on animal research are the "anything goes", the "on balance justification", the "moral dilemma", and the "abolitionist" positions (Nuffield Council on Bioethics 2005:244). The first one and the last one advocate a full support or a complete rejection of animal experimentation, respectively. The other two opinions are more prevalent: According to proponents of the "on balance justification", research is acceptable when the advantages outweigh the disadvantages, while harm is reduced. The "moral dilemma" view suggests that a conflict exists between two opposing goods, namely animal welfare *versus* research that is beneficial to humans and animals.

Animals participating in experimental biology research can be grouped according to a number of different categories, be it species, size, and emotional significance for humans, e.g., regarding the difference between purpose-bred and companion animals. These categories influence the judgments about the appropriateness of animal experimentation in society.

In particular, controversies ensue over the participation in experimental research of those animals that are most closely related to humans, i.e. non-human primates. In the following, the term primate will be used as an abbreviation for non-human primate and is therefore disjunctive with the term human. This topic does not necessarily include the research that relies on careful observation of wild animals in their natural environment. The life of these animals is rarely compromised by the observations of skilled scientists. Rather, the focus is on those captive ani-

mals that are taken out of their natural habitat, in part purpose-bred in captivity, and placed into artificial housing as a prerequisite for experimentation.

The paper is divided into seven sections: The next section presents background information on primates in research. The third and fourth sections present official regulations and guidelines for primate research that have already been established as well as recommendations that are compatible with these regulations and guidelines. The fifth section summarizes data on the extensive cognitive, social and moral capabilities of primates. Based on these, the sixth and seventh sections sketch ethical frameworks and minimum requirements for research involving primates.

2 Primates in experimental research

An estimated number of approximately 60,000 primates participate in research in the United States of America per year (American Society of Primatologists 2011). The primates studied in laboratories most frequently are Old World monkeys such as rhesus monkeys *(Macaca mulatta)* and cynomolgus monkeys (crab-eating or long-tailed monkeys; *Macaca fascicularis)* (American Society of Primatologists 2011).

According to a simplified and adapted classification, primates are divided into prosimians, monkeys, lesser apes, great apes, and humans, in the ascending order of their presumed evolutionary development (Institute for Laboratory Animal Research 1998; Weatherall et al. 2006). For example, prosimians include lemurs; monkeys comprise both New World monkeys, such as marmosets, capuchin monkeys, and squirrel monkeys, and Old World monkeys, such as macaque monkeys *(Macaca* species); and lesser apes encompass gibbons and siamangs. The so-called great apes *(Hominidae* family) include the species orangutan *(**Pongo*** genus*)*, gorilla *(Gorilla* genus*)*, chimpanzee *(Pan* **genus; e.g., common chimpanzee, namely** *Pan troglodytes)*, and bonobo *(Pan paniscus)*. Humans *(Homo* genus) are also part of the *Hominidae* family in evolution.

Primates are employed in several specific research areas, which can be assigned to the larger research endeavors of the neurosciences, the infectious diseases, or other fields (Weatherall et al. 2006). The motives for research in neuroscience emcompass both goals in basic and in applied neuroscience: Basic neuroscience examines the overall structure and function of the brain, whereas applied neuroscience focuses on disease processes and functional systems, such as on Parkinson's disease, Alzheimer's disease, stroke, addiction, and vision (Weatherall et al.

2006). Primates have been taken to successfully study numerous diseases (Leader and Stark 1987) whose highly complex human phenotypes cannot be adequately evaluated in other animal models. For example, various infectious diseases have been investigated, for which the research has already resulted in effective vaccines. Currently, such studies are concerned with malaria, tuberculosis, schistosomiasis, hepatitis B and C, and human immunodeficiency virus (HIV), which leads to the acquired immunodeficiency syndrome (AIDS). Interests in other fields of research with primates include reproduction, behavior, xenotransplantation, anatomy, pharmaceutical research and development, and gene therapy (Weatherall et al. 2006).

This overview results in the observation that primates participate in relatively few fields overall. In contrast, the range of studies in rodents such as mice and rats is much more heterogeneous and spans numerous biomedical disciplines and methodologies. This restriction on primate experiments may already be a consequence of a critical stance towards primate research and of reflections on choosing the most appropriate animal model.

3 Regulations and guidelines for animal and primate research

Official guidelines have been developed both for animal research in general and for research involving primates in particular. In Europe, animal protection in science has been regulated by the EU Directive 2010/63/EU (European Parliament 2010), which came into effect in 2010.

The EU Directive 2010/63/EU (European Parliament 2010) repeatedly emphasizes that experiments with primates are only allowed when they are inevitable in the sense that there are no other animal models available that allow meaningful conclusions for humans: primate experiments can only be performed when "there is scientific justification to the effect that the purpose of the procedure cannot be achieved by the use of species other than non-human primates" (European Parliament 2010: Article 8). The EU Directive places strict limitations on experiments with great apes: "[…] great apes shall not be used in procedures, subject to the use of the safeguard clause" (European Parliament 2010: Article 8), which specifies serious and life-threatening conditions. Furthermore, animals are not to be captured from the wild for experiments (European Parliament 2010: Article 9).

Together with dogs and cats, primates are privileged insofar as they receive a special treatment by the creation of an "individualized history file" (European Par-

liament 2010: Article 31). Aside from the technical and biomedical reasons for this, such a document does elevate these animals to the status of individual beings, which is unusual for laboratory animals and for the majority of wild, including vertebrate, animals. Primates thus get formally justified as individual beings with characteristic features of their own in the eyes of their veterinary technicians and caregivers. This regulation, to a certain extent, pays tribute to the emotional significance of selected species for humans.

In addition to the EU Directive (European Parliament 2010), primate welfare in Germany is reinforced by scientific societies and institutions such as the *Gesellschaft für Primatologie* (GfP; Society for Primatology) and the *Deutsches Primatenzentrum* (DPZ; German Primate Center). They collect and distribute information on primate research ethics and regulations and also issue their own statements on governmental directives and moral codes.

In the United Kingdom, the National Centre for the Replacement, Refinement and Reduction of Animals in Research developed the NC3Rs guidelines in support of replacement, refinement, and reduction (National Centre for the Replacement, Refinement and Reduction of Animals in Research 2006). Data regarding primate research was compiled in two major reports, namely the Weatherall and Bateson Reports (Weatherall et al. 2006; Bateson et al. 2011), which culminated in specific recommendations for the care of primates in research. These reports were funded by large and prominent institutions, such as the Medical Research Council, the Biotechnology and Biological Sciences Research Council, and the Wellcome Trust.

In the United States of America, where far-reaching regulations exist, the following organizations, among others, determine how primates are treated in laboratories (American Society of Primatologists 2011): The Public Health Service (PHS) issues the PHS Policy on Humane Care and Use of Laboratory Animals, according to the Health Research Extension Act, and requires an Institutional Animal Care and Use Committee (IACUC). The Institute for Laboratory Animal Research (ILAR) edits the ILAR Guide for the Care and Use of Laboratory Animals, which is published by the National Academy Press (Institute for Laboratory Animal Research 2010). The United States Department of Agriculture's Animal Plant and Health Inspection Service (APHIS) enforces the Animal Welfare Act, and the U.S. Food and Drug Administration (FDA) advices Good Laboratory Practice regulations. The American Association for the Accreditation of Labora-

tory Animal Care (AAALAC) offers voluntary membership and promotes adherence to guidelines.

It is an intriguing endeavor to check policies that govern experiments involving humans for their transferable relevance to animals. The Declaration of Helsinki (World Medical Association 2008) is a prominent international agreement for human experimentation. For animals, however, it is hardly applicable. The aim of the Declaration is the health and protection of humans, and much emphasis is laid on the concept of informed consent, which animals cannot give. Still, the Declaration states: "The welfare of animals used for research must be respected" (World Medical Association 2008:12). One appealing entry says: "At the conclusion of the study, patients entered into the study are entitled [...] to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits." (World Medical Association 2008:33) While this may not always be feasible for all in every animal study, the general standpoint may very well be transferred from humans to animals: Veterinary medicine should use the outcomes from animal experiments to promote the health and well-being of the same, and potentially other, species. On a related note, medications that have passed testing in pharmaceutical studies in humans are also conversely utilized for animals again to improve their health. In this way, humans and animals mutually benefit each other, albeit with an imbalance.

4 Recommendations for animal and primate research

In addition to the official regulations and guidelines, the scientific literature contains a number of recommendations regarding the appropriate and ethical treatment of animals and primates. In the following, some of these recommendations are summarized according to the stage of research where they become relevant:

A. Research planning

1. *Need:* Research proposals should be carefully reviewed regarding the real need to employ primates, as opposed to other animals or no animals at all (Bateson et al. 2011). Studies may be judged according to three criteria (Nuffield Council on Bioethics 2005:248; Bateson et al. 2011): 1. the scientific quality and importance of the research, 2. the probability of medical and public benefit, and 3. the likelihood of animal suffering.

2. *Replacement, refinement, and reduction:* The "3R" goals ought to be pursued, namely replacement, refinement, and reduction (National Centre

for the Replacement, Refinement and Reduction of Animals in Research 2006). For example, primates could benefit from novel non-invasive methods that are employed instead of invasive ones, such as advantages from neuroimaging with functional magnetic resonance imaging (fMRI) in comparison to recordings from implanted electrodes (Weatherall et al. 2006).

B. Research process with data collection from animals

3. *Experienced personnel:* Only experienced personnel are to carry out animal experimentation (Institute for Laboratory Animal Research 2010).

4. *Avoidance of pain:* Pain needs to be avoided, not only pain due to experiments, but also pain due to husbandry (Institute for Laboratory Animal Research 2009).

5. *Improvements in housing conditions:* Since primates possess a rich social life, single housing should be avoided (Weatherall et al. 2006). Cage fittings need to be optimized, e.g. by adherence to minimum cage sizes (Weatherall et al. 2006). Both outside access and visual stimulation are regarded as advantageous for primates (Weatherall et al. 2006).

6. *Respect for animals:* Animals ought to be treated with respect (American Psychological Association 1990).

C. Research analysis and communication of results

7. *Optimization of analysis:* The analysis of studies with primates should be maximized, e.g. by collaborative efforts, excellent infrastructure, high-quality publications, report of negative or inconclusive data, data-sharing, use of repositories for data, and knowledge transfer (Bateson et al. 2011).

8. *Realistic expectations:* The potential benefits of primate studies should be portrayed in a realistic manner. Over-exaggeration regarding advancements for humans should be discouraged (Bateson et al. 2011). This requirement does not only apply to the retrospective interpretation and discussion of data within the scientific community and to the general public, but also to the prospective portrayal of expected benefits during the earlier stages of research planning and grant writing.

5 Cognitive, social, and moral capabilities of primates

Primates are regarded as highly developed animals with cognitive, social, and moral capabilities. They are said to possess a social life, cognitive abilities such as intelligence and memory, and even personality (Research Group Comparative Differential and Personality Psychology 2011).

Neurobiology research heavily relies on experiments in primates (Weatherall et al. 2006; cf. section 2 above) because their cognitive abilities are most closely comparable to the ones found in humans. The similarities between humans and primates include tool use, greater communication skills than in other animals, and the formation of elaborate social relationships. When primates grow up in deprived environments, they develop behavioral patterns that remind of severe social isolation in humans. Primates are also said to possess quality of life with specific needs for well-being and differences in personality (Research Group Comparative Differential and Personality Psychology 2011).

Similarities between humans and apes exist in moral sentiments, social pressure, and judgment and reasoning, i.e., concerning those components of the social life that may be identified as the three levels of morality (De Waal 2006:166–175). Moral sentiments comprise "the capacity for empathy, a tendency for reciprocity, a sense of fairness, and the ability to harmonize relationships" (De Waal 2006:168), and the parallels between humans and primates are most pronounced in these areas. Morality in primates and humans alike may be represented by a Russian doll model (De Waal 2006:37–42): The inner core is the most prominent part that mediates an emotional contagion by an automatic emotional impact in a perception-action mechanism. The outer layers are cognitive empathy, which helps to understand others' emotions, and attribution, which means that others' perspectives can be fully adopted (De Waal 2006:39). While humans manifest a balance of all three factors, primates' moral capabilities encompass the full emotional contagion, but only partial cognitive empathy and even less labile attribution (De Waal 2006:40).

Primates can be ascribed empathy, consolation behavior (De Waal 2006:21–36), reciprocity, fairness, and gratitude (De Waal 2006:42–49). These talents are partially specific to great apes only: "The difference between monkey and ape empathy has been confirmed by systematic studies of a behavior known as 'consolation' […] Consolation is defined as reassurance by an uninvolved bystander to one of the combatants in a preceding aggressive incident. For example, a third party goes over to the loser of a fight and gently puts an arm around his or her shoul-

ders […] Consolation has thus far been demonstrated in great apes only." (De Waal 2006:33–35)

According to the definition of moral subjects and moral agents, i.e., "[m]oral agents are beings that are able to behave in a moral way and are liable to moral criticism for any failure to do so. Moral subjects are beings whose features should be taken into account in the behaviour of moral agents..." (Nuffield Council on Bioethics 2005:39), primates are then not only moral subjects, but also moral agents within their own community, albeit to a lesser degree than humans. The scientific observations and studies presented above demonstrate that research involving primates ends up in a paradox: The vast similarities to humans are the reason why primates are examined in research, and exactly those similarities warrant special treatments that primates deserve in their living environments in the research facilities.

6 Ethical frameworks for primate research

In bioethics, positions and arguments have been classified according to the entity that is the focus of their moral concern or moral value ascription. For a position of physiocentrism in bioethics, the appropriate criterion for consideration is the status as a natural entity or being. Biocentrism takes living beings into account, whereas pathocentrism focuses on living beings with the capacity to experience pain. Anthropocentrism considers humans as the most important moral subjects, moral agents, and moral patients, due to their species membership.

The pathocentric viewpoint has been widely adopted to argue against unjustified cruelty against animals, and accordingly, pain, suffering, and distress are to be avoided (European Parliament 2010: Article 23). Nevertheless, these criteria do not seem sufficient to do justice to the higher cognitive, social, and moral capabilities that some animal species, particularly primates, possess. Also, three criteria have been proposed to assess the moral acceptability of studies involving animals, namely the quality of research, the certainty of benefit, and animal suffering (Nuffield Council on Bioethics 2005:248; Bateson et al. 2011). These criteria do not refer to pain, but rather to suffering, a fact that implies a wider concept of negative influences on animal well-being than a restriction on pain perception only.

The moral status of animals depends in part on the complexity of their cognitive functions. The more complex the mental life of animals is, the more they can be hurt not only by actions that directly activate their pain perception, but also

by actions that interfere with the life normal to them. For example, social animals may be deprived by limiting their contact with others of the same species. This may, in humans, be comparable to the physical and psychological differences between punching the body and taking a favorite toy away. Therefore, every effort should be made for social animals to minimize distress and to take their inherent needs into account in order to enrich their living environments appropriately. Since primates possess advanced skills, attention to these issues is particularly essential, more so than for other animals. Nevertheless, a status distinction between primates and humans continues to appear justified because of the species separation for cognitive, social, and moral capabilities, which is revealed in cultural achievements and communicative abilities.

Animals may be said to possess five features to varying degrees, and these can be used to assess them both as moral subjects, moral agents, and moral patients: sentience, higher cognitive capacities (including communication, tool use, intelligence, and social behavior), the capacity to flourish, sociability, and possession of a life (Nuffield Council on Bioethics 2005:41). The concept of flourishing suggests that primates possess the aptitude to cultivate satisfying lives according to their own needs in their communities, an opportunity that may surpass that of other animals due to their higher cognitive, social, and moral capabilities. Humans should support this type of flourishing for primates in experimental settings and under artificial housing conditions as well.

7 Minimum requirements for experiments involving primates

The distinctive appeal of research on primates results from their close resemblance to humans on the large scale. On the one hand, the functioning of individual cells in humans can certainly be modeled by tissue explants, cell lines, or even bacteria. However, the interplay between different body systems, e.g., of the immune system with various organs in the body, can only be studied in models that are highly similar to humans. Consequently, at least these requirements for research on primates must be met:

1. The results cannot be obtained by other means, in particular not through research on animals with more limited cognitive abilities.

2. Insight into the examined condition or state is crucial for humans, as evidenced by the severity or prevalence of diseases (in the case of somatic diseases) or by identity questions (in the case of cognitive psychology research). At the same

time, humans would be burdened by the experiments out of proportion, i.e. because of the invasiveness or negative sequelae of the procedures.

3. The research carries a high promise of success. For example, prior knowledge or preliminary studies, in other animals, suggest a high probability that the involvement of primates will indeed achieve the intended aims.

4. The research is carried out according to the best practice standards that have widely been agreed upon (cf. sections 3 and 4 above). Pain, suffering, and distress are minimized, and instead, the primates are allowed to live mostly according to their own psychological and social needs.

In conclusion, animal species possess distinctive aptitudes that could be reflected in the ethical evaluation of their varying status as moral subjects, moral agents, and moral patients. The pathocentric position in bioethics accommodates and values this insight only partially because it rather emphasizes the avoidance of pain, suffering, and distress. In contrast, primates possess extraordinary cognitive, social, and moral capabilities, which closely relate them to humans, and therefore, primates may need to be ascribed a higher status as moral subjects in comparison to other animals. The ethical assessment of animal research should consider differential capabilities of animals to a higher degree than in the past.

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References

American Psychological Association (1990) Resolution on the use of animals in research, testing, and education: joint resolution adopted by the AAAS Board and Council, 19 February 1990: endorsed by the APA Board of Directors June 1990 & Council of Representatives August 1990. www.apa.org/about/governance/council/policy/bsa-resolution.aspx (16 September, 2011)

American Society of Primatologists (ASP) (2011) Research questions and answers: commonly asked questions about nonhuman primate research. www.asp.org/research/faq.html (8 September, 2011)

Bateson P et al. (2011) Review of research using non-human primates: report of a panel chaired by Professor Sir Patrick Bateson FRS. www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC008083 (16 September, 2011)

De Waal F (2006) Primates and philosophers: how morality evolved. Princeton University Press, Princeton and Oxford

European Parliament (2010) Directive 2010/63/EU of the European Parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes. eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:En:PDF (16 September, 2011)

Institute for Laboratory Animal Research (ILAR) (1998) The psychological well-being of nonhuman primates. The National Academies Press, Washington, DC

Institute for Laboratory Animal Research (ILAR) (2009) Recognition and alleviation of pain and distress in laboratory animals. The National Academies Press, Washington, DC

Institute for Laboratory Animal Research (ILAR) (2010) Guide for the care and use of laboratory animals: eighth edition. The National Academies Press, Washington, DC

Leader RW, Stark D (1987) The importance of animals in biomedical research. Perspectives in Biology and Medicine 30 (4): 470–485

National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) (2006) NC3Rs guidelines: primate accommodation, care and use. National Centre for the Replacement, Refinement and Reduction of Animals in Research, London. www.nc3rs.org.uk/downloaddoc.asp?id=418 (8 September, 2011)

Nuffield Council on Bioethics (2005) The ethics of research involving animals. Nuffield Council on Bioethics, London. www.nuffieldbioethics.org/animal-research/animal-research-chapter-downloads (11 December, 2011)

Research Group Comparative Differential and Personality Psychology (2011) Personality differences and social relationships in primates: projects of the research group Comparative Differential and Personality Psychology at Freie Universität Berlin, Germany, funded by the Deutsche Forschungsgemeinschaft DFG (German Research Foundation). www.primate-personality.net (8 September, 2011)

Weatherall D et al. (2006) The use of non-human primates in research: a working group report chaired by Sir David Weatherall FRS FMedSci. www.bprc.nl/BPRCE/L4/newsdownloads/The%20use%20of%20non-human%20primates%20in%20research%20-%20The%20Weatherall%20Report.pdf (16 September, 2011)

World Medical Association (WMA) (2008) Declaration of Helsinki: ethical principles for medical research involving human subjects. www.wma.net/en/30publications/10policies/b3/17c.pdf (11 December, 2011)

Relevant Similarity in the Light of Biomedical Experimentation[1]

Lara K. Kutschenko

Abstract

We need good reasons to justify the use and sacrifice of animals in research (if we want to allow animal-based research at all). The primary argument in favour of animal-based research in biomedicine is an epistemological one: animal models are considered necessary to better understand and intervene into human diseases because certain phenomena can only be studied *in vivo*. For example, it has been argued that mammalian models are necessary to better understand the aetiology of Alzheimer's disease (AD) because higher cognitive functions can only be studied in animals that are similar to humans with respect to their brain organisation. This argument attributes a specific epistemic power to the degree of similarity between the given experimental organism and human beings. In ethical discourses, however, the degree of similarity between animals and humans is used as an argument *against* the legitimacy of experimentation with (some) animals.

In this paper I take a closer look at the epistemological part of this purported dilemma: are animal models that are more similar to human beings generally better models than less similar ones? The reference to similarity usually is a reference to the phylogenetic relatedness of animals and humans. Yet, laboratory animals need to be experimentally standardised, modified and assessed in order to become animal models for human diseases. Thus, they do not only gain their value due to their naturalness as animals but also through the applied experimental practices that make them models. I therefore argue that we need to re-assess the epistemic power of similarity in light of experimentation.

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1 The paper draws on work from a research project on the history and philosophy of mouse models in research on Alzheimer's disease. The project is supported by a research grant (MAIFOR) of the University Medical Center Mainz that has been awarded to Lara Huber and me. I draw in this paper on some arguments regarding the epistemic specificity of the establishment of animal models in biomedicine that are further elaborated in an article entitled "Mutant Mice: Experimental organisms as materialised models in biomedicine" that I co-author with Lara Huber (forthcoming in Studies in History and Philosophy of Biological and Biomedical Sciences). Special thanks go to Xenia Paultre and Kristin Hagen for helpful comments on earlier drafts of this paper.

1 Introduction: Similarity – an ethical-epistemological dilemma?

The debate concerning the legitimacy of animal-based research is directed towards two main issues: Firstly, to what extent and on which grounds should it be allowed to use living beings as experimental resources? Secondly, how important is the knowledge that is obtained by animal-based research and could it also be generated by other means? The topic of this book raises a question that is associated with both of these issues: Does size matter? Is the use of large animals as biomedical models associated with specific ethical challenges and/or with a particular epistemic value?

My paper takes an epistemological perspective. Epistemological issues of animal-based modelling address questions such as what we can actually learn through animal-based research and how we can assess this. These are important questions also for non-philosophers, not least because legal and ethical issues inflict the assessment of the realisability and the desirability of research projects. The established laws and ethical guidelines for animal experimentation state that such experimentation should be allowed if and only if it is scientifically indispensable and not replaceable. Therefore, the question how we actually evaluate scientific indispensability of experiments is crucial. With regard to biomedical research, realisability and desirability of experiments are usually discussed in terms of *applicability* of the data that is derived from animal-based research in clinics, and, thus, to human patients. Francione (2007:241) characterises this as the "necessity issue" that "is empirical and asks whether the use of non-humans in experiments is required in order to gather statistically valid information that will contribute in a significant way to improving human health". Yet, this definition itself challenges the merely empirical character of the "necessity issue", that is to say, it is not self-evident against which standards and with which means the necessity of animal experimentation should be empirically evaluated. In particular, it is to be questioned why "statistically valid information" within the laboratory leads to clinically applicable data. Moreover, what it means to "contribute in a significant way to improving human health" is fairly vague – how and with respect to which outcome parameters (for example, prolonged life-span versus quality of life) should this be measured? What should be the threshold for significance?[2]

2 These issues are of crucial importance, not only for animal experimentation but for biomedicine more generally (cf. Kutschenko 2012 for a discussion of the ramifications of these issues regarding the evaluation of clinical trials).

Another problem to empirically answering the necessity issue is the difficulty – and at the same time the requirement – to assess the applicability of animal-derived results *before* the experiments are performed. This heavily challenges ethical evaluations of animal-based research in biomedicine since it is simply impossible to evaluate the indispensability of results before the actual results are obtained. Still, possible scenarios and their outcomes can be described and discussed in order to facilitate critical reflection and ethical evaluation (Düwell 2008:233). Such an evaluation is, however, based on *possible* outcomes rather than the *actual* epistemic power of the animal-based research in question. It follows that we do not only have to address the question against which standards we should evaluate the outcomes of animal-based research, we must also ask which scenarios are possible and which factors will facilitate their realisation.

The question "Does size matter?" seems to suggest that size may be a relevant factor. In this paper, I critically examine in how far the similarity between animals and the target system of biomedical research, namely human beings, could predict the success of extrapolations. Interestingly, the similarity of experimental animals to humans plays an equally important though inverted role in ethical and epistemological debates. In ethical ones, similarity is chiefly discussed with respect to the mental capacities of animals and the consequences these have for their moral status and/or their suffering due to experimentation. Here, a high degree of similarity seems to be unfavourable.[3] With regard to the epistemological question – if results in animal-based research are applicable to humans – a high degree of similarity can, however, very well be favourable, namely if it facilitates such extrapolations. It is this purported tension between epistemological and ethical issues regarding similarity that I aim to revisit. Let me stress that the argument I want to make in this paper is *not* that similarity matters in ethics but that even if it did matter, this would not automatically give rise to an ethical-epistemological dilemma regarding the use of "more similar" animals in biomedical research, because phylogenetic similarity does not necessarily matter epistemically.

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3 This argument is contested from both an ethical as well as an animal welfare perspective (see also other papers in this edited documentation). In ethics, the question to what extent similarity matters largely depends on the ethical theory applied. From an animal welfare perspective, one might argue that it is better to experiment with animals that we can easier relate to because this can help to satisfy the needs of the given animal. Both of these issues are entangled with an epistemological issue that this paper is concerned with: How can we assess similarity in biological and biomedical experimentation at all? With respect to the above mentioned ethical and animal welfare interjections the question arises how to compare suffering or mental capacities of different (human and non-human) animals.

2 Similarity and analogous reasoning in biomedicine

To attribute a particular epistemic power to similarity between animal models and humans means to assume that by performing experimentation on organism O^1 we can learn more about organism O^2 than by using experimental systems, models or simulations that are less similar to O^2 in the relevant aspect. Note that this argument combines the idea of analogous reasoning – experimenting with O^1 so as to learn something about O^2 – with the notion of relevant similarity – experimenting with O^1 because it is similar to O^2 in relevant aspects. For example, mammalian models are attributed a particular epistemic value for the characterisation of cognitive decline in humans. Decline of cognitive functioning is thought to be best studied in animals that resemble humans with respect to their brain organisation. While non-mammalian animals like fruit flies can very well be used for a large-scale screening of drugs that dissolve protein agglomerations, mammalian models, and especially transgenic mice, are regarded to play crucial roles for the characterisiation of the aetiology of neurodegenerative diseases like Alzheimer's disease (AD) (cf., e.g. Woodruf-Pak 2008; Morrissette et al. 2009).[4]

The conjunction of similarity and analogous reasoning seems to be a particularly attractive strategy if little is known about the target system, "in the hopes that the various similarities between them [model and target system, LKK] will include the ones that are relevant, given the sorts of things they [the researchers, LKK] would like to find out about the target system", as Wendy Parker (2009:494) has pointed out. It is compelling to argue that the reference to overall similarity reflects not necessarily a general criterion to evaluate the power of a model but rather a lack of knowledge of more fine-grained criteria. This is certainly an answer that could also be applied to the question "Does size matter?". However, this answer is somewhat unsatisfactory. As a scientist one prefers not to rely on "hopes that the various similarities (…) will include the ones that are relevant" (ibid.) but one wants to have good and preferably verifiable reasons for assuming that a certain similarity is indeed relevant.

At this point, it may be important to introduce some terminological specifications: I consider human beings to be the target system, that is to say the field of application of animal-based modelling in biomedicine.[5] It should be noted that the target

4 Yet, mice are not only used because of the similarity of mouse and human brain organisation. In the third part of this paper, I examine further factors that impact on the selection of experimental organisms.

5 In veterinary medicine, the target system is, thus, the animal patient. In this paper, I am limiting my analysis to human biomedicine but I think that my conceptual analysis could also be fruitfully applied to veterinary science and general biology. This may be particularly interesting to single out the differences between animal-based modelling in sciences with different epistemic aims and fields of application.

system is not the same as the target of modelling, and the model system is not the same as the means of modelling. In my reading, humans are the target system, the specific pathogenic process is the target of modelling, the animal that is experimented on is the means of modelling, and the resulting animal model is the model system or field of experimentation. In biomedicine, means (experimental organisms) and targets of modelling (pathogenic processes) are selected with regard to both clinical relevance and experimental potentiality. Thus, in the process of animal-based modelling, a specific pathogenic process is examined within a given field of experimentation (the animal model). The validity of the results obtained by this endeavour are, however, not only evaluated with respect to their epistemic power regarding explanations and predictions that work for the model system, but also with respect to their epistemic power for the target system (that is, in biomedicine, the human patient).

Parker (2009) points out that it may be easier to extrapolate the results gained within the field of experimentation if the model system and the target system are materially more similar to each other. **This leads us to the question what similarity** actually is and how to assess degrees of similarity.

According to Susan Sterrett (2009) epistemic power was first attributed to similarity within Euclidean geometry. Here, similarity is the proportionality of the lengths of line segments in geometrical shapes and degrees of similarity are the ratios of different lengths of line segments to each other. Later, Newton recycled the notion of similarity for the purpose of describing physical systems and phenomena like velocity or force. Sterrett (2009:801, her italics) argues in this context that "in order to generalize the notion of similarity from geometry to natural science, *both* the notion of ratio *and* the notion of shape must be generalized." Ratio was no longer restricted to lengths of line segments in geometrical figures but could involve other observables of physical systems such as time and mass. I think that we can learn at least two lessons from Sterrett's analysis regarding the expansion of the notion of similarity with respect to our discussion: Firstly, the notion of similarity is context-sensitive[6], that is to say similarity in geometry is not the same as similarity in physics, and thus, it is not self-evident what similarity in the life sciences could be; and secondly, in order to make use of degrees of similarity, it is necessary to transform the observables into measurable units.

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6 The context-sensitivity of similarity was most prominently introduced and discussed by Nelson Goodman (1972) who stated that "[c]ircumstances alter similarities" (ibid.:445). For a critical discussion of his and other philosophical approaches to similarity, especially with regard to their application to cognitive science, see Decock and Douven (2011).

For instance, the application of similarity in the natural sciences necessitated the organisation of time, mass and other observables into a system and their specification as (measurable) dimensions (e.g. seconds and grams as defined within the standardised *Système International d'Unités)*.

The context-sensitivity and dimensionality of similarity need to be regarded against the background of the history of model-based experiments within different sciences. As Thomas Brandstetter (2011) has recently illustrated, engineering sciences had a crucial impact on shifting the focus of scientists from maximising the ontological similarity of model and target system, that is to say, that model and target system *are* similar in terms of identity, to the characterisation of relevant conditions that are necessary to extrapolate results from the model experiment to the target system, that is to say, that model and target system *function* similarly with respect to the examined trait.

When considering the similarity between animal models and human beings in biomedicine, similarity has been chiefly discussed in terms of conserved mechanisms with respect to the phylogenetic relationship between animals and humans (cf, e.g. Burian (2005 [1993]); Weber 2005; but also Shanks and Greek 2009 who use the very argument to make the case against animal experimentation for biomedical uses).[7] Interestingly, the notion of "conserved mechanism" relates to both ontological and functional similarity. The fact that there *are* no significant genetic alterations between a biological mechanism in two organisms, that is to say, the mechanism under study is genetically homologous in model and target system, lends credibility to the assumption that the results obtained in the model experiment regarding the *function* of the mechanism under study can be applied with respect to the target system. If we compare this notion of biological, namely phylogenetic similarity, with the notions of similarity in physics and engineering sciences, the question arises to what extent biological models epistemically differ from physical and mechanical ones: for instance, Richard Burian (2005 [1993]:24, his italics) notes that

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7 The phylogenetic relationship of two organisms is characterised by the generations that have passed since their last common ancestor. This timeframe is seen to correlate with genetic, structural, functional and morphological alterations between the two organisms. Usually, phylogenetic relationships are mapped as pedigrees (for a critical evaluation, see O'Malley 2010). Importantly, not only organisms have been grouped to species and mapped according to their natural history. Also, the natural history of single proteins has been reconstructed: The alterations that come along with evolution are not randomly distributed because some alterations have lethal or at least unfavourable effects and are therefore not passed on to the next generation. Certain processes therefore vary more than others. If they are kept quite stable between two organisms they are referred to as "conserved".

> *biological knowledge is knowledge of large numbers of particular systems that cannot be identically prepared*. As such, it cannot, in principle, be derived from a body of laws plus initial or boundary conditions. Substantive knowledge of evolutionary history, of alternative biological mechanisms, of phylogenies, and so on, is needed to evaluate the power of a given result.

Drawing on this, Marcel Weber (2008:181) argues that "[a]ny extrapolations from model organisms are only reliable to the extent that the mechanisms under study have the same evolutionary origin in the model organisms and in humans".

Extrapolations are, to quote Daniel Steel (2008:3), inferences when "one begins with some knowledge of a causal relationship in one population[8], and endeavors to reliably draw a conclusion concerning that relationship in a distinct population". Steel argues that the degree of similarity (in terms of phylogenetic relatedness) between a population of organism O^1 and a population of O^2 is *not* necessary for obtaining reliable extrapolations if the differences between O^1 and O^2 are known. To this end, he stresses the possibility of comparative process tracing in both populations of organisms to achieve the relevant knowledge that allows for extrapolating results from one organism to another one that is not that similar (Steel 2008:78–100). The crucial point here is that the reference to natural similarities established by evolutionary links can be trumped through elaborate experimental practices, that is to say the reliability of extrapolations need not depend on a close phylogenetic relationship between model and target system. The reason for this lies in the fact that while biology and biomedicine make use of experimental organisms these are not just natural organisms. As Cameron Shelley (2010:297) points out:

> […] the mouse is not the whole of the model. The entire regime to which the mouse is subjected comprises part of the model also. That is [in the case of examining mice in the Porsolt Forced-Swim Test, LKK], the administration of stimulant, the cylinder of water, the starting and stopping conditions of the test, are all part of the model too.

It is this theme that I would like to scrutinise further in this paper.

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8 As Steel rightly points out, experiments in biology and biomedicine are always performed in a group of more or less heterogeneous animals and humans. Importantly, Steel's notion of population does not necessarily correspond to its meaning within evolutionary theory but is primarily pragmatic in character, that is to say, it refers to the result of directed grouping practices of researchers.

In animal models (model systems) for diseases that only occur in humans (target systems), the pathological features (targets of modelling) not only need to be assessed, they have to be introduced into the experimental organism (means of modelling). While the animal itself is not technically constructed but develops biologically, there are many experimental steps that indeed mimic engineering. Also, for the purpose of modelling a human disease the very disease needs to be characterised in terms of assessable parameters in the first place. For instance, it is important to know which features are actual hallmarks of the disease in order to identify good targets of modelling. However, a greater degree of this additional measure of similarity, namely completeness of representation – here in terms of the model system exhibiting all hallmarks of the given disease – may not necessarily be of experimental and epistemic merit. Researchers have stressed the value of so-called incomplete models for complex diseases such as Alzheimer's disease. For instance, in the context of testing new diagnostics, the epistemic value of a model cannot be measured with respect to its mirroring the actual human disease in all its complexity, as Alzheimer researchers stress (Radde et al. 2008:S73):

> Since tau lesions appear also in many other neurodegenerative diseases, the generation of specific compounds which either bind to NFTs [Neurofibrillary tangles, LKK] or Aβ [Amyloid β plaques, LKK] is of great importance for *in vivo* imaging and diagnostic purposes. Cross-β structure and congophilic birefringence are two characteristics of plaques and tangles, thus complicating the design of lesion-specific tracers. The availability of models for only the Aβ lesions and models for the tau lesions is therefore of great value for identifying tracers that target either tauopathy or Aβ-amyloidosis.

Note that Radde et al. talk, like biomedical researchers commonly do, about models and not about animals, although they are referring to mice. As outlined above, I submit that the reference to animal models (model systems) instead of animals (means of modelling) is more than a linguistic difference and has strong implications for the similarity question.

3 Standardising, modifying and assessing animal models

Animal-based modelling in biomedicine refers, in a first approximation, to experimentation with a subset of standardised organisms in order to extrapolate the obtained results or apply the tested methods to humans. Three aspects are of central importance, namely (at least some steps of) experimentation with the *whole organism* (rather than for instance research on animal-derived cell-lines) that takes place in a *laboratory* (thereby excluding so-called field studies) and that aims to draw conclusions that can be *applied to humans*. Biology and biomedicine make use of animals in very different ways. Let me briefly place the animal models that I discuss in this paper in a broader context (see Figure 1):

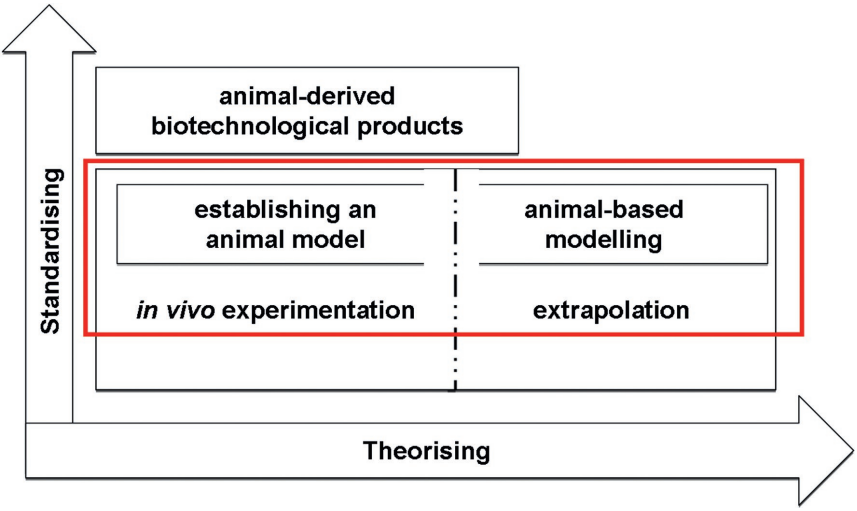

Figure 1: Different modes of using animals as experimental resources. Three modes of uses are delineated along the axes of standardising and theorising: animal-derived biotechnological products, in vivo experimentation and extrapolation. There is no clear cut between experimentation and extrapolation. Note that the degree of standardising and theorising varies not only between but also within the different subsets. The red box marks practical and theoretical issues addressing a particular subset of experimentation and extrapolation aspects regarding "animal models".

Two essential epistemic practices of researchers are addressed: animal-based experimentation and extrapolation (which I introduced in the previous section). Note the dotted line in Figure 1 indicates an interrelation between establishing an animal model and animal-based modelling: Experimental practices that are needed to gen-

erate standardised laboratory animals go hand in hand with the use of these animals as materialised models. In other words, animal models need to be fitted to the question of interest to be models at all – and this fitting is guided by theoretical assumptions, for instance regarding the selection of hallmarks of a disease to be modelled, which is realised through experimental practices. Three sets of experimental practices can be distinguished: (1) standardising the organism, (2) introducing the modifications of interest, and (3) assessing the effects of the introduced modifications:

(1) *Standardising.* Researchers can make use of already standardised, well-characterised laboratory animals as well as standardised procedures for their handling. Standardisation can refer to genetic homogenisation that uses inbred strains to reduce inter-individual variability due to genetic differences. Also, nutrition and other environmental conditions are standardised in order to reduce inter-individual variability due to physiological differences (Gaudillière 2006:30). These standardisation techniques are necessary conditions for the generation of reproducible results. Karin Knorr-Cetina (1999:27) has described the associated laboratory practices as "epistemically advantageous for the pursuit of science" because of the "detachment of natural objects [the organisms, LKK] from their natural environment and their installation in a new phenomenal field [the laboratory, LKK] defined by social agents". This "detachment" is enforced by the introduction of specific modifications into the organisms.

(2) *Modifying.* Researchers use a variety of devices to introduce modifications. Again, we can distinguish between genetic and physiological ones. Genetic modifications comprise methods of either randomly introducing mutations, for example via radiation, or by selectively targeting the genome, for example by generating vectors that carry specific mutations or additional genes and introducing these into the organism. Physiological modifications include differences in the handling of the organism—say a particular diet or an enriched environment. Both, genetic and physiological modifications are of epistemic value if and only if they are recognised as alterations, that is to say, they need to be controlled against a standardised "wild type" and they need to be assessed as potential difference-making factors. As Rachel Ankeny (2007:50 f.) notes, using such controls necessitates

> at least two forms of idealization: the choice of a wild type (which provides concrete laboratory instantiations of the organism, permitting comparison, for instance, to particular mutant strains) and data summarizing descriptive devices (such as wiring or cell lineage diagrams).

The introduction of modifications is therefore designed and performed with reference to established standards and according to the screening and storing techniques that are at hand (see also Leonelli 2008).

(3) *Assessing*. Researchers can choose between diverse assessment tools and protocols. The choice and abundance of these tools have a huge impact on the epistemic power that is attributed to a given animal model. Assessment (including operationalisation, measurement and visualisation) of parameters plays a pivotal role for the evaluation of the validity of animal models. If we want to attribute the status of a model to an experimental animal it is not enough for the animal *to have* the traits of interest; it is also necessary that the researcher is able to *assess them as such*. This is – depending on the experimental set-up – nontrivial. Consider the assessment of memory deficits in mice: It is impossible to assess them in the same ways as is done in humans, namely using neuropsychological tests. If we want to evaluate in how far the mouse model represents memory deficits as they are conceptualised within neuropsychology, we will not only have to evaluate the particular mouse model but also the general validity of the methods used to assess these deficits in mice in relation to the methods used for humans (see also Sullivan 2010). Consequently, empirical questions (Does this mouse model exhibit memory deficits?) have to be evaluated in light of methodological questions (Is this method adequate for measuring memory deficits?), and these, again, need to be related to epistemological questions (How can the information we receive by applying this method help us explain, predict and understand memory deficits in humans?).

Choosing organisms and methods necessitates quite some laboratory experience and skilled judgement with respect to all three sets of experimental practices (standardising, modifying, assessing). These – to a certain degree historically contingent yet epistemically relevant – decisions regarding "the right organism for the job" (Lederman and Burian 1993) have been a focal point in the discussion on the history, philosophy and sociology of organism-based research in biology and (bio-)medicine. The choice of an organism will influence in one way or another "what we know about functions or diseases" (Bynum 1999:400) or how we will conceive of concepts like "disease specificity" (op cit.:401). Moreover, the right organism not only needs to be found but, as we have seen, also has to be modified and founded in the laboratory in order to be chosen at all.[9] As a consequence, model organisms differ so much from non-laboratory organisms that they *are*

9 I refer here to Sophia Efstathiou's (2009) notion of "found science" that relates, comparable to "found art", to the process of finding the ordinary and founding it in the context of science.

something else entirely. In technical terms, they are attributed a specific *ontological status* between natural object (organism) and artefact or instrument (materialised model), whereby the naturalness and craftedness of animal models are matters of degrees. Nicole Karafyllis (2003) has therefore characterised animal models as hybrid "biofacts".[10] The technicity of animal models is inserted into the organism, grows with it and seemingly disappears.

In this context, it is important to differentiate between two technology-related aspects of animal models. The first one is rather unchallenged, namely that animal models are technically modified. Marcel Weber (2005:174) calls this strongly cumulative activity of researchers "preparative experimentation" to denote "the large amount of work that goes into developing the materials that are needed for research – work that is not primarily aimed at discovering new phenomena or testing theories". The second issue refers to the tool-like character of animal models. In this respect, there is more debate regarding the degree to which animal models can be considered tools or instruments in the strict sense of the word (cf. Weber 2005:169–173). For the purpose of this paper it is sufficient to say that animals are experimentally modified to be better models for the targets of interest and that these modifications do not necessarily aim for a greater similarity to the target but to increase the assessability of its representational relation to the target.

4 Conclusion

Experimental practices involved in establishing animal models for human diseases mark the difference between "animal" and "animal model". The take-home-message of this paper is that if we are concerned with the epistemic value of similarity in terms of "Does a greater degree of similarity between the animal model and human target help us to gain better results?", it matters that we are talking of animal *models* and not of animals in more general terms. Similarity in biomedical research cannot be reduced to similarity in nature. In research contexts, any feature in question needs to be controllable and assessable first of all. What is more, if there are methodological possibilities to ensure the representational relation between model and target with respect to the question of interest, these are not *per se* less trustworthy if they do not presuppose natural material similarities. This brings us back to the initial question whether size matters. The answer

10 I would like to thank Mathias Gutmann for pointing me to the fact that Karafyllis' notion should better be called "zoofact" in order to signify the hybrid status between the technical artifact and the living (ζῷον) rather than the lifeform (βίος).

that my epistemological analysis implies is: It depends. There certainly are cases in which the size of the animal model is a relevant factor, for instance if diagnostic instruments or surgical practices are tested that necessitate a certain size of the biological material. These cases represent biomedical issues that have much in common with physical and mechanical problems in engineering sciences. When it comes to the question of whether a given disease can successfully be modelled in an animal, the representational relation is much more difficult to evaluate, especially if the disease does not spontaneously affect non-human animals. In this context research on conserved mechanisms can be of major importance, but as I have pointed out, the reference to phylogenetic similarity alone cannot warrant extrapolations from animal to human. To attribute the status of a model to an experimental animal it is not enough for the animal to have the traits of interest; it is also necessary that the researcher is able to assess them as such. Accordingly, the traits modelled need only be the same as those observed in humans when they are relevant to the very question the researcher aims to answer. Consequently, to argue in favour of biomedical research on primates *just because* they are our closest relatives is a spurious endeavour not only from an ethical but also from an epistemological point of view. The example illustrates why the similarity between human beings and animal models does not automatically give rise to an insurmountable dilemma of epistemic value versus ethical concerns. This may be one more reason for directing the attention of scientists to research into methods that avoid or at least refine animal experimentation and the attention of philosophers to the very concept of "refinement".

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References

Ankeny RA (2007) Wormy Logic: Model Organisms as Case-Base Reasoning. In: Creager ANH, Lunbeck E, Wise MN (eds) Science without Laws: Model Systems, Cases, Exemplary Narratives. Duke University Press, Durham – London, pp 46–58

Brandstetter T (2011) Täuschend ähnlich – Bemerkungen zur Geschichte des Modellexperiments. Berichte für Wissenschaftsgeschichte 34:207–233

Burian RM (2005 [1993]) How the Choice of Experimental Organism Matters. Epistemological Reflections on an Aspect of Biological Practice. In: Burian RM (2005) The Epistemology of Development, Evolution and Genetics. Cambridge University Press, Cambridge, pp 11–28

Bynum WF (1990) "C'est un malade": Animal Models and Concepts of Human Diseases. J Hist Med Allied Sci 45:397–413

Decock L, Douven I (2011) Similarity After Goodman. Rev Philos Psychol 2:61–75

Düwell M (2008) Bioethik. Methoden, Theorien und Bereiche. JB Metzler Stuttgart – Weimar

Efstathiou S (2009) The use of "race" as a variable in biomedical research. PhD thesis in Philosophy (Science Studies), University of California, San Diego, eScholarship http://escholarship.org/uc/item/18s69193 (17 February 2012)

Francione, GL (2007) The Use of Nonhuman Animals in Biomedical Research: Necessity and Justification. J Law Med Ethics 35:241–48

Gaudillière J-P (2006) La médecine et les sciences. XIXe–XXe siècles. Éditions La Découverte, Paris

Goodman N (1972) Seven Strictures on Similarity. In: Problems and Projects. Bobbs-Merill, Indianapolis – New York, pp 437–446

Karafyllis NC (ed) (2003) Biofakte – Versuch über den Menschen zwischen Artefakt und Lebewesen. Mentis, Paderborn

Knorr-Cetina K (1999) Epistemic Cultures. How the Sciences Make Knowledge. Harvard University Press, Cambridge/MA – London

Kutschenko LK (2012) Diagnostic misconceptions. A closer look at clinical research on Alzheimer's disease. J Med Ethics 38:57–59

Lederman M, Burian RM (1993) Introduction: The Right Organism for the Job. J Hist Biol 26:235–37

Leonelli S (2008) Performing Abstraction: Two Ways of Modelling Arabidopsis Thaliana. Biol Philos 23:509–28

Morrissette DA, Parachikova A, Green KN, LaFerla FM (2009) Relevance of Transgenic Mouse Models to Human Alzheimer Disease. J Biol Chem 284:6033–6037

O'Malley MA (ed) (2010) Special Issue: The Tree of Life. Biol Philos 25:441–736

Parker WS (2009) Does Matter Really Matter? Computer Simulations, Experiments, and Materiality. Synthese 169:483–96

Shanks N, Greek CR (2009) Animal Models in Light of Evolution. Brown Walker, Boca Raton

Shelley C (2010) Why test animals to treat humans? On the validity of animal models. Stud Hist Phil Biol Biomed Sci 41:292–299

Steel DP (2008) Across the Boundaries. Extrapolation in Biology and Social Science. Oxford University Press, New York

Sterrett SG (2009) Similarity and Dimensional Analysis. In: Meijers A (ed) Philosophy of Technology and the Engineering Sciences. Elsevier, Amsterdam, pp 799–824

Sullivan JA (2010) Reconsidering 'Spatial Memory' and the Morris Water Maze, Synthese 177:261–83

Weber M (2005) Philosophy of Experimental Biology. Cambridge University Press, Cambridge/MA

Woodruf-Pak DS (2008) Animal models of Alzheimer's disease: therapeutic implications. J Alzheimers Dis 15:507–521

Does size matter – considerations of importance for choice of animal species in a transgenic model for Alzheimer's disease

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Abstract

The pig *(Sus scrofa)* is being used increasingly in neuroscience research. The question is: does size matter for this development? To model a particular human disease, it is essential to choose the animal species most suitable for complete replication of the disease, thereby optimizing the validity of the model. In the present paper, we present considerations of importance for our choice of animal species for a transgenic Alzheimer's disease model, leading to the selection of the pig. The considerations include availability of pig breeds, the life cycle of pigs, pros and cons of the pig brain size and complexity for a higher degree of comparability as well as the possibility of porcine genetic modification. Further, a brief description of Alzheimer's disease in terms of genetic foundation, neuropathological hallmarks and clinical manifestation is provided. We conclude that the size of the pig – including effects derived of body size – does matter for the choice of animal species for the Alzheimer's disease model. In particular, the longer pig longevity combined with the larger size and greater complexity of the pig brain compared to rodents enables a more direct translation to human brain function, and a continuing development in cognitive testing of pigs produces porcine alternatives as models for human neurodegenerative diseases.

1 Introduction

Translational research is the process of transforming basic research outcomes into clinical application (Wehling 2008). Animal models are core components in translational research for evaluating disease etiology, drug efficacy and safety or therapeutic capacities. Traditionally, biomedical research has been conducted on small animal species with the vast majority being mice. Rodents have been considered advantageous due to significant fertility and feasible requirements concerning housing facilities. However, in several cases rodents have not been suitable for replicating human diseases, and non-human primates such as macaques, vervet monkeys and marmosets (Carlsson et al. 2004) have been used widely due to their

genetic and functional similarities with humans. However, non-human primates are expensive and difficult to obtain and maintain, and ethical concerns regarding the use of primates in biomedical research (Goodman and Check 2002) have resulted in closing of colonies of research monkeys in several European countries during the last decade. Besides, complex cognitive abilities need not exclusively be present in non-human primates (Emery and Clayton 2004).

More than 40 years have passed since the domestic pig *(Sus scrofa)* was first proposed as an experimental animal for human medical research (Bustad and McClellan 1965), and the use of the pig in biomedical research is now well established particularly for surgical or physiological purposes (Tumbleson and Schook 1996). Recently, pigs have also increasingly been used within neuroscience research (Lind et al. 2007), and several brain diseases have been fully or partially modeled, such as Parkinson's disease (Mikkelsen et al. 1999), multiple sclerosis (Singer et al. 2000), seizures (Marchi et al. 2007) and acute ischemic stroke (Sakoh et al. 2000; Imai et al. 2006). The question is: does size matter for this development? In the present chapter, we present considerations of importance for our choice of animal species for a model of Alzheimer's disease. It is always essential to choose the most suitable animal species to model a particular human disease in order to replicate the disease most completely and thereby to obtain the highest degree of validity. Our aim is to account for the arguments leading us to select the pig as the model species for a transgenic animal model of Alzheimer's disease.

2 Availability of pig breeds and knowledge about the pig

The pig was domesticated from the wild boar around 9,000 years ago (Porter 1993; Giuffra et al. 2000) and a vast number of different breeds have been developed. Agricultural pig breeds possess the advantage of having been widely investigated in their capacity as a meat producing farm animals. They are readily available and inexpensive. In addition, available information on the prevention and treatment of porcine infections benefits the use of pigs as laboratory animal species (Hansen et al. 1997). Today, the worldwide production of pork approaches 100,000 tons and the current stock exceeds 800 mio animals (Danish Agriculture & Food 2010), primarily based on a number of commercially available breeds genetically selected to optimize meat production. In these breeds, females give birth to large litters, often exceeding 16 piglets (Danish Agriculture and Food 2009). Typically, the piglets are weaned at 4 weeks of age, allowing sows to give birth to more than two litters per year. The young pigs grow fast and reach puberty

at 5–6 months for the agricultural breeds. Commercially produced pigs are slaughtered at a body weight of approximately 100 kg, which is reached before they are 6 months old. Depending on the breeds the body weight of a mature female approximates 200 kg and the mature males are even larger.

3 Body size

Because of an adult body weight in excess of 200 kg, commercial agricultural pigs are most commonly used for short-term research studies at a weight of less than 40 kg. This restriction sets the upper age limit of research pigs at only 15–16 weeks of age, which in nature corresponds to the time of weaning, and accordingly, animals of these ages are (pre-) pubertal. The introduction of minipig breeds (England 1966; Haring et al. 1966; Panepinte 1996) has abated the previous problem with the size of the pig. Mature minipigs weigh 40–80 kg, and reach puberty within 3–4 months of age, and this reduction in size results in substantially less space requirements and easier handling. The Yucatan and Hanford breeds have an adult body weight of 70–90 kg, whereas the Göttingen, Sinclair and Yucatan micropig breeds have an adult weight of 35–55 kg. Several laboratory breeds have been developed in China, where for instance Xiang and Wuzhishan minipigs are commonly used for experimental purposes (Fan et al. 2003). With an adult body weight of 40–80 kg, much *in vivo* experimental instrumentation used for clinical or preclinical research is applicable for pigs (e.g. positron emission tomography (PET), magnetic resonance imaging (MRI), functional magnetic resonance imaging (fMRI), electroencephalography, and electrocardiography.

4 Life cycle

The life cycle of pigs accommodate pros and cons for modeling human neurodegenerative diseases. The pig is easily bred with a short gestation period (115 days) and large litter size compared with primates. The life span of pigs is probably up to 20 years, which is long compared with rodent species. Compared to rodents, the long juvenile period of pigs may present translational advantages for studies of neurodevelopment and plasticity.

5 The brain

Not only the pig as a whole, but also the larger size of the pig *brain* compared to rodents, facilitates the use of human clinical equipment such as conventional brain scanners. Additionally, the larger complexity of the pig brain causes a more straightforward translation to human brain function: The weight of the adult pig brain ranges from 80 to 180 g, depending on body size and breed (Herre 1936), which is significantly larger that the rat brain weighing around 2 g, and comparable to the brain mass of several nonhuman primate species traditionally used for experimental purposes. In the mature Danish Landrace pig, the total number of neocortical neurons is 430 million, and approximately 325 million in the Göttingen minipig (Jelsing et al. 2006). In comparison, the total number of neocortical neurons in rat brain is 21 million (Korbo et al. 1993), versus the 19–23 billion neurons in the human cerebral cortex (Pakkenberg and Gundersen 1997). Comparison of the overall shape of brains of different species can be done using the dimensionless isomorphy factor, which is a coefficient for monitoring brain shape independent of size (Mayhew 1992; Mayhew et al. 1996). The isomorphic value for the pig brain is around 50, whereas the formalin fixated human brain reaches a value of around 65 (Pakkenberg and Gundersen 1997); for the rat brain, the value is less than 10 (Nieuwenhuys et al. 1998). The pig brain cortical surface more closely resembles human gyrencephalic neocortex (Hofman 1985) compared with the lisencephalic rodent brain. The extent and pattern of the cortical convolution pattern of the pig brain has been described in detail in a number of studies (Stephan 1951; Kruska 1970). However, the assessment of comparability to the human brain has been rather discrepant. This is further complicated by the apparent variability between individuals in pigs, as well as variability between breeds and genders (Herre 1936; Stephan 1951; Kruska and Rohrs 1974). Therefore, although the pig brain in several ways enables more straight translation to the human brain compared to rodents, further research is warranted to investigate the pig brain in more detail anatomically in order to facilitate comparison with the human brain.

Other implications for using the pig as a model in neuroscience are the homology of neurotransmitter systems, where studies of the porcine brain have reported comparability to the human brain (reviewed by Lind et al. 2007). Similar receptors (e.g. Rosa-Neto et al. 2004a, b; Jakobsen et al. 2006; Cumming et al. 2007), enzymes (e.g. Brust et al. 2004; Parker et al. 2005; Jensen et al. 2006) and transporters (Brust et al. 2003) as in the human brain have been identified in the pig.

Interestingly though, in the dopamine system it has not been possible to detect typical human transporters in the pig brain (Minuzzi et al. 2006).

Much of the *in vivo* experimental instrumentation used for clinical or preclinical research has already been applied to pigs (e.g. positron emission tomography (PET), magnetic resonance imaging (MRI), functional magnetic resonance imaging (fMRI), electroencephalography, and electrocardiography. Several stereotaxic instruments for precise localisation of the target tissue by use of three-dimensional coordinates have been developed (e.g. Marcilloux et al. 1989; Saito et al. 1998; Bjarkam et al. 2009), and a number of stereotaxic atlases of the pig brain are available (e.g. Yoshikawa 1968; Felix et al. 1999; Watanabe et al. 2001).

Despite incomplete characterisation of the pig brain, it can be said to resemble the primate brain with respect to morphology, histology, brain development, and transmitter systems, and may thus present advantages compared with the use of rodents for modeling human neurodegenerative diseases, in particular in case of disorders that are age dependent. In humans, neurodegenerative disorders may affect cognitive abilities in particular, and it is thus very valuable to investigate these aspects in brain disease models, and several brain disorders have been either fully or partially modeled in the pig (Kornum and Knudsen 2011). Interest in characterisation of pig cognition is increasing, and several promising types of task for use in studies of pig cognition have been identified (Gieling et al. 2011; Kornum and Knudsen 2011). Pigs are able to learn classical and operant conditioning tasks (e.g. Baldwin 1969; Baldwin and Meese 1979) and have proven easy to train. Much has been learned about cognitive abilities in pigs (Ferguson et al. 2009), however experience is limited, and results have not been replicated, resulting in a lack of validated, translational behavioural paradigms that are specially suited to evaluate specific aspects of pig cognition.

6 Genetic modification

Inbred strains of rodents have been available for decades, providing the advantages of fixing the genotype within a strain. Until recently, such pigs have not been available and consequently, the inter-individual variation among pigs has been more pronounced compared to inbred rodent strains. However, with the development of cloning techniques (e.g. Schmidt et al. 2010), the potential of producing genetically identical pigs is growing. The entire pig genome sequence and many other genomic resources will be available in the near future. Importantly, effi-

cient and precise techniques for the genetic modification of pigs are being established, facilitating the generation of tailored disease models (Aigner et al. 2010). The development of transgenic pigs as disease models was launched by Petters et al. (1997) establishing a porcine retinitis pigmentosa model. Later, attempts of modeling Huntington's disease in a transgenic porcine model were made (Uchida et al. 2001), and a porcine model for cystic fibrosis (CF) provides a pivotal tool for research in the pathology and therapy of the disease (Rogers et al. 2008a, b). Furthermore, attempts to develop a pig model for diabetes mellitus have been made (Umeyama et al. 2008). Besides these examples, the use of transgenic pigs as disease models has been limited (Kraft et al. 2005; Hao et al. 2006; Renner et al. 2008, 2010), probably due to the lack of appropriate methods for producing transgenic pigs as compared with the well-characterised genetic modifications of rodents (Matsunari and Nagashima 2009). Much effort is, however, currently being put into pig transgenesis, and the techniques are being refined and becominge more efficient (Schmidt et al. 2010).

7 A porcine model for Alzheimer's disease

The discovery of disease causing mutations in human patients suffering from Alzheimer's disease has led to an increasing interest in the development of transgenic animal models for Alzheimer's disease. Alzheimer's disease is the most frequent cause of neurodegenerative dementia characterised by a severe progressive course of disease with extensive cognitive decline, and by a unique pathology. The disease is named after the German psychiatrist Alois Alzheimer (Kraepelin 1910), who was the first to report a case of intellectual deterioration with the distinctive histological findings of senile plaques and neurofibrillary tangles (Alzheimer 1907). AD is considered a multifactorial disease, but in certain rare families Alzheimer's disease occurs as an autosomal dominant disorder with onset at 40 to 50 years of age, which is earlier than in the population in general, where disease onset is at more than 60 years of age (Eurocode 2009). Mutations causing the disease are located in the amyloid precursor protein gene (APP) on chromosome 21 (Chartier-Harlin et al. 1991; Goate et al. 1991), and in the presenilin 1 and 2 genes on chromosome 14 and 1, respectively (Alzheimer's disease collaborative group 1995; Rogaev et al. 1995; Sherrington et al. 1995). These mutations increase the intracellular production of the neurotoxic amyloid beta protein, Aβ (Scheuner et al 1996), which is normally removed from the brain. In Alzheimer's disease, Aβ is accumulated, and there is general agreement that the Aβ accumulation initiates the pathogenic process

leading to neuritic plaques and formation of intraneuronal neurofibrillary tangles and, eventually, neuron loss (Hardy and Allsop 1991). During the last 20 years, various combinations of the mutated Alzheimer's disease-genes have been used to develop transgenic murine models expressing the disease phenotype. Important knowledge about the disease process has been established by these models, but none have developed all characteristic neuropathological and behavioural deviations (reviewed by Göts et al. 2004; Kobayashi and Chen 2005; Duyckaerts et al. 2008). Further, the phenotype is often unpredictable and diverges between different background strains. Transgenic mice expressing mutant human APP develop many neuropathological features of Alzheimer's disease, including amyloid plaques and inflammatory changes (Games et al. 1995; Hsiao et al 1996; Benzing et al. 1999), but neurofibrillary tangles and widespread neuronal loss have not been observed in these models (Takeuchi et al. 2000). Co-expression of mutant human tau genes may cause these models to develop neurofibrillary tangles (Oddo et al. 2003). The relevance of this tau pathology with respect to Alzheimer's disease is, however, unclear, since the tau mutation *per se* causes neurofibrillary tangles in transgenic mice, whereas in humans, tau mutations are associated with a frontotemporal dementia, which is clinically and neuropathologically distinct from Alzheimer's disease (Hogg et al. 2003). A more equivalent model for Alzheimer's disease may be created in an animal evolutionarily closer to humans, and the pig is considered an attractive non-primate candidate (Chen et al. 2007).

It is not known whether the pig develops Alzheimer's disease spontaneously. In Denmark, a pig's age does rarely exceed four to five years, and there has been no documentation of demented pigs. In a series of 12 elderly sows (age 10–12 years), no neuropathological changes in terms of neurofibrillary tangles or amyloid plaques were found, but immunohistochemical staining has shown the presence of Aβ1-42 in the neuronal cytoplasm (IE Holm: personal communication), which has been shown to precede the development of neuritic plaques in transgenic mice (Oddo et al. 2003). A study by Smith et al. (1999) has shown accumulation of Aβ in pig brains after diffuse brain trauma. Likewise, accumulation of Aβ in axons as a result of head trauma has been shown (Chen et al. 2004). Combined with the similarity of the porcine and human hippocampus (Holm 1995), several factors thus suggest that the pig may prove suitable as an animal model for Alzheimer's disease.

8 Diagnosing Alzheimer's disease

Alzheimer's disease produces a multidimensional behavioural syndrome with dementia being a hallmark symptom. Loss of memory is the first and most severe symptom (Santacruz and Swagerty 2001). Alzheimer's disease is characterised by a process that may be categorised according to clinical stages. These stages proceed in a continuum with normal aging, but are qualitatively different (Petrella et al 2003). In patients, a practical approach to the diagnosis of dementia begins with the clinical recognition of a progressive decline in memory, a decrease in the patient's ability to perform activities of daily living, psychiatric problems, personality changes and problem behaviours. Physical examination and laboratory tests are recommended to rule out specific reversible causes of dementia, for instance hearing or vision deficits, hypothyroidism, vitamin B12 deficiency or depression (Santacruz and Swagerty 2001). Clinical criteria used for diagnosing Alzheimer's disease have been formulated in the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA) criteria (McKhann et al. 1984) for possible or probable Alzheimer's disease and in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria (American Psychiatric Association 2000) for dementia of the Alzheimer type. According to DSM-IV, clinical examination should include assessment of cognitive domains, including speech (aphasia), motor memory (apraxia), sensory recognition (agnosia) and complex behaviour sequencing (executive functioning). Although the Mini-Mental State Examination (MMSE) (Folstein et al. 1975) is not diagnostic of dementia and Alzheimer's disease, it is useful for assessing cognitive function and documenting subsequent decline. The MMSE can detect cognitive impairment by evaluating orientation, attention, recall, language and ability to follow commands. The diagnosis is dependent on the presence of both characteristic clinical symptoms and specific neuropathological changes, so the diagnosis cannot be decisively made while the patient is still alive. The gross appearance of the brain of Alzheimer's disease patients includes coarse atrophy with expanded sulci and ventricles, primarily affecting the parietal and temporal lobes. Microscopic changes include lesions in terms of the presence of amyloid (senile) plaques and neurofibrillary tangles in these areas of the brain (Jellinger and Bancher 1998), which are the neuropathological hallmarks of the disease (Ball et al. 1997).

It may be argued that animal models in Alzheimer's disease research do not provide a high degree of face validity since the core-symptoms described in DMS-IV (aphasia, apraxia, agnosia, and executive functioning) (American Psychiatric Association

2000) cannot readily be produced in an animal model. However, being a disease of sound behavioural rationale in humans, an analogy to a porcine model would reasonably include behavioural assessment of disease progress. Embracing hallmark symptoms of Alzheimer's disease patients, an approximation of symptoms can be produced in animals.

9 Conclusion

From the present review, it appears that the body and brain size is a relevant factor in the choice of species for an animal model of Alzheimer's disease and has been an important factor for the choise of the pig rather than rodents. Interconnected to the relatively large body size is longevity and more complex brain, and in conjunction these three factors suggest that pigs enable a more direct translation to human brain function than rodents. Concurrently, the cognitive abilities of the pig are well-developed and are being investigated, and a continuing development in cognitive testing of pigs is in progress, which helps in the production of relevant and valid porcine alternatives as models for human neurodegenerative diseases.

We conclude that in the search for a suitable animal model for Alzheimer's disease, the pig offers several advantages compared to traditional rodent models. Some of these are due to the size of this animal. Hence, size does matter when choosing animal species for human disease models within neuroscience. Size is, however, not the sole factor leading us to favor the pig as an animal model for Alzheimer's disease. The cognitive abilities of the pig in conjunction with the availability of transgenic methods, as well as the vast amount of knowledge already accumulated about the pig, are other important factors for choosing it. The potential of this species as a model for neurodegenerative diseases such as Alzheimer's disease is great, which – combined with the need for an alternative to non-human primates – is why we advocate using this species in translational research within the field of neuroscience.

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References

Aigner B, Renner S, Kessler B, Klymiuk N, Kurome M, Wünsch A, Wolf E (2010) Transgenic pigs as models for translational biomedical research. J Mol Med (Berl) 88:653–664

Alzheimer A (1907) Über eine eigenartige Erkrankung der Hirnrinde. Allgemeine Zeitschrift für Psychiatrie und Psychisch-Gerichtliche Medizin 64:146–148; English translation in: Arch Neurol (1967) 21:109–110

Alzheimer's Disease Collaborative Group (1995) The structure of the presenilin 1 (S182) gene and identification of six novel mutations in early onset AD families. Nat Genet 11: 219222

American Psychiatric Association (2000) Diagnostic and statistical manual of mental disorders. Fourth edition, Washington, DC

Baldwin BA (1969) The study of behaviour in pigs. Br Vet J 125:281–288

Baldwin BA, Meese GB (1979) Social behaviour in pigs studies by means of operant conditioning. Anim Behav 25: 497–507

Ball M, Braak H, Coleman P, Dickson D, Duyckaerts C, Gambetti P, Hansen K, Hyman B, Jellinger K, Markesbery W, Perl D, Powers J, Price J, Trojanowski JQ, Wisniewski H, Phelps C, Khachaturian Z (1997) Consensus recommendations for the post-mortem diagnosis of Alzheimer's disease. Neurobiol Aging 18:S1–S2

Benzing WC, Wujek JR, Ward EK, Shaffer D, Ashe KH, Younkin SG, Brunden KR (1999) Evidence for glialmediated inflammation in aged APPsw transgenic mice. Neurobiol Aging 20:581–589

Bjarkam CR, Cancian G, Glud AN, Ettrup KS, Jørgensen RL, Sørensen JC (2009) MRI-guided stereotaxic targeting in pigs based on a stereotaxic localizer box fitted with an isocentric frame and use of SurgiPlan computer-planning software. J Neurosci Meth 183:119–126

Brust P, Zessin J, Kuwabara H, Pawelke B, Kretzschmar M, Hinz R, Bergman J, Eskola O, Solin O, Steinbach J, Johannsen B (2003) Positron emission tomography imaging of the serotonin transporter in the pig brain using [11C](+)-McN5652 and S-([18F]fluoromethyl)-(+)-McN5652. Synapse 47:143–151

Brust P, Vorwieger G, Walter B, Fuchtner F, Stark H, Kuwabara H, Herzau M, Opfermann T, Steinbach J, Ganapathy V, Bauer R (2004) The influx of neutral amino acids into the porcine brain during development: a positron emission tomography study. Brain Res Dev Brain Res 152:241–253

Bustad LK, McClellan RO (1965) Use of pigs in biomedical research. Nature 208:531–535

Carlsson H-E, Schapiro SJ, Farah I, Hau J (2004) Use of Primates in Research: A Global Overview. Am J Primatol 63:225–237

Chartier-Harlin MC, Crawford F, Houlden H, Warren A, Hughes D, Fidani L, Goate A, Rossor M, Roques P, Hardy J, Mullan M (1991) Early-onset Alzheimer's disease caused by mutations at codon 717 of the β-amyloid precursor protein gene. Nature 353:844–846

Chen X-H, Siman R, Iwata A, Meaney DH, Trojanowski JQ, Smith DH (2004) Long-term accumulation of amyloid-b, b-secretase, presenilin-1, and caspase-3 in damaged axons following brain trauma. Am J Pathol 165:357–371

Chen K, Baxter T, Muir WM, Groenen MA, Schook LB (2007) Genetic resources, genome mapping and evolutionary genomics of the pig (Sus scrofa). Int J Biol Sci 3:153–165

Cumming P, Møller M, Benda K, Minuzzi L, Jakobsen S, Jensen SB, Pakkenberg B, Stark AK, Gramsbergen JB, Andreasen MF, Olsen AK (2007) A PET study of effects of chronic 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") on serotonin markers in Göttingen minipig brain. Synapse 61:478–487

Danish Agriculture & Food 2010: http://www.lf.dk/Tal_og_Analyser/Aarstatistikker/Statistik_svin/Statistik_svin_2010.aspx (28 February 2012)

Danish Agriculture and Food 2009: http://vsp.lf.dk/Publikationer/~/media/Files/PDF%20-%20Aarsberetning%20VSP/VSP_aarsberetning_dk_2009.ashx (28 February 2012)

Duyckaerts C, Potier M-C, Delatour B (2008) Alzheimer disease models and human neuropathology: similarities and differences. Acta Neuropathol 115:5–38

Emery NJ, Clayton NS (2004) The mentality of crows: convergent evolution of intelligence in corvids and apes. Science 306:1903–1907

England DC (1966) Early history and objectives of miniature swine development. In: Bustad LK, McClellan RO (ed) Swine in biomedical research. Pacific Northwest Laboratory, Battelle Memorial Institute, Richland, WA, pp 765–767

Eurocode (2009) European Collaboration on Dementia: WP7-Prevalence rates. Luxembourg, Alzheimer, Europe

Fan B, Yang SL, Liu B, Yu M, Zhao SH, Li K (2003) Characterization of the genetic diversity on natural populations of Chinese miniature pig breeds. Anim Genet 34:465–466

Felix B, Leger ME, Albe-Fessard D, Marcilloux JC, Rampin O, Laplace JP (1999) Stereotaxic atlas of the pig brain. Brain Res Bull 49: 1–137

Ferguson SA, Gopee NV, Paule MG, Howard PC (2009) Female mini-pig performance of temporal response differentiation, incremental repeated acquisition, and progressive ratio operant tasks. Behav Process 80:28–34

Folstein M, Folstein S, McHugh P (1975) Mini-mental state. A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 12:189–198

Games D, Adams D, Alessandrini R, Barbour R, Borthelette P, Blackwell C, Carr T, Clemens J, Donaldson T, Gillespie F, Guido T, Hagopian S, Johnson-Wood K, Khan K, Lee M, Leibowitz P, Lieberburg I, Little S, Masliah E, McConlogue L, Montoya-Zavala M, Mucke L, Paganini L, Penniman E, Power M, Schenk D, Seubert P, Snyder B, Soriano F, Tan H, Vitale J, Wadsworth S, Wolozin B, Zhao J (1995) Alzheimer-type neuropathology in transgenic mice overexpressing V717F -amyloid precursor protein. Nature 373:523–527

Gieling ET, Nordquist RE, van der Staay FJ (2011) Assessing learning and memory in pigs. Anim Cogn 14:151–173

Giuffra E, Kijas JMH, Amarger V, Carlborg Ö, Jeon J-T, Andersson L (2000) The origin of the domestic pig: independent domestication and subsequent introgression. Genetics 154:1785–1791

Goate A, Chartier-Harlin MC, Mullan M, Brown J, Crawford F, Fidani L, GiuVra L, Haynes A, Irving N, James L, Mant R, Newton P, Rooke K, Roques P, Talbot C, Pericak-Vance M, Roses A, Williamson R, Rossor M, Owen M, Hardy J (1991) Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature 349:704–706

Goodman S, Check E (2002) The great primate debate. Nature 417:684–687

Göts J, Streffer JR, David D, Schild A, Hoerndli F, Pennanen L, Kurosinski P, Chen F (2004) Transgenic animal models of Alzheimer's disease and related disorders: histopathology, behavior and therapy. Mol Psychiatr 9:664–683

Hansen AK, Farlov H, Bollen P (1997) Microbiological monitoring of laboratory pigs. Lab Animal 31:193–200

Hao YH, Yong HY, Murphy CN, Wax D, Samuel M, Rieke A, Lai L, Liu Z, Durtschi DC, Welbern VR, Price EM, McAllister RM, Turk JR, Laughlin MH, Prather RS, Rucker EB (2006) Production of endothelial nitric oxide synthase (eNOS) over-expressing piglets. Transgenic Res 15:739–750

Hardy J, Allsop D (1991) Amyloid deposition as the central event in the aetiology of Alzheimer's disease. Trends Pharmacol Sci 12:383–388

Haring F, Gruhn R, Smidt D, Scheven B (1966) Miniature swine development for laboratory purposes. In: Bustad LK, McClellan RO (ed) Swine in biomedical research. Pacific Northwest Laboratory, Battelle Memorial Institute, Richland Washington, pp 769–774

Herre W (1936) Untersuchungen an Hirnen von Wild- und Hauschweinen. Verhandlungen der Deutschen Zoologischen Gesellschaft: 201–211

Hofman MA (1985) Size and shape of the cerebral cortex in mammals. I. The cortical surface. Brain Behav Evol 27:28–40

Hogg M, Grujic ZM, Baker M, Demirci S, Guillozet AL, Sweet AP, Herzog LL, Weintraub S, Mesulam MM, LaPointe NE, Gamblin TC, Berry RW, Binder LI, de Silva R, Lees A, Espinoza M, Davies P, Grover A, Sahara N, Ishizawa T, Dickson D,Yen S-H, Hutton M, Bigio EH (2003) The L266V tau mutation is associated with frontotemporal dementia and Pick-like 3R and 4R tauopathy. Acta Neuropathol 106:323–336

Holm IE (1995) The hippocampal region of the domestic pig. Doctoral thesis, Aarhus University Press, Århus, DK

Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G (1996) Correlative memory deficits, Ab elevation, and amyloid plaques in transgenic mice. Science 274:99–102

Imai H, Konno K, Nakamura M, Shimizu T, Kubota C, Seki K, Honda F, Tomizawa S, Tanaka Y, Hata H, Saito N (2006) A new model of focal cerebral ischemia in the miniature pig. Neurosurgery 104:123–132

Jakobsen S, Pedersen K, Smith DF, Jensen SB, Munk OL, Cumming P (2006) Detection of alpha(2)-adrenergic receptors in brain of living pig with C-11-yohimbine. J Nucl Med 47:2008–2015

Jellinger KA, Bancher C (1998) Neuropathology of Alzheimer's disease; a critical update. J Neural Transm (Suppl) 54:77–95

Jelsing J, Nielsen R, Olsen AK, Grand N, Hemmingsen R, Pakkenberg B (2006) The postnatal development of neocortical neurons and glial cells in the Göttingen minipig and the domestic pig brain. J Exp Biol 209:1454–1462

Jensen SB, Olsen AK, Pedersen K, Cumming P (2006) Effect of monoamine oxidase inhibition on amphetamine-evoked changes in dopamine receptor availability in the living pig: a dual tracer PET study with [(11)C]harmine and [(11)C]raclopride. Synapse 59:427–434

Kobayashi DT, Chen KS (2005) Nehavioral phenotypes of amolyid-based genetically modified mouse models of Alzheimer's disease. Genes Brain Behav. 4:173–196

Korbo L, Andersen BB, Ladefoged O, Moller A (1993) Total numbers of various cell types in rat cerebellar cortex estimated using an unbiased stereological method. Brain Res 609:262–268

Kornum BR, Knudsen GM (2011) Cognitive testing of pigs (Sus scrofa) in translational biobehavioural research. Neurosci Biobehav R 35:437–451

Kraepelin E (1910) In: Psychiatrie: Ein Lehrbuch für Studierende und Ärzte. Barth, Leipzig, pp 593–632

Kraft TW, Allen D, Petters RM, Hao Y, Peng YW, Wong F (2005) Altered light responses of single rod photoreceptors in transgenic pigs expressing P347L or P347S rhodopsin. Mol Vis 11:1246–1256

Kruska D (1970) Untersuchungen an Gehirnen von Wild- und Hausschweinen. Z Anat Entwicklungs 131:291–324

Kruska D, Rohrs M (1974) Comparative-quantitative investigations on brains of feral pigs from the Galapagos Islands and of European domestic pigs. Z Anat Entwicklungs 144:61–73

Lind NM, Moustgaard A, Jelsing J, Vajta G, Cumming P, Hansen AK (2007) The use of pigs in neuroscience: modeling brain disorders. Neurosci Biobehav Rev 31:728–751

Marchi N, Angelov L, Masaryk T, Fazio V, Granata T, Hernandez N, Hallene K, Diglaw T, Franic L, Najm I, Janigro D (2007) Seizure-promoting effect of blood-barriere disruption. Epilepsia 48:732–742

Marcilloux JC, Rampin O, Felix MB, Laplace JP, Albe-Fessard D (1989) A stereotaxic apparatus for the study of the central nervous structures in the pig. Brain Res Bull 22:591–597

Matsunari H, Nagashima H (2009) Application of genetically modified and cloned pigs in translational research. J Reprod Dev 55:225–230

Mayhew TM (1992) A review of recent advances in stereology for quantifying neural structure. J Neurocytol 21:313–328

Mayhew T, Mwamengele G, Dantzer V, Williams S (1996) The gyrification of mammalian cerebral cortex: quantitative evidence of an isomorphic surface expansion during phylogenetic and ontogenetic development. J Anat 188:53–58

McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984) Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRA work group under the auspices of Department of Health and Human Services task force on Alzheimer's disease. Neurology 34:939–944

Mikkelsen M, Moller A, Jensen LH, Pedersen A, Harajehi JB, Pakkenberg H (1999) MPTP-induced Parkinsonism in minipigs: a behavioral, biochemical, and histological study. Neurotoxicol Teratol 21:169–175

Minuzzi L, Olsen AK, Bender D, Arnfred S, Grant R, Danielsen EH, Cumming P (2006) Quantitative autoradiography of ligands for dopamine receptors and transporters in brain of Gottingen minipig: comparison with results in vivo. Synapse 59:211–219

Nieuwenhuys R, ten Donkelaar HJ, Nicholson C (1998) The Central Nervous System of Vertebrates. Springer, Berlin

Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kayed R, Metherate R, Mattson MP, Akbari Y, LaFerla FM (2003) Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. Neuron 39:409–421

Pakkenberg B, Gundersen HJ (1997) Neocortical neuron number in humans: effect of sex and age. J Comp Neurol 384:312–320

Panepinte LM (1996) Miniature swine breeds used worldwide in research. In: Tumbleson ME, Schook LB (ed) Advances in swine in biomedical research, Vol. 2. Plenum Press, New York, pp 681–691

Parker CA, Matthews JC, Gunn RN, Martarello L, Cunningham VJ, Dommett D, Knibb ST, Bender D, Jakobsen S, Brown J, Gee AD (2005): Behaviour of [C-11]R()- and [C-11]S(+)-rolipram in vitro and in vivo, and their use as PET radiotracers for the quantificative assay of PDE4. Synapse 55:270–279

Petrella JR, Coleman RE, Doraiswamy PM (2003) Neuroimaging and early diagnosis of Alzheimer's disease: A look to the future. Radiology 226:315–336

Petters RM, Alexander CA, Wells KD, Collins EB, Sommer JR, Blanton MR, Rojas G, Hao Y, Flowers WL, Banin E, Cideciyan AV, Jacobson SG, Wong F (1997) Genetically engineered large animal model for studying cone photoreceptor survival and degeneration in retinitis pigmentosa. Nat Biotechnol 15:965–970

Porter V (1993) Pigs: A handbook to the breeds of the world. Bodmin, Cornwall, Helm

Renner S, Kessbler B, Herbach N, von Waldthausen DC, Wanke R, Hofmann A, Pfeifer A, Wolf E (2008) Impaired incretin effect in transgenic pigs expressing a dominant negative receptor for glucose-dependent insulinotropic polypeptide in the pancreatic islets. Reprod Fertil Dev 20:82

Renner S, Fehlings C, Herbach N, Hofmann A, von Waldthausen DC, Kessler B, Ulrichs K, Chodnevskaja I, Moskalenko V, Amselgruber W, Göke B, Pfeifer A, Wanke R, Wolf E (2010) Glucose intolerance and reduced proliferation of pancreatic beta-cells in transgenic pigs with impaired glucose-dependent insulinotropic polypeptide function. Diabetes 59:1228–1238

Rogaev EI, Sherrington R, Rogaeva EA, Levesque G, Ikeda M, Lyang Y, Chi H, Lin C, Holman K, Tsuda T, Mar L, Sorbi S, Nacmias B, Piacentini S, Amaducci L, Chumakov I, Cohen D, Lannfelt L, Fraser PE, Rommens JM, St George-Hyslop P (1995) Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's type 3 gene. Nature 376:775–778

Rogers CS, Abraham WM, Brogden KA, Engelhardt JF, Fisher JT, McCray PBJ, McLennan G, Meyerholz DK, Namati E, Ostedgaard LS, Prather RS, Sabater JR, Stoltz DA, Zabner J, Welsh MJ (2008b) The porcine lung as a potential model for cystic fibrosis. Am J Physiol Lung Cell Mol Physiol 295:L240–L263

Rogers CS, Hao Y, Rokhlina T, Samuel M, Stolz DA, Li Y, Petroff E, Vermeer DW, Kabel AC, Yan Z, Spate L, Wax D, Murphy CN, Rieke A; Whitworth K, Linville ML, Korte SW, Engelhardt JF, Welsh MJ, Prather RS (2008a) Production of CFTR-null and CFTR-DeltaF508 heterozygous pigs by adeno-associated virus-mediated gene targeting and somatic cell nuclear transfer. **J Clin Invest 118:1571–1577**

Rosa-Neto P, Doudet DJ, Cumming P (2004a) Gradients of dopamine D1- and D2/3-binding sites in the basal ganglia of pig and monkey measured by PET. Neuroimage 22:1076–1083

Rosa-Neto P, Gjedde A, Olsen AK, Jensen SB, Munk OL, Watanabe H, Cumming P (2004b) MDMA-evoked changes in [11C]raclopride and [11C]NMSP binding in living pig brain. Synapse 53:222**–233**

Saito T, Bjarkam CR, Nakamura M, Nemoto T (1998) Determination of stereotaxic coordinates for the hippocampus in the domestic pig. J Neurosci Meth 80:29–36

Sakoh M, Rohl L, Gyldensted C, Gjedde A, Ostergaard L (2000) Cerebral blood flow and blood volume measured by magnetic resonance imaging bolus tracking after acute stoke in pigs: comparison with [(15)O]H(2)O positron emission tomography. Stroke 31:1958–1964

Santacruz K, Swagerty D (2001) Early Diagnosis of Dementia. Am Fam Physician 63:703–713

Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N, Bird TD, Haardy J, Hutton M, Kukull W, Larson E, Levy-Lehad E, Viitanen M, Peskind E, Poorkaj P, Schellen-

berg G, Tanzi R, Wasco W, Lannfelt L, Selkoe D, Younkin S (1996) Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nat Med 2:864–870

Schmidt M, Kragh PM, Li J, Du Y, Lin L, Liu Y, Bøgh IB, Winther KD, Vajta G, Callesen H (2010) Pregnancies and piglets from large white sow recipients after two transfer methods of cloned and transgenic embryos of different pig breeds. Theriogenology 74:1233–1240

Sherrington S, Rogaev EI, Liang Y, Rogaeva EA, Levesque G, Ikeda M, Chi H, Lin C, Li G, Holman K, Tsuda T, Mar L, Foncin JF, Bruni AC, Montesi MP, Sorbi S, Rainero I, Pinessi L, Nee L, Chumakov I, Pollen D, Brookes A, Sanseau P, Polinsky RJ, Wasco W, Da Silva HAR, Haines JL, Pericak-Vance MA, Tanzi RE, Roses AD, Fraser PE, Rommens JM, St George- Hyslop PH (1995) Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. Nature 375:754–760

Singer BA, Tresser NJ, Frank JA, McFarland HF, Biddison WE (2000) Introduction of experimental allergic encephalomyelitis in the NIH minipig. J Neuroimmunol 105:7–19

Smith DH, Chen XH, Nonaka M, Trojanowski JQ, Lee VM, Saatman KE, Leoni MJ, Xu BN, Wolf JA, Meaney DF (1999) Accumulation of amyloid beta and tau and the formation of neurofilament inclusions following diffuse brain injury in the pig. J Neuropathol Exp Neurol 58:982–992

Stephan H (1951) Vergleichende untersuchungen über den Feinbau des Hirnes von Wild- und Haustieren (nach Studien am Schwein und Schaf). **Zool Jahrb Allg Zool 71:487–586**

Takeuchi A, Irizarry MC, Duff K, Saido TC, Hsiao Ashe K, Hasegawa M, Mann DM, Hyman BT, Iwatsubo T (2000) Age-related amyloid beta deposition in transgenic mice overexpressing both Alzheimer mutant presenilin 1 and amyloid beta precursor protein Swedish mutant is not associated with global neuronal loss. Am J Pathol 157:331–339

Tumbleson ME, Schook LB (eds) (1996) Advances in swine in biomedical research. Plenum Press, New York, NY

Uchida M, Shimatsu Y, Onoe K, Matsuyama N, Niki R, Ikeda JE, Imai H (2001) Production of transgenic miniature pigs by pronuclear microinjection. Transgenic Res 10:577–582

Umeyama K, Watanabe M, Nagashima H (2008) Expression of dominant-negative mutant hepatocyte nuclear factor 1 alpha transgene leads to impaired islet development and diabetes in pigs. In: Program of Swine in Biomedical Research Conference. San Diego, USA, abstract 54

Watanabe H, Andersen F, Evans SM, Gjedde A, Cumming P, The DaNeX Study Group (2001) MR-based statistical atlas of the Göttingen minipig brain. Neuroimage 14:1089–1096

Wehling M (2008) Translational medicine: science or wishful thinking? J Transl Med 6:31

Yoshikawa T (1968) Atlas of the brains of domestic animals. The brain of the pig. University of Tokyo Press, Tokyo

The right question and the corresponding animal model in light of the 3 R's

Brigitte von Rechenberg

1 The 3 R's (Reduce, Refine and Replace)

Asking the right question in relation to experimental animal research is probably one of the most delicate and difficult aspects especially in translational fields, where no standard models are available. While research related to mice and rats seem quite standardised in basic sciences, this becomes more difficult in applied research, such as biotechnology, biomaterials or medical devices, where usually larger animals are warranted. In absence of standards it is of even higher importance to ask the right questions, select the correct species and use the appropriate animal model. Apart from the research question, parallel issues of animal welfare, animal numbers and justification for use of animals for research have to be balanced including expertise to perform animal research, especially in large animals.

The 3 R's have been around for many years, usually in combination with *in vitro* cell culture work to replace *in vivo* experiments with animals. However, there is not much literature around to extend the 3 R's also to the animal work *per se* and the question arises whether this is really possible? While *in vitro* work is highly important to test biocompatibility issues in context with using foreign materials, it will never completely replace the need for animal experiments. The complex nature of the immune system and the entire organism and its answer to foreign materials will never be mimicked by *in vitro* cell cultures, no matter how sophisticated they are. In addition, translation of *in vitro* work to the real situation in the organism is not straightforward, but often very complex.

2 Translation of results *in vitro* to *in vivo*

One of the open questions for *in vitro* work starts with the use of the correct cell types. Are cell lines really representative for the real life organism? Or does their change to become cell lines also change their overall behavior such that primary cell lines should be better used? An example from our own research with supraparamagnetic ion oxide nanoparticles (SPIONs) may serve as illustration: the aim of the *in vitro* cell cultures with coated SPIONs was to establish biocompatibility for later use as local drug delivery in the joint. Since joint structures are composed

of cartilage, synovial membrane including joint capsule structures and bone, we chose to use primary chondrocytes, osteoblasts and synoviocytes as testing system *in vitro*. The up-take of SPIONs over time, as well as the dosage titration was different for each cell type (Schöpf et al. 2005), which was also later confirmed by *in vivo* application (Schulze et al. 2005, 2006). In addition, the coating and functionalization with either fluorescent dyes or plasmids changed their behavior *in vitro* and *in vivo* (Galuppo et al. 2006; Hellstern et al. 2006; Kamau et al. 2006; Schulze et al. 2005) Especially the change of plasmid functionalization could not be foreseen and ended somewhat as a surprise despite all the pervious cell culture work (Galuppo et al. 2006; Kamau et al. 2006).

Another danger with *in vitro* cell culture work may be related to interpretation and conclusions drawn from this work for the *in vivo* clinical situation. An example is the significance to metallic or polyethylene wear particles in conjunction with aseptic loosening of metallic devices, such as hip or knee prosthesis. Clinically retrieved material of the surrounding tissue of failed metallic implants revealed interface membranes congested with macrophages filled with metallic wear particles (Figure 1).

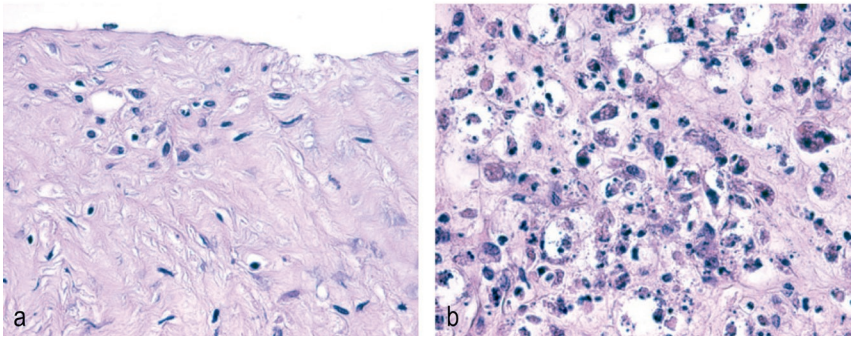

Figure 1: An interface membrane is pictured after experiments with titanium alloys in a primary stable (a) and unstable (b) condition. While controls (a) show a relatively unreactive fibrous tissue membrane, the unstable implants generated a lot of wear particles which are phagocytosed by macrophages. Furthermore, other inflammatory cells such as lymphocytes and plasma cells are visible.

Subsequent extensive *in vitro* cell culture work then showed that wear particles had a significant and deleterious effect on various cell types depending on size, amount and composition. Although this is certainly true it is definitively premature and incorrect to draw the conclusion that wear particles are the *cause* of aseptic loosening. Rather, the wear particles are just one attribution to a complex cascade in the living organism,

where biomechanical instability and subsequent micromotion, corrosion and tribo-corrosion of the metallic implant and cellular response including acidosis and regulation of cytokines and inflammatory mediators within the local environment of the medical device played a major role (El-Warrak et al. 2001, 2004; Hodgson et al. 2001).

Therefore, in order to draw the correct conclusions, it essential to ask the correct research question from the beginning, and this relates to *in vitro* and *in vivo* work.

3 Asking research questions, how and when?

The art to ask the right question relates to many aspects, among them the goal to reduce animal numbers, to justify the animal's suffering and finally, whether it is feasible to combine questions and research aspects in order to apply animal welfare issues. My doctorate thesis supervisor once taught me that "if you ask *one* research question at a time, you may be lucky to get *one* answer and at least 10 new questions…". After years of research I may add out of my own experience, *if negative answers also allow an answer and correct interpretation, it may have been a good research question*. Too many times I have seen in reviewing experiments that only positive answers allowed interpretation and if the answer was negative no conclusion could be drawn. One of my own, early experiences may serve as a negative sample: When testing a new device for sealing the annulus fibrosus in the spinal disk for its biocompatibility and functionality, it was directly implanted into surgically damaged disks in sheep. At the end the results indicated inflammatory responses of the disk environment, but it was unclear whether the material itself or the failing resistance to mechanical load and thus, dispersion of the material throughout the spinal canal was responsible for this negative response. Another, often erroneously combined research question is the test of a new biomaterial as a bone filler directly implanted in critical size long bone defects. Failure is always related to non-union with or without failure of implants. If biocompatibility issues were not solved beforehand in a non-loaded experimental situation, no conclusions can be drawn whether failure was related to biocompatibility issues of the new biomaterial, the relative bulk of the material, missing mechanical stability or a combination thereof. Bone reacts similarly in cases of bio-incompatibility, failure of implant and non-union: it produces a lot of fibrous tissue with more or less numerous inflammatory cells. But there is no definitive cut-off in cell numbers to assess whether failure was related to mechanical problems or material properties.

In both cases the unfortunate combination of research questions aimed at reducing animals and costs resulted exactly in the opposite: high research costs without results, no reduction of numbers and animals died for nothing!

Often animal models that were useful and validated for one research question suddenly are shifted and used for other research questions, where the model does not really serve well. For example, nowadays, biocompatibility of new materials is tested quite frequently with their subcutaneous or intramuscular implantation in the dorsum of rats. While this heterotopic model is well validated for tests aiming at bone induction (therefore implantation at a non-bone environment to study the potential of the material), it does not really make sense for biocompatibility. A capsule is formed around the material in most of the cases, mainly due to mechanical issues and the fact that the environment recognizes it as irritation. As capsule formation, however, is one of the main arguments to determine biocompatibility, this is not a good model for this purpose. If biocompatibility of material should be tested for later use in bone, it is of much more value to use and implant the material directly in bone, where capsule formation is really indicative of problems related to biocompatibility.

Other important questions related to biomaterials in combination with local drug delivery are dose related or how the biomimetic substances or mesenchymal stem cells can be optimally applied (Meinel 2001; Meinel et al. 2003). There, titration studies *in vitro* have to be the basis for later *in vivo* application, which then still have to encircle the optimal dose requirement by using a low and a high dose tactic to reach the goal (Meinel et al. 2003; Arrighi et al. 2009). Even though *in vitro* studies may have been promising, *in vivo* results may be jeopardized by unexpected problems related to bulk erosion of the material and/or burst release issues of the drug. Therefore, the behavior and degradation of new materials also has to be studied over time such that the reaction of intermediate products during degradation can be followed and no surprises come up later in clinical trials. Moving forward *step by step* and including appropriate controls is of paramount importance (Figure 2).

Last, it is also very important to accurately assess cellular reactions of the local tissue towards the new material, not only over time but also at various zones within or close to the material. While new bone formation may be seen in some zones, right beside it there may a foreign body reaction, which could be detrimental to the overall healing. So assessing only new bone formation of materials is not enough and does not give the full answer (Figure 3).

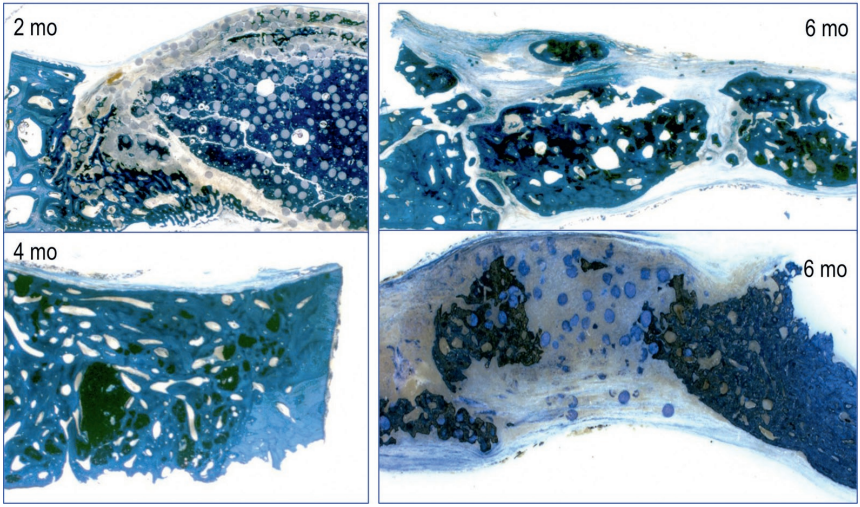

Figure 2: Application of calcium phosphate cement over time (2, 4 and 6 months) is illustrated in histology sections of non-decalcified bone samples of a skull defect in sheep. While up to 4 months results with new bone formation look quite promising, at 6 months almost all cement is resorbed and replaced by fibrous tissue. Although the exact reason is unknown at this point, it most likely has more to do with general healing capacity of the skull bone than the cement itself, which works very well in other locations of the body and is commercially available in the mean time for filling defects in ling bones.

This careful approach may look in the beginning as if more animals will be used through increasing group numbers (controls, various time points). However, at the end usually results can be validated and the animals have been wisely used justifying their suffering and giving their lives.

4 Animal species

Choosing the right animal species is important and is also related to the research question. While small laboratory rodents (mice, rats) may serve well to study basic mechanisms of signal transduction, or regeneration and repair mechanisms in bone or cartilage especially with the possibility of using transgenic strains, they usually don't serve well for testing medical devices or bone induction with new bio-scaffolds. Good results with bone biomimetics in laboratory rodents including the rabbit cannot necessarily be repeated in larger animals including humans (see the history of bone morphogenic protein, BMP). Question of the ease of use, access to appropriate facilities, costs, but also size, overall metabolisms *including ethics* have to be carefully weighed against each other. In European countries the

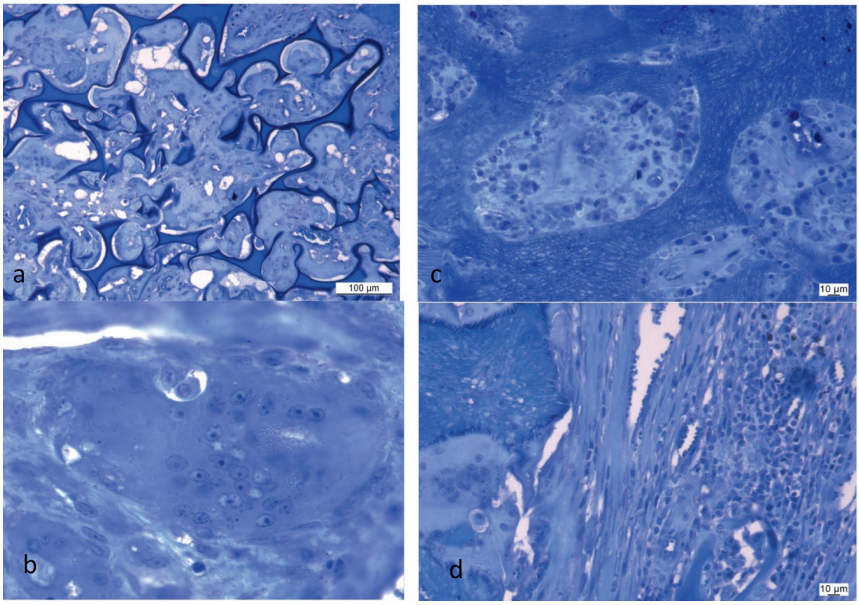

Figure 3: Assessment of a silk matrix as bone replacement in the drill hole model in sheep at 2 months shows different cellular reactions depending on the zone. While in the middle of the scaffold the materials cavities are infiltrated by macrophages and multinuclear foreign body cells directly at the surface of the remaining silk (a, b), at the periphery the material is already digested and shows matrix degradation as well as infiltration with lymphocytes and plasma cells. At the transition of the material to the adjacent bone, a fibrous capsule with a high number of lymphocytes indicates immune reaction to the foreign material. Later, at 4 and 6 months these reactions subsided. The immune reaction could be traced to the incomplete washout of a solvent during processing of the scaffolds, which later could be successfully resolved.

use of companion animals, such like dogs or horses, or non-human primates is a very sensitive issue and will be clearly weighed higher in the future – rightfully so! Since the "dignity of animals" has become part of the animal welfare legislation, it will be much more difficult to justify their use.

Last but not least, ethics of animal use should also include the handling of animals and the emotional attachment of animal technician and/or researchers to the experimental animals. For example, sheep and goats can often be used interchangeably for research questions in bone or cartilage. But then, while sheep are usually much easier to handle and keep in herds to their benefit of well-being, goats don't always work well in groups (attack each other violently in smaller areas). In addition, goats are much more personable and attached to human beings and vice versa. Therefore, the emotional strain for the people involved is much higher when it comes to sacrifice of the animals.

5 Possible animal models

In view of all points raised above, two animal models in sheep are presented that take all these considerations into view. The first, so called "drill hole model" serves well for testing biocompatibility issues for materials tested in bone replacement studies (Nuss et al. 2006). An 8mm drill hole is placed bilaterally in the proximal and distal humerus and femur of sheep at locations where no risk of fracture or other unnecessary stress is involved for the animals (Figure 4).

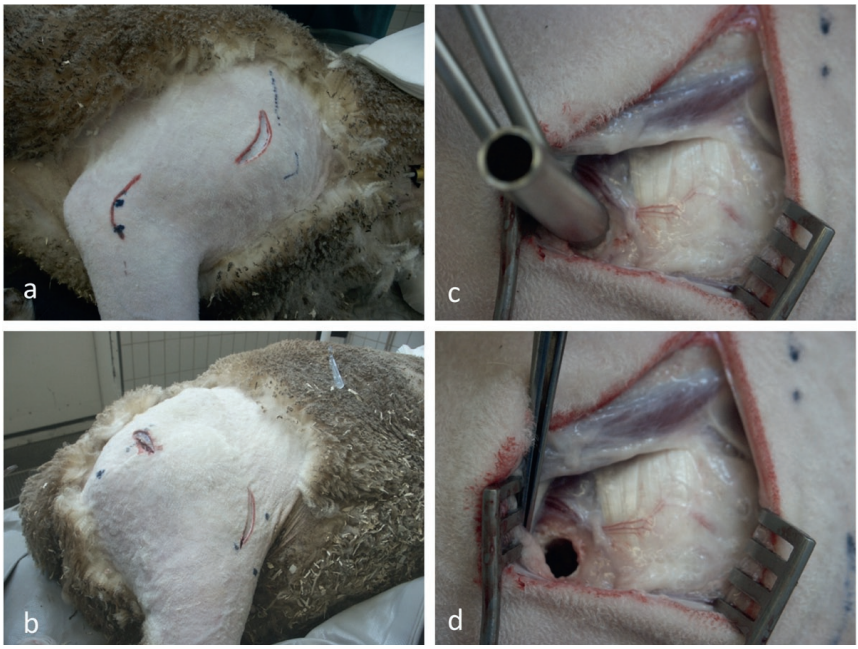

Figure 4: The drill hole model is illustrated, where the approach and the size of the wounds are demonstrated in the proximal and distal humerus (a) and femur (b). A standardised drill and drill guide (c) are used to prepare the 8mm drill hole (d) with minimal injury to the surrounding soft tissues.

No additional implants are used and the materials are subjected to only physiological load of normal cancellous bone. It is an ideal model for screening of new materials where suffering of the animals can be kept to a minimum while intra- and inter-individual differences can be studied optimally (Figure 5). If done properly and with the use of modern analgesics the animals get up right after surgery without any impairment of ambulation or other behavior. If the new materials

prove worthy, only the best are taken further and applied in a tibia defect model, where additional implants guarantee stable fixation. Thus, suffering can be kept to a minimum and also the use of animal numbers undergoing a more stressful animal model (tibia defect).

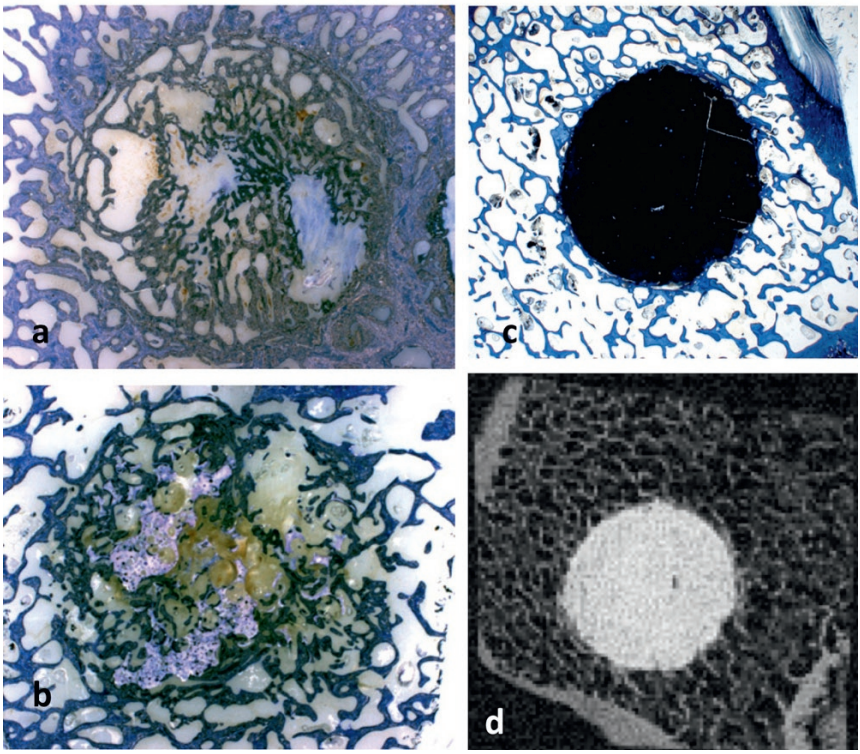

Figure 5: Histological assessment (a–c) and microradiography (d) of the different materials within the same animal shows impressive differences of material degradation and new bone formation.

Another animal model along this line is the pelvic model in sheep to test osseointegration of novel metal implants into bone (Ferguson et al. 2008; Langhoff et al. 2008). There up to 18 (dental) implants can be tested in one animal allowing also parallel insertion of implants that are evaluated for histology and biomechanical removal torque or push/pull out tests (Figure 6). Like in the drill hole model, if adequately performed, the suffering of animals can be kept to a minimum and animals can quickly lead a normal life at 2-3 days after surgery until their time point of sacrifice. Again, only the best metal surfaces should be selected for testing in a

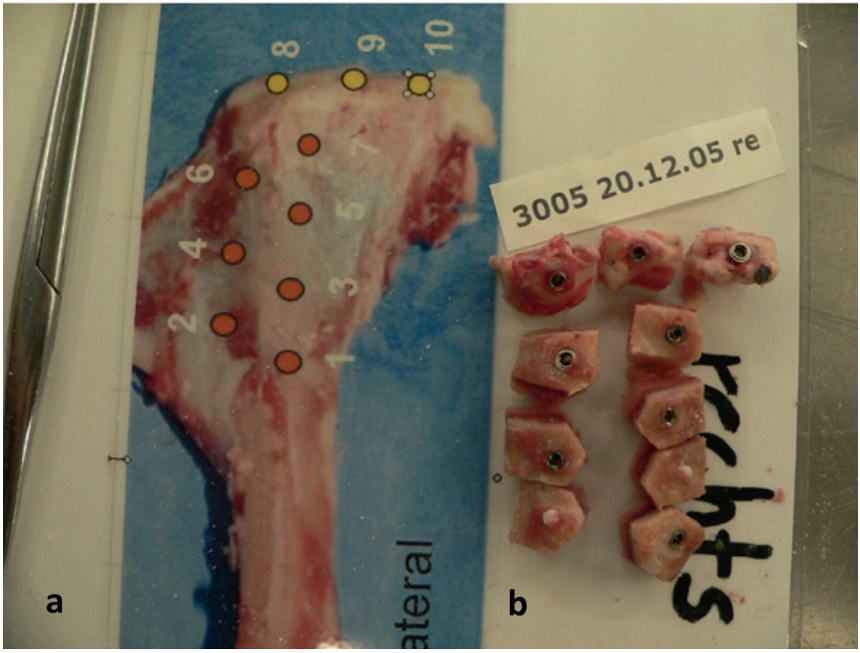

Figure 6: The picture shows the pelvic sheep model with several implant locations (a) and the samples prepared for histology or torque removal test after sacrifice (b).

more challenging animal model, such as intra-oral application of dental implants or joint replacement (hip, stifle).

Last but not least, if a critical size defect is used in sheep, the size of the defect in the animal has to be carefully selected. A 4 cm defect in humans does not relate directly to the same size in the sheep. A 4 cm defect in the sheep is a high challenge in a sheep tibia, which is much smaller (ca. 60% of a human tibia) and therefore, may not be the correct model. Problems with internal fixation may well override the original research question and jeopardize the overall result. Complications with refracturing of tibiae from 25–50%, or even up 100% are common, but are seldom reported in the literature (author has these numbers as critical reviewer from internal, not published reports) which – of course – are an inacceptable number in view of animal welfare. If a 1 cm defect is selected in sheep and the periosteum is removed at the time of surgery, it is a sufficient large size defect to test the new material under conditions, which are not so harmful to the animals. If the material proves worthy, the best performing could be selected an applied in a larger defect of 2.5cm, that is even more

challenging but still can be applied with appropriate internal fixation without asking for high complication numbers. In view of the different size of tibia in human and animals, this defect will prove as sufficiently large in relation to the overall body size to validate the use of this new material in humans (Figure 7).

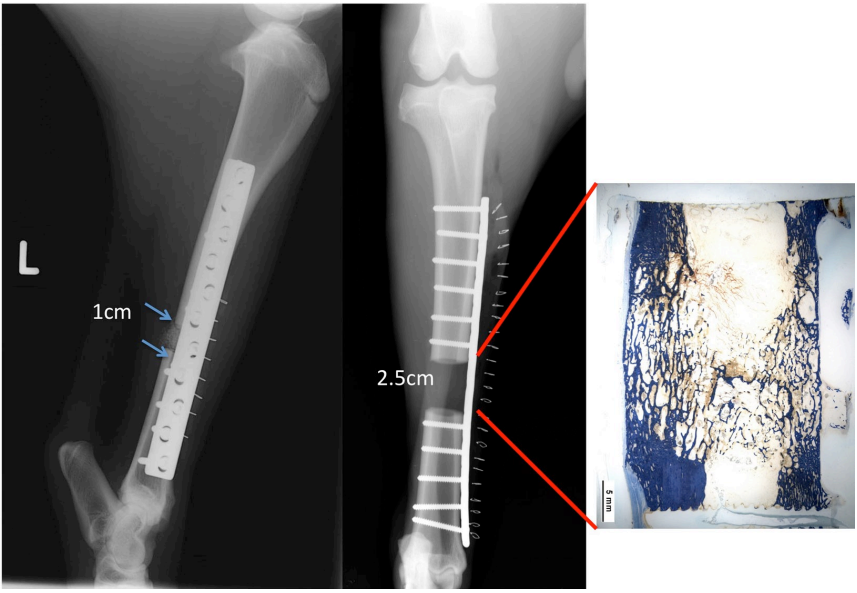

Figure 7: The picture shows the tibia defect model with an 1 cm and 2.5 cm defect. At the right the histological section is shown where bone healing can be assessed.

6 Expertise in larger animal models

Performing animal models in larger animals as sheep, goats or pigs (and of course if it cannot be avoided in dogs) requires expertise not only as a researcher, but also as a surgeon or anesthetist and as a facilitator of animal management from the beginning to the end of the experiment. Especially tibia defect models as described above are challenging and require a lot of expertise for additional casting of the limbs, maintenance of the sheep in suspension systems, clinical follow-up and analgesia regimens. Therefore, it is the firm belief of the author that only veterinary specialists should be allowed to perform these types of experiments. There, veterinary specialists (www.ebvs.org.) of the European Colleges

of Veterinary Surgery (ECVS), Anesthesia and Analgesia (ECVAA), and Laboratory Animal Medicine (ECLAM) should be entitled to conduct experimental surgeries in larger animals than small laboratory rodents (Auer et al. 2007).

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References

Arrighi I, Mark S, Alvisi M, von Rechenberg B, Hubbell JA, Schense JC (2009) Bone healing induced by local delivery of an engineered parathyroid hormone prodrug. Biomaterials, doi: S0142-9612(08)00992-7 [pii]10.1016/j.biomaterials.2008.12.023

Auer JA, Goodship A, Arnoczky S, Pearce S, Price J, Claes L, von Rechenberg B, Hofmann-Amtenbrinck M, Schneider E, Müller-Terpitz R, Thiele F, Rippe KP, Grainger DW (2007) Refining animal models in fracture research: seeking consensus in optimising both animal welfare and scientific validity for appropriate biomedical use. BMC Musculoskelet Disord 8:72, doi: 1471-2474-8-72 [pii]10.1186/1471-2474-8-72

El-Warrak AO, Olmstead M, Apelt D, Deiss F, Noetzli H, Zlinsky K, Hilbe M, Bertschar-Wolfsberger R, Johnson AL, Auer J, von Rechenberg B (2004) An animal model for interface tissue formation in cemented hip replacements. Vet Surg 33:495–504

El-Warrak AO, Olmstead ML, von Rechenberg B, Auer JA (2001) A review of aspetic loosening in total hip prosthesis. Vet Comp Orthop Traumatol 3:115–124

Ferguson SJ, Langhoff JD, Voelter K, von Rechenberg B, Scharnweber D, Bierbaum S, Schnabelrauch M, Kautz AR, Frauchiger VM, Mueller TL, van Lenthe GH, Schlottig F (2008) Biomechanical comparison of different surface modifications for dental implants. Int J Oral Maxillofac Implants 23:1037–1046

Galuppo LD, Kamau SW, Steitz B, Hassa PO, Hilbe M, Vaughan L, Koch S, Fink-Petri A, Hofman M, Hofman H, Hottiger MO, von Rechenberg B (2006) Gene expression in synovial membrane cells after intraarticular delivery of plasmid-linked superparamagnetic iron oxide particles – a preliminary study in sheep. J Nanosci Nanotechnol 6:2841–2852

Hellstern D, Schulze K, Schopf B, Petri-Fink A, Steitz B, Kamau S, Hilbe M, Koch-Schneidemann S, Vaughan L, Hottiger M, Hofmann M, Hofmann H, von Rechenberg B (2006) Systemic distribution and elimination of plain and with Cy3.5 functionalized poly(vinyl alcohol) coated superparamagnetic maghemite nanoparticles after intraarticular injection in sheep in vivo. J Nanosci Nanotechnol 6:3261–3268

Hodgson AWE, Mischler S, Virtanen S, El-Warrak AO, von Rechenberg B (2001) Degradation of CoCrMo hip implants - a corrosion, wear and clinical analysis. European Cells and Materials 2:70–71

Kamau SW, Hassa PO, Steitz B, Petri-Fink A, Hofmann H, Hofmann-Amtenbrink M, von Rechenberg B, Hottiger MO (2006) Enhancement of the efficiency of non-viral gene delivery by application of pulsed magnetic field. Nucleic Acids Res 34:e40

Langhoff JD, Voelter K, Scharnweber D, Schnabelrauch M, Schlottig F, Hefti T, Kalchofner K, Nuss K, von Rechenberg B (2008) Comparison of chemically and pharmaceutically modified titanium and zirconia implant surfaces in dentistry: a study in sheep. Int J Oral Max Surg 37:1125–1132, doi: 10.1016/j.ijom.2008.09.008

Meinel L (2001) Delivery of insulin like growth factor I for bone repair, Dept. of Pharmacy, Swiss Federal Institute of Technology, Zürich, p 110

Meinel L, Zoidis E, Zapf J, Hassa P, Hottiger MO, Auer JA, Schneider R, Gander B, Luginbuehl V, Bettschart-Wolfisberger R, Illi OE, Merkle HP, von Rechenberg B (2003) Localized insulin-like growth factor I delivery to enhance new bone formation. Bone 33:660–672

Nuss KM, Auer JA, Boos A, von Rechenberg B (2006) An animal model in sheep for biocompatibility testing of biomaterials in cancellous bones. BMC Musculoskelet Disord 7:67

Schöpf B, Neuberger T, Schulze K, Petri A, Chastellain M, Hofmann M, Hofmann H, von Rechenberg B (2005) Methodology description for detection of cellular uptake of PVA coated superparamagnetic iron oxide nanoparticles (SPION) in synovial cells of sheep. J Magnetic Materials and Magnetism 293:411–418

Schulze K, Koch A, Petri-Fink A, Steitz B, Kamau S, Hottiger M, Hilbe M, Vaughan L, Hofmann M, Hofmann H, von Rechenberg B (2006) Uptake and biocompatibility of functionalized poly(vinylalcohol) coated superparamagnetic maghemite nanoparticles by synoviocytes in vitro. J Nanosci Nanotechnol 6:2829–2840

Schulze K, Koch A, Schöpf B, Petri A, Steitz B, Chastellain M, Hofmann M, Hofmann H, von Rechenberg B (2005) Intraarticular application of superparamagnetic nanoparticles and their uptake by synovial membrane-an experimental study in sheep. J Magnetic Materials and Magnetism 293:419–432

Predictive validity of animal models and the question of size

Orsolya E. Varga

1 Introduction

The importance of selecting the right animal model in all kinds of animal research cannot be overemphasised, and in preclinical research it is crucial. The model selection process aims to find those species/strains from which translation of results to humans is most straightforward. This article[1] provides an introduction to the concept of validity of animal models, focusing on the relation of body size and the predictive validity of animal models for the study of human diseases.

There are three main arguments for the use of animals in biomedical experiments: 1) unlike cell or organ cultures they provide the complex biological system needed for certain studies, 2) it is possible to control and standardise genetic and environmental influence on laboratory animals in order to guarantee reproducible results in experimentation, and 3) it is possible to apply invasive sampling and interventions in ways that are not ethically, legally and practically possible in research with human subjects. In research, animal models are used to test new hypotheses, to discover basic pathomechanisms and to develop new treatments.

It is hardly questioned in our society that there is a continuous need for new drugs, and therefore for new Research and Development (R&D) incentive. The Pharmaceutical Research and Manufacturers of America estimates that it costs more than $1 billion on average to bring a new drug to market, and that only one in 10,000 medicines tested may reach commercialization (PhRMA 2011). The vast majority of drug candidates are sorted out before clinical trials, and only 8–12% of drugs tested in clinical trials will be finally approved. Drugs have to go through 3 different types of clinical trials before approval. Phase 1 clinical trials are done to evaluate the safety, and Phase 2 is to test the efficacy of a drug or treatment. These first two phases regularly involve less than 100 people. There are more patients enrolled in Phase 3 trials which are for gathering statistical proof of efficacy, while continuing to monitor safety. Phase 3 trials often aim to compare the efficacy of the novel treatment to a well-established one on the market.

1 I would like to thank to Anna Olsson and Peter Sandoe for their guidance on this research topic. I would like to address special thanks to Kristin Hagen for her support and advice in improving this paper.

Every phase has high attrition (reduction) rate, however, in fact, the majority of drug candidates fall out in Phase 2 trials. An analysis has found that Phase 2 success rates fell from 28% in 2006 and 2007 to just 18% in 2008 and 2009, according to the Centre for Medicines Research, which examined trials undertaken by 16 drugmakers (representing approximately 60% of global R&D spending) (Arrowsmith 2011b). Phase 2 success rates are lower than at any other phase of development, although success rates depend on therapeutic areas and the types of molecules (Arrowsmith 2011a). To understand the low success rate the failures were analysed: 51% (44 out of 87) were due to insufficient efficacy, 29% (25 out of 87) were due to strategic reasons and 19% (17 out of 87) were due to clinical or preclinical safety reasons (Arrowsmith 2011a). One may wonder why so many drugs seem to be appropriate in preclinical studies but fail in human trials. Haberman believes that the poorly predictive animal models constitute a major cause of drug attrition. In his opinion the major reason for why animal models are poorly predictive is that we understand relatively little about normal and disease biology (Haberman 2009). Therefore, the selection of appropriate animal models is crucial in drug development.

Rodents, especially mice, dominate biomedical research. Mice are the most commonly used animal models, and are widely considered to be the best model of inherited human disease since their genetic background can be easily modified. However, one may question if rodents are really universal models. There are several cases when results from mice could not be extrapolated to humans (Friese et al. 2006; Gawrylewski 2007). Many believe that the current rodent exclusivity forms an obstacle to scientific progress, and bigger body size would widen research possibilities in a technical sense (Vilahur et al. 2011).

Large animal models are not as widely used as mice, flies, or nematodes. The main reasons for this are the high price of individual animals and the obvious need for space to maintain these animals (Purcell 2010). However, some argue that investing into large animals would reduce research costs because using large animals could enhance the development speed and increase the probability of the successful development of new therapeutics offering enhanced efficacy and reduced adverse effects (Henze et al. 2010).

The aim of this paper is to discuss the relation of body size and the validity of animal models with special focus on their predictive value. I here use the most conventional definition of large animals: main domesticated livestock species, such as cows, sheep, goats, pigs, and occasionally horses. Although primate models or

companion animals such as dogs and cats are also "large animals" in terms of size, they receive special attention since having their unique set of attributes and issues as animal models. Rodents are considered small animals in the literature.

2 Selecting the right animal model

The idea of animal models is based on the August Krogh principle that for every defined physiological problem, there is an optimally suited animal that would most efficiently yield an answer (Jorgensen 2001). A modern definition describes an animal model as "...a living organism with an inherited, naturally acquired, or induced pathological process that in one or more respects closely resembles the same phenomenon occurring in man. Thus, animal models should not be expected to be ideal, nor to be universally suited to all foreseeable uses..." (Wessler 1976). Researchers have to select those animal models which are relevant to their research questions. For example, HIV vaccines can be tested only in chimpanzees; the sole model animal species allowing replication of the virus. However there are several research questions which may be studied in a huge variety of species. In these cases, in fact, scientists mostly prefer the cheaper and well established models in their research (Coid 1978).

As Rand (2009) has noted, the selection of a species should not be based solely on availability, familiarity, or cost. The readily available, familiar, or inexpensive species may not provide the genetic, physiologiccal or psychological facets needed for the proposed project. Instead of giving specific rules for the choice of the best animal model, Rand lists without ranking, several factors which may contribute to model selection. For clarity, these factors are grouped here into four main categories: factors related (1) to the appropriateness of the selected animal models, (2) to the animal, (3) to financial and other pragmatic issues, and (4) ethical factors which may influence the model selection.

(1) With regard to the appropriateness of an animal model Rand suggests to consider its appropriateness as an analog, background knowledge of its biological properties, the possibility to generalize the results obtained with it, and the transferability of information. The bigger the similarity between an animal model and human subjects, and the higher the generalizability and transferability of results, the better the selected model.

(2) Animal related factors mostly concern biological characteristics of animals such as genetic aspects, life span, age, sex and size, current diseases or conditions

of animals, ease of and adaptability to experimental manipulation, and other special features. As mentioned above, mice are often considered the ideal models due to their small size, easy breeding, short life-span, and large amount of phenotypic variation. Large animals tend to live longer and their reproduction rate is lower. These specific biological features of mice and also other rodents facilitate and influence their use as research models, and this selection highly influences our scientific knowledge as such. For example, what we know about genetics is based on data generated from 'manageable species' like fruit flies, worms, mice.

(3) The pragmatic issues in the choice of the appropriate animal model are about the costs and availability of animals, and husbandry issues such as consultation with veterinary experts, customary practice, housing availability and husbandry expertise.

(4) The ethically relevant factors to consider in choice of animal models concern, for example, the ecological risks if hazardous components are used in the experimentation, and how stressful the experimentation is for the animals.

3 Classification of animal models according their validity

In preclinical research the major question is to what extent the results from experiments with animals can be translated to humans, or with other words, how valid the animal models are. The validity of the chosen animal models should have priority in the model selection. Animal activists often claim that no animal species is a valid model for humans due to differences in metabolism, physical appearance, etc. (Shanks 2007; Shanks et al. 2009). The opposite view is that animals can be valid models of human physiology, at least in a limited sense. Validity of models is a complex concept. An animal model is described as valid if it "resembles the human condition in aetiology, pathophysiology, symptomatology and response to therapeutic interventions" (Van Dam et al. 2006). Usually, this general validity is broken down into three aspects: predictive validity (performance in the test predicts performance in the modelled condition), face validity (phenomenological analogy with the modelled condition) and construct validity (the model has a sound theoretical rationale) (van der Staay 2006).

Over the last few years several initiatives have been launched to encourage the use of more accurate animal models in both industrial and academic research. European and US authorities have published guidelines which identify the key characteristics of an approved animal model and list criteria which, if met, demonstrate

a model's suitability (cross-species comparison taking into account the target, its structural homology, distribution, signal transduction pathways and the nature of pharmacological effects); these are to be addressed by those seeking approval or a licence for drugs or biological products (Committee for Medicinal Products for Human Use (CHMP) 2007; U.S. Department of Health and Human Services Food and Drug Administration 2009). Several voluntary initiatives by researchers and industry point in the same direction, including the STRAIT initiative for more sophisticated, consensus-based validity criteria governing preclinical animal studies of stroke (Regenberg et al. 2009), and the ongoing MATRICS, TURNS and CNTRICS programmes to improve research into therapy for schizophrenia (Markou et al. 2009).

Essentially, these initiatives promote a more sophisticated way of delivering construct and face validity. However, when the results of an animal study are intended to be translated into human treatments (preclinical research), the ultimate proof of a model's value is its predictive validity. While face and construct validity are primarily theoretical considerations, predictive validity involves the calculation of a number of statistical parameters in a validation process (Bhogal et al. 2008). Thus, predictive validity of animal models can be calculated retrospectively, after obtaining data from humans (Willner 1991; Worth et al. 2002).

4 Predictive validity and body size

Predictive validity of species/strains can be examined either case-by-case or systematically. Comparative studies often compare the validity of species or strains and then recommend or don't recommend their use. These studies are based on selected examples, and may be influenced by the authors' personal experience. For example, a review of atherosclerosis and thrombosis studies compared the usefulness of small and large animals. The review concluded that there is a room for both, although the translation from rodent studies to humans have failed several times, and therefore the use of more promising large animals is necessary before clinical trials (Vilahur et al. 2011). As Garth Whiteside has noted, comparison of large datasets from preclinical efficacy studies and human trials is complex, and the translatability cannot be described as "worked", "didn't work", or "failed"; deeper analysis is needed (Whiteside et al. 2008). We have to take the effort to understand to what extent our models used in preclinical studies are predictive.

If we take the concept of quantitative interpretation of predictive validity, predictive validity can be calculated as a correlation between performance in the model and performance in the target. Usually, this type of correlation is defined as a numeric coefficient between one and minus one. If the coefficient is near zero, then the correlation is weak, meaning that the performance of the drug in humans cannot be predicted on the basis of the model. If a coefficient is near one or minus one, then the correlation is strong, meaning that the performance of the drug in humans can be predicted quite accurately on the basis of the model. Regarding this concept the question is where to draw the line in validity: which degree of predictability must animal models reach in order to be predictively valid. There are two basic approaches of validity arguments in the literature: fixed threshold and relative threshold arguments (Shelley 2010).

The fixed threshold predictive validity argument says that animal models are predictively valid exactly if their correlation with results in human tests exceeds a fixed value (Shelley 2010). For example, Greek argues that this value is 0.9 (or -0.9) in biomedical sciences, and since animal models don't reach it, in their opinion, animal models fail to have predictive validity (Greek et al. 2011).

The relative threshold predictive validity argument, instead of evaluating animal models against a fixed threshold, is based on a comparison. For example, validity of animal research is often compared to the validity of cell or tissue cultures. In one of these studies in vitro cell cultures were compared to neonatal mice for measuring the infectivity of five genotype 2 isolates of Cryptosporidium parvum. The study concluded that in vitro cell culture was equivalent to the "gold standard" mouse infectivity for measuring the infectivity of Cryptosporidium parvum and should therefore be considered a practical and accurate alternative for assessing oocyst infectivity and inactivation. However, due to the high levels of variability displayed by all assays, a final verdict on their validity was not given (Rochelle et al. 2002).

Given data from humans and different species, the predictive validity of animal models could thus in principle be objectively compared by calculation of their correlation coefficient. This calculation requires quantitative information about treatment effects both in animal and human subjects. First, systematic reviews of animal and human studies have to be prepared to get comparable outcomes, and then the correlation coefficient can be calculated. One may think that the comparison of correlation coefficients from different animal models is a straightforward step, since most diseases are studied on several animal models, including small

and large animals. In other words, there is no theoretical obstacle in comparison of large and small animals' predictive validity. For example, the metabolic syndrome, which is a complex human lifestyle related disorder, has been studied on 78 animal models from 11 species (Varga et al. 2010), including for instance llamas and leptin deficient mice. However, since preclinical animal studies are very diverse, systematic reviews often simply cannot be performed. The species effect can only be examined if studies report the same outcomes and are measured in the same way.

Methodological inconsistency among studies, such as different screening tests applied in preclinical tests and clinical trials (Lerman et al. 2007), or different study design used (Sena 2007; Macleod et al. 2009; Sena et al. 2010) are well known problems of preclinical research. Besides these methodological problems, the retrospective evaluation of predictive validity of animal models is further complicated by statistical weaknesses. Animal studies regularly report data from few animals, and usually such studies are not repeated by independent other laboratories.

To put it simply, if we want to know whether large animal are better (more predicative) models for a particular question than small animals we have to systematically analyze the relevant human and animal studies. At the same time, the diversity among preclinical animal studies may effectively prevent achieving a clear conclusion with this method.

5 Trends to improve predictive validity of animal models

If it were possible to analyze the predictive validity of established animal models, this would help to select better and to design future models. Since the predictive value of each animal model can hardly be assessed objectively, no systematic feedback exists. However, there are other forward approaches used to improve the predictive value of animal models: 1) selecting animal models with better face validity or 2) designing animal models with better construct validity (Haberman 2009).

5.1 Face validity and body size

The classical approach of model selection in preclinical research focuses on the face validity (sufficient similarities between the phenotype of the animal and symptoms of the human disorder). **One way to improve the predictive value of models**

is to move toward large animals (e.g. pigs) which better recapitulate the human phenomena than mouse models. The body size as similarity can be interpreted at two levels: one as meaning the physical appearance, the largeness; the other as the bigger genetic similarity from an evolutionary point of view. (For clarity, it has to be noted that there is no direct connection between body size and genetic proximity: larger animals are not closer but those species commonly referred as 'large animals' in biomedicine vs. rodents; see below.) Both interpretations have significance in model selection.

The physical size of animals matters in certain cases: there are "prominent differences in body size between species, which affect their appropriateness as a model for certain experiments" (Kuzmuk et al. 2011). A guide by the FDA recommends considering principles about body size when developing animal studies for cardiovascular devices (U.S. Department of Health and Human Services 2011). It is easy to understand that size does matter in certain fields; and large animals cannot be replaced by smaller ones. For example, an obvious advantage of large model animals concerns the practical possibilities for biotechnical instrumentation. Cannulation of blood and lymph vessels (e.g. the thoracic duct) allows one to sample continuously or intermittently the large number of cells in circulation. To study nutrition, metabolism, and development of the immune system in the fetus, chronic catheterization techniques are often necessary to investigate maternal and fetal blood flow in the animals (Hodgson and Coe 2005).

The evolutionary viewpoint assumes that bigger genetic overlap between animals and humans leads to more valid animal models and higher probability of translational success of their experimental results. Although in the phylogenetic sense rodents are closer to humans than domestic artiodactyls (such as cattle or pigs), the genetic homology within domestic species is also significant (Cooper et al. 2003). However, in science, it is not of great importance whether there is a two per cent or 30 per cent difference in the genome sequence; the main barrier is caused by just one or a few genes which are relevant for the research questions. Homology in genes and phylogenetic proximity are obviously connected, but not the same thing. For example, a study investigated the similarity between the insulin promoters in several mammal species such as humans, cows, pigs, mice, rats. The study concluded that within mammals, the dog stands out due to its much greater homology to humans in this respect. Rodent promoters (important in diabetes studies) are markedly different from the human promoters, and the study has urged caution in extrapolating data from rodent promoter studies to the etiology and therapy of diabetes (Hay et al. 2006).

The genetic homology of humans is the best with non-human primates; with chimpanzees it is more than 98%. Because of similarities, non-human primate animal models are believed to be better suited than other animal models to investigate most human disorders. For examples, chimpanzees and humans are the only two species that are susceptible to hepatitis-C infection, and no other suitable animal model currently exist to test a prophylactic vaccine. However, the use of primates is constrained by special moral and pragmatic concerns. Although a large proportion of the public supports animal research, the attitude towards research on primates is the opposite, probably because they resemble us. The species specific (risk of extinction) and financial concerns, as well as the complexity, training and expertise required are also obstacles to the wide use of non-human primates. Non-human primates are often 'replaced' by the less similar but more accessible and less costly large animal models, such as the porcine model, which fairly reproduces human symptoms in many cases (Vilahur et al. 2011).

Although the evolutionary approach (gene level) is very promising in model selection, it has to be noted that the link between face and predictive validity is not very strong. Minor differences in the genetic makeup of two otherwise very similar species can result in very different responses to drugs and diseases (Greek et al. 2011).

5.2 Construct validity and body size

The second approach for improving the predictive value of animal models is to design animal models which represent the human condition of interest well (Willner 1991). Besides the fact that genetic research is providing genetic information, not only from humans, but also from animals traditionally used as models, genetics has opened the door for new ways of designing humanized models. This knowledge is applied to produce specific transgenic animals or knockouts, which better mimic the physiological complexity of human disease than existing models (Greek et al. 2011). Genetically modified mice are universally used in science but genetically altered pigs, sheep, etc. are also increasingly available.

Although humanized models have important roles in research, translating results from genetically altered animals (mostly mice) to humans is not 'straightforward'. For example, research on mice mutant in Fmr1 or MeCP2 (the gene for methyl CpG binding protein 2, mutated in Rett syndrome), has promisingly suggested possible new therapies based on detailed understanding of the biochemical pathology. Indeed, the effects of the mutation of this gene on synaptic plasticity are well

described at the biochemical and cellular levels. However, the ultimate impact of these changes on the functions of specific neural circuits and how these explain the observed behaviors or psychopathology remain obscure, and drug development is still a future promise (Mitchell et al. 2011).

6 Conclusion – size does matter?

Three different types of validity are regularly distinguished in the literature: predictive-, face-, and construct validity. In preclinical research the aim is to select predictive animal models of which results can be translated to humans. However, the predictive capacity of each model can only be determined when data are also available from human clinical trials. In model selection, considering the face- and the construct validity of animal models can help.

Body size has impacts on both face and construct validity. Since our typically chosen large animal models can be 'genetically' more closely related to humans than rodents, bigger body size intimates genetic proximity (face validity) and therefore better predictive validity in certain areas of research. So far genetically modified small animals (as examples of construct validity) have not proven to be more predictive than traditional ones. However, while genetically altered large animals are still rare in research, recent advances in transgenic technology which allow creation of genetically altered large animals, may lead to animal models with higher face and construct validity than either rodents or traditional large animals.

To conclude, body size does matter in preclinical studies, but apart from a few research fields where large body size is an obvious technical requirement or advantage, it is not clear to what extent the typically chosen large animal models are more predictive than small ones.

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References

Arrowsmith J (2011a) Trial watch: Phase II failures: 2008–2010. Nat Rev Drug Discov 5:328–329

Arrowsmith J (2011b) Trial watch: Phase III and submission failures: 2007–2010. Nat Rev Drug Discov 2:87–87

Bhogal N, Balls M (2008) Translation of new technologies: from basic research to drug discovery and development. Curr Drug Discov Technol 3:250–262

Coid CR (1978) Symposium: Tests in laboratory animals – are they valid for man? Selection of animals suitable for biomedical investigations. J R Soc Med 9:675–677

Committee for Medicinal Products for Human Use (CHMP) (2007) Guideline on Strategies to Identify and Mitigate Risks for First-Inhuman Clinical Trials with Investigational Medicinal Products. European Medicines Agency, London. EMEA/CHMP/SWP/28367/07

Friese MA, Montalban X, Willcox N, Bell JI, Martin R, Fugger L (2006) The value of animal models for drug development in multiple sclerosis. Brain:1940–1952

Gawrylewski A (2007) The Trouble With Animal Models. The Scientist 7:44

Greek R, Shanks N (2011) Complex systems, evolution, and animal models. Stud Hist Philos Biol Biomed Sci 4:542–544

Haberman AB (2009) Approaches to Reducing Phase II Attrition Overview. CHI Insight Pharma Reports:160

Hay CW, Docherty K (2006) Comparative analysis of insulin gene promoters: implications for diabetes research. Diabetes 12:3201–3213

Henze DA, Urban MO (2010) Large Animal Models for Pain Therapeutic Development. In: Kruger L, Light AR (eds) Translational Pain Research: From Mouse to Man. CRC Press, Boca Raton, chpt 17

Hodgson DM, Coe CL (eds) (2005) Perinatal Programming: Early Life Determinants of Adult Health & Disease. Taylor & Francis, London

Jorgensen B (2001) August Krogh and Claude Bernard on Basic Principles in Experimental Physiology. Bioscience 1:59–61

Kuzmuk KN, Schook LB (2011) Pigs as a Model for Biomedical Sciences. In: Rothschild, MF, Ruvinsky A (eds) The Genetics of the Pig. CAB International, Wallingford, pp 426–436

Lerman C, LeSage MG, Perkins KA, O'Malley SS, Siegel SJ, Benowitz NL, Corrigall WA (2007) Translational research in medication development for nicotine dependence. Nat Rev Drug Discov 9:746–762

Macleod M R, Fisher M, O'Collins V, Sena ES, Dirnagl U, Bath PM, Buchan A, van der Worp HB, Traystman RJ, Minematsu K, Donnan GA, Howells DW (2009) Reprint: Good laboratory practice: preventing introduction of bias at the bench. Int J Stroke 1:3–5

Markou A, Chiamulera C, Geyer MA, Tricklebank M, Steckler T (2009) Removing obstacles in neuroscience drug discovery: the future path for animal models. Neuropsychopharmacology 1:74–89

Mitchell KJ, Huang ZJ, Moghaddam B, Sawa A (2011) Following the genes: a framework for animal modeling of psychiatric disorders. BMC Biol:76

PhRMA (2011) Drug Discovery And Development. http://www.phrma.org/research/drug-discovery-development (22 February 2012)

Purcell S (2010) We're Gonna Need a Bigger Lab: Large Animal Models in Research. From http://www.benchfly.com/blog/were-gonna-need-a-bigger-lab-large-animal-models-in-research (22 February 2012)

Regenberg A, Mathews DJ, Blass DM, Bok H, Coyle JT, Duggan P, Faden R, Finkel J, Gearhart JD, Hillis A, Hoke A, Johnson R, Johnston M, Kahn J, Kerr D, King P, Kurtzberg J, Liao SM, McDonald JW, McKhann G, Nelson KB, Rao M, Siegel AW, Smith K, Solter D, Song H, Sugarman J, Vescovi A, Young W, Greely HT, Traystman RJ (2009) The role of animal models in evaluating reasonable safety and efficacy for human trials of cell-based interventions for neurologic conditions. J Cereb Blood Flow Metab 1:1–9

Rochelle PA, Marshall MM, Mead JR, Johnson AM, Korich DG, Rosen JS, De Leon R(2002) Comparison of in vitro cell culture and a mouse assay for measuring infectivity of Cryptosporidium parvum. Appl Environ Microbiol 8:3809–3817

Sena E, van der Worp HB, Howells DW, Macleod MR (2007) How can we improve the preclinical development of drugs for stroke? Trends Neurosci 30:433–439

Sena ES, van der Worp HB, Bath PM, Howells DW, Macleod MR (2010) Publication bias in reports of animal stroke studies leads to major overstatement of efficacy. PLoS Biol 8(3): e1000344

Shanks N, Greek R, Greek J (2009) Are animal models predictive for humans? Philosophy, ethics, and humanities in medicine (PEHM) 4:2

Shanks N, Greek R, Nobis N, Swingle-Greek J (2007) Do Animal Experiments Predict Human Responses? Skeptic 13:2–9

Shelley C (2010) Why test animals to treat humans? On the validity of animal models. Stud Hist Philos Biol Biomed Sci 3:292–299

US Department of Health and Human Services, FDA, Center for Devices and Radiological Health (2011) Guidance for Industry and FDA Staff: General Considerations for Animal Studies for Cardiovascular Devices. White Paper. Rockville

US Department of Health and Human Services Food and Drug Administration (2009). Guidance for Industry, Animal Models — Essential Elements to Address Efficacy Under the Animal Rule. Food and Drug Administration. G:\8324dft.doc

Van Dam D, De Deyn PP (2006) Drug discovery in dementia: the role of rodent models. Nat Rev Drug Discov 11:956–970

van der Staay FJ (2006) Animal models of behavioral dysfunctions: basic concepts and classifications, and an evaluation strategy. Brain Res Rev 1:131–159

Varga O, Harangi M, Olsson IA, Hansen AK (2010) Contribution of animal models to the understanding of the metabolic syndrome: a systematic overview. Obes Rev 11:792–807

Vilahur G, Padro T, Badimon L (2011) Atherosclerosis and thrombosis: insights from large animal models. J Biomed Biotechnol: 907575

Wessler S (1976) Introduction: What is a model? Animal Models of Thrombosis and Hemorrhagic Diseases, NIH, Bethesda

Whiteside GT, Adedoyin A, Leventhal L (2008) Predictive validity of animal pain models? A comparison of the pharmacokinetic-pharmacodynamic relationship for pain drugs in rats and humans. Neuropharmacology 5:767–775

Willner P (1991) Methods for Assessing the Validity of Animal Models of Human Psychopathology, pp 1–23

Worth AP, Balls M (2002) The principles of validation and the ECVAM validation process. Altern Lab Anim:15–21

The dog – an alternative animal model for haematopoietic malignancies

Barbara C. Rütgen, Ilse Schwendenwein, Sabine E. Essler and Armin Saalmüller

1 Scientific background and relevance

Malignant lymphoma encompasses all neoplastic changes originating from lymphatic tissue. It represents a systemic disease of juvenile or mature B, T, Large Granular Lymphocytes (LGL) or unclassified lymphocytes, resulting in solid tumours in lymphatic organs (especially lymph node and spleen), bone marrow or other tissues. In dogs, it was first described in 1871 (Kessler 2005). Regarding clinical symptoms and pathology, this neoplasia represents a very heterogeneous group of tumours. The original source of the neoplastic cells is the peripheral lymphatic tissue followed by secondary infiltration into other organs. In theory, lymphomas have to be distinguished from lymphatic leukaemia, because in the latter no solid tumours occur. Nevertheless the differentiation between these two diseases is hampered by dissemination of malignant cells via the bloodstream.

In dogs, lymphomas are the most common tumours (Kessler 2005), apart from neoplasias originating in skin and mammary gland. With 90% of all canine haematopoietic tumours and 13–33 cases per year in 100,000 individuals, lymphomas have a similar incidence in dogs compared to humans (16-30/100,000 individuals; Hahn et al. 1994). Most commonly middle-aged animals (6.3–7.7 years) are affected (Kessler 2005). The frequency of cases is dependent on breed and family (Modiano et al. 2005) but not on sex (Kessler 2005). In contrast to cats, mice, birds or cattle, viral infection has not been proven to cause lymphomas in dogs (Greenlee et al. 1990). Symptoms are extremely variable and depend primarily on location and stage of disease at first presentation. Chemotherapy is the most commonly used therapy in dogs suffering from this disease and allows 77% of the patients to achieve complete remission with an average life expectancy of 265 days after diagnosis (Kessler 2005).

T-cell lymphomas seem to have a poorer prognosis than B-cell lymphomas (Teske 1994; Kessler 2005). Recent studies showed that dogs with aggressive lymphoproliferative disease were not all suffering from T-cell lymphomas but included some diffuse large Burkitt-type B-cell subtypes (Raskin 2004a) indicating that phenotype is not the only important prognostic marker (Ponce et al. 2004). Further-

more, T-cell lymphomas showing "mixed phenotypes" (e.g. $CD3^+$ with aberrant expression of $CD79\alpha^+$) are well documented in humans and dogs, suggesting that they may occur more frequently than previously thought (Thomas et al. 2001).

2 Characterisation, staging, and prognosis of canine lymphoma

Because of the heterogeneity of the disease, it is important to differentiate malignant tissue at cellular level for reliable prognosis and therapy. The World Health Organization (WHO) classification used in human diagnosis is based on morphological and cytological features and allows predictions for tumour development and prognosis for the chosen therapy. For the first time in the 1990s, cytological and morphological data from canine lymphoma were matched with the human classification system (Greenlee et al. 1990; Teske 1994; Millner et al. 1996; Fournel-Fleury et al. 1997), leading to the first WHO classification for lymphoma in veterinary medicine (Valli et al. 2002; Gilson and Séguin 2003; Raskin 2004b). However, to build a proper diagnosis in clinical practice, morphological and cytological findings are no longer considered sufficient. Using monoclonal antibodies (mAb), 38% of analysed lymphomas can be identified as T-cell type but in most cases no association between immunophenotypic and cytological characteristics can be observed (Greenlee et al. 1990; Teske 1994; Kessler 2005). In 2011, the WHO classification system was adapted for canine patients (Valli et al. 2011), thereby enabling a more precisely defined characterisation of various entities. Nevertheless, to fulfil all criteria for the characterisation of haematopoietic neoplasms in animals, additional data considering the identification and characterisation of cytogenetic and molecular aberrations are necessary. Recent publications underline the similarity of molecular changes in dogs and humans, strengthening the qualification of the dog as a powerful model for human haematopoietic malignancies such as lymphoma (Cruz Cardona et al. 2011; Suter et al. 2011; Thomas et al. 2011; Wolfesberger et al. in press).

In routine diagnosis, immunophenotyping using flow cytometry (FCM) represents a fundamental state-of-the-art method for diagnosis. In the Central Laboratory of the University of Veterinary Medicine Vienna, a diagnostic protocol with 17 mAbs was established in 2005, and since then more than 120 samples of canine lymphoma cells have been analysed. By means of assessing the expression level of these extra- and intracellular markers, this technique allows to immunophenotype lymphocytic populations floating in single cell suspension. Furthermore, var-

ious types of sample material, for example whole blood, bone marrow, fine needle aspiration (FNA) material and cerebrospinal fluid (CSF) or effusions can be used for FCM analyses. The characterisation of lymphocyte populations of different haematopoietic lineages and the discrimination between B-, T-, or "mixed cell" (B- and T-cell marker expression) are the main reasons for performing this analysis. To date, this differentiation has only a small impact on chemotherapeutic protocols, but is important for the prognosis of the disease (Teske 1994; Kessler 2005). By applying FCM analysis, canine lymphoproliferative diseases are characterised in-depth allowing the recently updated WHO classification including immunophenotype and molecular properties to be customized for canine samples more precisely.

In recent years, molecular techniques have also become available for the diagnosis of canine lymphoproliferative disorders. Polymerase chain reaction (PCR) for antigen receptor rearrangement (PARR) is a method used for the detection of clonal lymphocyte populations based on the assessment of diversity within the complementary determining region 3 (CDR3) of the immunoglobulin heavy chain variable region genes (IgH) and the T cell receptor gamma (TCRγ) genes (Burnett et al. 2003; Lana et al. 2006; Kisseberth et al. 2007). At present, PARR is routinely done in the Central Laboratory of the University of Veterinary Medicine representing an additional suitable method for distinguishing reactive from neoplastic populations of lymphocytes. This technique allows to overcome the limitations of FCM analysis because of low cell concentrations given in many source materials like whole blood, bone marrow, FNA material, CSF and different kinds of effusions. Compared to FCM, PARR is more suitable for the diagnosis of ambiguous cases of haematopoietic malignancies, for assessing minimum residual disease (MRD), and enables retrospective studies based on formalin-fixed paraffin-embedded (FFPE) material.

3 The dog as a large animal model for human lymphoma

Canine lymphoma represents a useful translational model to study the pathogenesis and treatment of lymphoma as dogs share extensive genome homology and a common environment with humans. Because companion dogs have shared a common environment with humans for thousands of years confronting them with the same pollution, dog cancers capture the 'essence' of the problem of human cancer in a manner not possible with other animal model systems, thus making them a powerful experimental model. Additionally, they are outbred individuals and

develop the disease spontaneously like humans. (Vail and MacEwen 2000; Starkey et al. 2005; Greenlee et al. 1990; Teske 1994; Fournel-Fleury et al. 1997; Thomas et al. 2003; Fosmire et al. 2007; Breen et al. 2008; Rowell et al. 2011). As the remission time of lymphomas is much shorter in dogs than in humans, the resulting compressed clinical course of disease reduces the time required to perform longitudinal studies. With a look to improve treatment, it is generally easier to test novel therapies, drugs and compounds at earlier time points in the course of disease in dogs than in human patients, supporting the increasing importance of the dog as a spontaneous, clinically relevant, large animal model of Non-Hodgkin's Lymphoma in humans.

4 Availability of research tools in haematopoietic diseases

It is very important to use the same consistent material throughout a research project. For investigations of comparative lymphoma biology and for new lymphoma treatments for translational research, well documented and characterised canine lymphoma/leukaemia cell lines for comparative studies are required. Establishment of leukaemia and lymphoma cell lines is known to be difficult (Drexler 2001), and therefore detailed investigations of canine lymphoma are hampered by the restricted availability of such cell lines. Also in humans, the success rates for leukaemia/lymphoma cell line establishment are poor and depend on sampling site and treatment status (Drexler 2001). A few years ago, the only three canine lymphoma/leukaemia cell lines were the CL-1 (Momoi et al. 1996), GL-1 (Nakaichi et al. 1996) and OSW (Kisseberth et al. 2007) cell line. The best characterised of these is the T-cell lymphoma cell line OSW. To overcome the lack of a well-characterised B-cell lymphoma cell line the working group "canine oncology" at the Institute of Immunology at the University of Veterinary Medicine Vienna started its research in 2005. The focus was to improve the cell culture conditions for primary canine lymphoma samples with the goal of expanding the generally short cell culture time to long-term culture and cell line development. In 2008, we established the novel canine diffuse large B-cell line CLBL-1 (Rütgen et al. 2010) which now serves as an important novel tool for translational *in vitro* and *in vivo* research on canine lymphomas. This cell line was established without any growth factor supplementation and shows positive staining in FCM for CD11a, CD79αcy, CD45, CD45RA, MHC II. PCR analysis for TCR-γ and immunoglobulin heavy chain (IgH) gene rearrangements yielded a monoclonal result for the IgH gene. Further characterisation of the cell line is currently under investigation with

respect of radiosensitivity together with gene expression profiling by quantitative real-time PCR (qPCR) assays for tumorigenic important genes playing key roles in cell proliferation, apoptosis, lymphopoiesis, lymphocyte differentiation, cell survival, proliferation, differentiation and tumorigenicity.

The value of the spontaneous canine patient model also depends on the availability of rodent models that can reproduce the disease as it occurs in dogs. Development of animal models that recapitulate the natural history of cancers and their clinical response to therapy is an important prerequisite for rapid bench-to-bedside translation of anticancer therapies (Mitsiades et al. 2003). Established cell lines should also be tested for their tumorigenicity in an *in vivo* model to strengthen their therapeutic potential. Immunodeficient mice injected intravenously or subcutaneously with cells of the CLBL-1 cell line develop multicentric lymphoma as observed in canine patients (manuscript in preparation, Rütgen et al.). These xenograft experiments represent an *in vivo* model of canine diffuse large B-cell lymphoma (DLBCL) that closely resembles the disease as it occurs in dogs, thus giving the opportunity for more accurate preclinical evaluation of investigational therapies against lymphomas. The long term goal of these scientific activities is in "comparative oncology" with the final aim of a better understanding of lymphomas in dogs and humans and to strengthen the dog as an alternative animal patient model for haematopoietic malignancies.

5 Initiative for the better understanding of lymphomas in dogs and humans

Besides the research activities mentioned above, our canine oncology group is intensively engaged in the founding and organisation of the "European Canine Lymphoma Network" (http://www.eu-can-lymph.net, 7 March 2012). This action strives to create a network of medical and veterinary research groups with international credits in diagnosis, management and research on human and canine lymphoid leukaemia and lymphoma. The general importance of a comparative approach is highlighted by current EU-funded research programmes like LUPA (http://www.eurolupa.org, 7 March 2012) focusing on the genetic basis of some canine diseases compared to humans. Recently, the Comparative Oncology and Genomics Consortium (http://www.ccogc.net, 7 March 2012), a network of US leading experts in human and canine oncology, has validated the canine model for the study and management of human cancer. The aims of the network are the standardisation of diagnostic protocols among laboratories active in the canine

field, coordinated strategies for the development of genetic, cytogenetic and immunophenotypic diagnostic and prognostic biomarkers for dogs, and dedicated cooperation among human and veterinary scientists that aim at the harmonisation of analytical protocols and research strategies.

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References

Breen M, Modiano JF (2008) Evolutionarily conserved cytogenetic changes in hematological malignancies of dogs and humans — man and his best friend share more than companionship. Chromosome Res 16:145–54

Burnett RC, Vernau W, Modiano JF, Olver CS, Moore PF, Avery AC (2003) Diagnosis of canine lymphoid neoplasia using clonal rearrangements of antigen receptor genes. Vet Pathol 40:32–41

Cruz Cardona JA, Milner R, Alleman AR, Williams C, Vernau W, Breen M, Tompkins M (2011) BCR-ABL translocation in a dog with chronic monocytic leukemia. Vet Clin Pathol 40/1:40–47

Fosmire SP, Thomas R, Jubala CM, Wojcieszyn JW, Valli VE, Getzy DM, Smith TL, Gardner LA, Ritt MG, Bell JS, Freeman KP, Greenfield BE, Lana SE, Kisseberth WC, Helfand SC, Cutter GR, Breen M, Modiano JF (2007) Inactivation of the p16 cyclin-dependent kinase inhibitor in high-grade canine non-Hodgkin's T-cell lymphoma. Vet Pathol 44:467–78

Drexler HG (2001) The leukemia-lymphoma cell line facts book. Academic Press, San Diego, CA

Fournel-Fleury C, Magnol JP, Bricaire P, Marchal T, Chabanne L, Delverdier A, Bryon PA, Felman P (1997) Cytohistological and immunological classification of canine malignant lymphomas: comparison with human non-Hodgkin's lymphomas. J Comp Pathol 117:35–59

Gilson SD, Séguin B (2003) Hematopoietic Neoplasia. In: Slatter D (ed) Textbook of Small Animal Surgery, 3rd edition. Saunders, Philadelphia, pp 2394–2405

Greenlee PG, Filippa DA, Quimby FW, Patnaik AK, Calvano SE, Matus RE, Kimmel M, Hurvitz AI, Lieberman PH (1990) Lymphomas in dogs. A morphologic, immunologic, and clinical study. Cancer 66:480–490

Hahn KA, Bravo L, Adams WH, Frazier DL (1994) Naturally occuring tumors in dogs as comparative models for cancer therapy research. In Vivo 8:133–143

Kessler M (2005) Kleintieronkologie – Diagnose und Therapie von Tumorerkrankungen bei Hunden und Katzen, 2nd edition. Parey, Blackwell Wissenschaft, pp 477–486

Kisseberth WC, Nadella MVP, Breen M, Thomas R, Duke SE, Murahari S, Murahari S, Kosarek CE, Vernau W, Avery AC, Burkhard MJ, Rosol TJ (2007) A novel canine lymphoma cell line: a translational andcomparative model for lymphoma research. Leukemia Res 31:1709–1720

Lana SE, Jackson TL, Burnett RC, Morley PS, Avery AC (2006) Utility of Polymerase Chain Reaction for Analysis of Antigen Receptor Rearrangement in Staging and Predicting Prognosis in Dogs with Lymphoma. J Vet Intern Med 20:329–334

Milner RJ, Pearson J, Nesbit JW, Close P (1996) Immunophenotypic classification of canine malignant lymphoma on formalin-mixed paraffin wax-embedded tissue by means of CD3 and CD79a cell markers. Onderstepoort J Vet Res. 63:309–313

Mitsiades CS, Mitsiades NS, Bronson RT, Chauhan D, Munshi N, Treon SP, Maxwell CA, Pilarski L, Hideshima T, Hoffman RM, Anderson KC (2003) Fluorescence imaging of multiple myeloma cells in a clinically relevant SCID/NOD in vivo model: biologic and clinical implications. Cancer Res 63:6689–6696

Modiano JF, Breen M, Burnett RC, Parker HG, Inusah S, Thomas R, Avery PR, Lindblad-Toh K, Ostrander EA, Cutter GC, Avery AC (2005) Distinct B-cell and T-cell lymphoproliferative disease prevalence among dog breeds indicates heritable risk. Cancer Res 65:5654–5661

Momoi Y, Okai Y, Watari T, Goitsuka R, Tsujimoto H, Hasegawa A (1997) Establishment and characterization of a canine T-lymphoblastoid cell line derived from malignant lymphoma. Vet Immunol Immunopathol 59:11–20

Nakaichi M, Taura Y, Kanki M, Mamba K, Momoi Y, Tsujimoto H, Nakama S (1996) Establishment and characterization of a new canine B-cell leukemia cell line. J VetMed Sci 58:469–471

Ponce F, Magnol JP, Ledieu D, Marchal T, Turinelli V, Chalvet-Monfray K, Fournel-Fleury C (2004) Prognostic significance of morphological subtypes in canine malignant lymphomas during chemotherapy. Vet J 167:158–166

Raskin RE (2004a) Canine lymphoid malignancies and the clinically relevant WHO classification. In: Proceedings of the 22nd Annual ACVIM Forum. Ontario Content Management Corp, pp 632–633

Raskin RE (2004b) Clinical relevance of World Health Organization classification of lymphoid neoplasias in dogs. Vet Clin Pathol 32:151

Rowell JL, McCarthy DO, Alvarez CE (2011) Dog models of naturally occurring cancer. Trends Mol Med 17:380–388

Rütgen BC, Hammer SE, Gerner W, Christian M, Guija de Arespacochaga A, Willmann M, Kleiter M, Schwendenwein I, Saalmüller A (2010) Establishment and characterization of a novel canine B-cell line derived from a spontaneously occurring diffuse large cell lymphoma. Leukemia Res 34:932–938

Starkey MP, Scase TJ, Mellersh CS, Murphy S (2005) Dogs really are man's best friend – canine genomics has applications in veterinary and human medicine! Briefings in Functional Genomics & Proteomics 4:112–128

Suter SE, Small GW, Seiser EL, Thomas R, Breen M, Richards KL (2011) FLT3 mutations in canine acute lymphocytic Leukemia. BMC Cancer 11:38

Teske E (1994) Canine malignant lymphoma: a review and comparison with human non-Hodgkin's lymphoma. Vet Q 16:209–219

Thomas R, Smith KC, Gould R, Gower SM, Binns MM, Breen M (2001) Molecular cytogenetic analysis of a novel high-grade canine T-lymphoblastic lymphoma demonstrating co-expression of CD3 and CD79a cell markers. Chromosome Res 9:649–657

Thomas R, Smith KC, Ostrander EA, Galibert F, Breen M (2003) Chromosome aberrations in canine multicentric lymphomas detected with comparative genomic hybridisation and a panel of single locus probes. Brit J Cancer 89:1530–1537

Thomas R, Seiser EL, Motsinger-Reif A, Borst L, Valli VE, Kelley K, Suter SE, Argyle D, Burgess K, Bell J, Lindblad-Toh K, Modiano JF, Breen M (2011) Refining tumor-associated aneuploidy through 'genomic recoding' of recurrent DNA copy number aberrations in 150 canine non-Hodgkin Lymphomas. Leukemia Lymphoma 52:1321–1335

Vail DM, MacEwen EG (2000) Spontaneously occurring tumors of companion animals as models for human cancer. Cancer Invest 18:781–792

Valli VE, Jacobs RM, Parodi AL, Parodi AL, Vernau W, Moore PF (2002) WHO Histological classification of Hematopoetic Tumors of domestic animals. Sec Ser Vol VIII, Armed Forces Institute Pathology, Washinhgton D.C.

Valli VE, San Myint M, Barthel A, Bienzle D, Caswell J, Colbatzky F, Durham A, Ehrhart EJ, Johnson Y, Jones C, Kiupel M, Labelle P, Lester S, Miller M, Moore P, Moroff S, Roccabianca P, Ramos-Vara J, Ross A, Scase T, Tvedten H, Vernau W (2011) Classification of Canine Malignant Lymphomas According to the World Health Organization Criteria. Vet Pathol 48:198–211

Wolfesberger B, Tonar Z, Fuchs-Baumgartinger A, Walter I, Skalicky M, Witter K , Thalhammer JG, Pagitz M, Kleiter M (in press) Angiogenic markers in canine lymphoma tissues do not predict survival times in chemotherapy treated dogs. Res Vet Sci, http://dx.doi.org/10.1016/j.rvsc.2011.04.018

Transmissible Spongiform Encephalopathy research in the original species and in laboratory mice[1]

Katy E. Beck

1 Introduction

'Transmissible spongiform encephalopathies' (TSEs) encompass a group of fatal neurodegenerative diseases that can manifest as sporadic, infectious or dominantly inherited disorders (Collinge 2001) and are so-called because of the sponge-like lesion that they cause in the Central Nervous System (CNS) of affected individuals. They are also known collectively as prion diseases since a pathogenic and self-replicating isoform of the prion protein, a host encoded glycoprotein of unknown function, is implicated as the sole infectious agent responsible for disease (Bolton et al. 1982; Oesch et al. 1985; Prusiner 1992). TSEs affect animals, i.e., scrapie in small ruminants, bovine spongiform encephalopathy (BSE) in cattle, transmissible mink encephalopathy (TME) in mink and chronic wasting disease (CWD) in mule deer and elk. They also affect humans i.e. variant and sporadic Creutzfeldt – Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), kuru and fatal familial insomnia (FFI). The aim of this paper is firstly to discuss the ethical rationale behind the animal based research that has been so fundamental to this field in light of the policy, public health and basic scientific research questions that have been raised, and secondly to discuss advances in our research that aim to minimise the future requirement for the use of animals.

2 An introduction to TSEs

In order to justify and to value the animal based research that has been carried out in the TSE field, it is ideal to begin with a brief history of the most prominent of these diseases so as to understand the threats they have posed to animal and human health and the scope of scientific questions that they have raised.

Of all known TSEs, classical scrapie, which affects sheep and goats, is the longest identified as it has been endemic to the UK sheep population for several centu-

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ries (Brown and Bradley 1998). It was formally determined as being 'transmissible' in 1936 (Cuillé and Chelle 1936; 1938) after which much research followed using small ruminants to determine the fundamental characteristics of the disease including permissible routes of inoculation and the anatomic distribution of the disease following natural and experimental infections. It is notable that in its long history, classical scrapie has never been found to be a human pathogen and so these early experiments mostly concerned the veterinary community until, in the mid 20th century, parallels were made with a human disease, kuru, identified in Papua New Guinea where ritual endocannabolism was practiced (Gajdusek and Zigas 1957; Hadlow 1959). The neuropathology of kuru was in turn reported to be comparable with Creutzfeld-Jakob disease (Klatzo et al. 1959). In 1961 the first experimental transmissions of classical scrapie to laboratory mice were reported (Chandler 1961).

Public perception of TSEs grew rapidly from the late 1980s following the identification of cattle with lesions resembling the spongiform change of classical scrapie in the brain (Wells et al. 1987), a disease termed bovine spongiform encephalopathy (BSE). The BSE epidemic that followed saw over 180,000 UK cattle diagnosed with the disease. These findings posed an immense challenge to the veterinary and scientific community and also to policy makers who could not rapidly ascertain the public health risk that BSE posed. In the first instance the cause of the epidemic had to be determined so that it could be contained and controlled. Epidemiological studies suggested that the feeding of ruminant derived protein in the form of meat and bone meal (MBM) containing a scrapie-like infectious agent was likely responsible, following a change in rendering practices in the late 1970s (Wilesmith et al. 1988). The subsequent ban of MBM in animal feed saw a gradual decline in the disease but many significant questions remained regarding its transmission and pathogenesis (Simmons et al. 2008).

The hypothesis that BSE was derived from classical scrapie also meant that it became more prudent to understand and attempt to eradicate this disease in sheep. Earlier experiments with scrapie had shown the incubation period of TSEs could be lengthy and so it was entirely conceivable that BSE infectivity could have entered the human food chain via animals in the pre-clinical phase of BSE early in the epidemic, or even before the first clinical cases were identified. Indeed BSE has since been linked to the human TSE, variant CJD, presumed to have occurred because of the ingestion of BSE-contaminated meat products and to TSEs in other species including cats and ungulates (Wyatt et al. 1991; Bruce et al. 1997; Cunningham et al. 2004), also through consumption of contaminated feed.

3 TSE diagnosis

An overarching requirement for all TSE research is that of effective disease diagnosis and classification. The early era of research, when the nature of the infectious agent was unknown, relied upon the histopathological identification of disease-specific vacuolation in the CNS of affected individuals as proof of infection. There are now several co-existing theories regarding the cause of TSEs but all infer a role for the prion protein, a host encoded glycoprotein (PrP^{C}) of unknown function. The infectious entity has been termed a prion (Prusiner 1982).

A fundamental property of prions is their ability to manifest as distinct 'strains' which present a reproducible phenotype when serially passaged in a given species (Bruce 1993). As such it was initially thought that prions carried a genome and were viruses or virinos (Dickinson and Outram 1988). However this theory has lacked any subsequent identification of an informational molecule such as a nucleic acid component to the prion agent. The alternative and more widely accepted hypothesis is that prions are proteinaceous infectious particles that are created by a conformational change in PrP^{C} to PrP^{Sc}, a pathogenic isoform of the same protein with increased resistance to protease digestion (Pan et al. 1993). According to this 'conformational hypothesis', PrP^{Sc} acts a template to which further PrP^{C} monomers are forced to convert to the pathogenic isoform and prion strain diversity can be attributed to the different tertiary structures that PrP^{Sc} monomers can attain (reviewed: Collinge and Clarke 2007). PrP^{Sc} therefore represents a marker of infection which can usually be detected in infected tissues by immunochemical methods and this detection can precede clinical and histopathological signs.

4 Models of TSE – what species to use for animal TSE research?

The unique nature of the infectious agent in TSEs limits the methodological approach to research. It is still not possible to readily isolate and quantify or characterise the agent *in vitro,* as is possible for most conventional pathogens. Only bioassay can be used to conclusively detect infectivity or to define strains. Bioassay can be defined as a method of determining the concentration, activity, or effect of a potentially harmful substance (micro-organism, biochemical, chemical) by testing its effect on a living organism and comparing this with the activity of an agreed standard.

For bioassay, donor animals can be natural TSE cases or experimental cases (in the natural host or laboratory animals). Recipient animals can be the same species as the donor, i.e., cattle-cattle or a different species, i.e., cattle-sheep or ruminant-mouse. The choice of donor and recipient species presents an interesting ethical consideration: if the donor and recipient are both species in which a given TSE can naturally occur then the experiment can be considered a model of that disease in the animals which could naturally succumb and the results could therefore be considered to directly benefit those species. If however the donor and/or the recipient are not species which would naturally become infected (i.e. rodents), then the experiment becomes an animal model of disease.

The 'refinement' element of the 3Rs principle (**reduction, refinement and replacement**) **dictates that where possible the least sentient species should be used for** research. However in an ethical debate it may be prudent to ask: if two sentient species are available (as is applicable to both rodents and the natural host species) is it more ethical to use the host species? In this disease paradigm mice seemingly have everything to lose and nothing to gain. Nevertheless the many benefits of utilising the mouse bioassay are discussed below.

Fundamentally, the decision as to which species is used for research into animal TSEs (i.e. those whose original host is an animal) depends upon the type of experiment undertaken, the aim being to produce results that directly represent, or can be extrapolated most closely to, the behavior of the agent under natural conditions. The decision is of particular relevance for risk-based policy making. As a caveat to any bioassay, it is essential that results are considered in the context of natural risk; if a TSE agent transmits experimentally, it cannot necessarily be concluded that it would under natural conditions or to the same extent (Simmons et al. 2008).

Bioassays are also inevitably governed by financial and time constraints, (particularly where policy may be implicated) and also ethical considerations in line with the Animals (Scientific) Procedures Act 1986 and implementation of the 3Rs and experiments must represent a balance of these considerations. As such the decision has to be made to use original species or rodents, or both.

TSE studies broadly fall into one of a number of groups:

(1) Transmission studies which determine whether an agent transmits inter- or intra-species and if so, to which species, with what titre of inoculum, via what route and with what clinical signs. The use of original host species is particularly advantageous due to easier translation of research findings even though the cost is high and study length can be prolonged. Large animal models are also prov-

ing useful for human TSE research, particularly for risk assessments of transmission of variant CJD via blood transfusion. In this paradigm sheep orally dosed with BSE are a good model as the pathogenesis of the resultant disease resembles humans with variant CJD and their size means that experimental intravenous transfusions can be carried out using medically relevant volumes of blood/blood components and via the same techniques as in routine blood transfusion practice (McCutcheon et al. 2011);

(2) Pathogenesis studies which investigate the route/mechanism and progress of infection and include determination of the anatomic location of infected and diseased tissues in natural or experimental cases. In food species such studies are critical in determining tissues that are suitable to enter the human and animal feed chains;

(3) Strain identification and characterisation studies which involve the identification of TSE strains based on the phenotype they present. Where a potential new strain is identified, its phenotypic characteristics must be identified. An important and possibly complicating aspect to such studies is the potential for mixtures of strains to co-exist and the ease with which they may be discriminated.

5 The mouse bioassay in TSE research

When research using the original host species is directly translational, why then do so many TSE studies employ a murine bioassay component? The most obvious advantages are the higher genetic standardization of mice and reduced costs, predominantly in terms of animal housing and study length. Historically the bioassay has most frequently used panels of 3 inbred wild-type mouse lines: RIII, C57BL/6 and VM. RIII and C57BL/6 mice share the same prion *(Prnp)* genotype, whilst in VM mice the genotype differs at 2 codons, which can affect the resultant disease phenotype (Westaway et al. 1987; Bruce et al. 1991). An overview to the traditional approach to wild-type mouse bioassay is shown in Figure 1A.

Murine bioassays can effectively complement the variety of TSE studies discussed in section 4 both as a diagnostic tool and for strain typing. For example, in transmission and pathogenesis studies, mouse bioassays are commonly used to investigate infectivity in host tissues and to determine agent titre (Andréoletti et al. 2011; Simmons et al. 2011). They can be particularly useful when the host represents a natural case of TSE and so may be fallen stock in which tissue quality may not be optimal for direct diagnosis/interpretation.

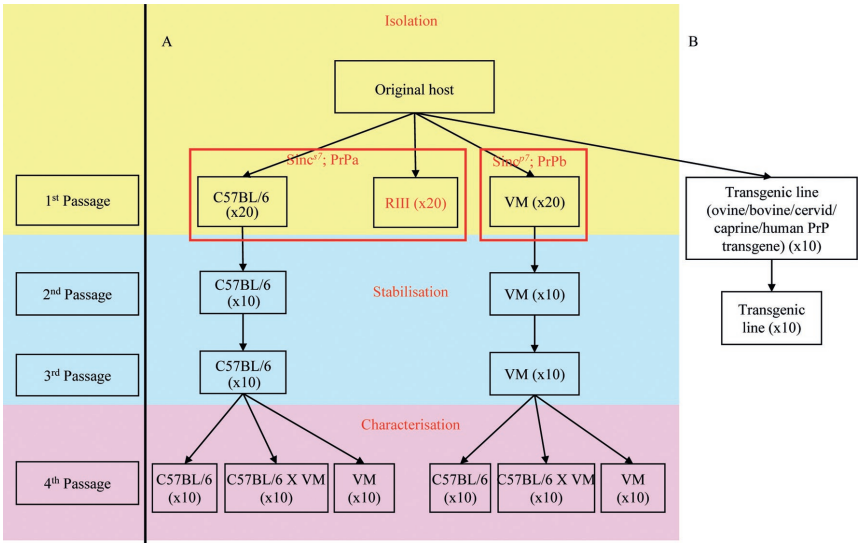

Figure 1: Overview of the mouse bioassay. A: For traditional bioassay a TSE isolate from a host species is inoculated into a panel of wild-type mice, 20 mice per line, via intracerebral (i.c) and intraperitoneal (i.p) routes (1st passage). Mice are monitored and euthanised at a pre-determined clinical end point. Brain material from the first histopathologically positive mouse is used to further inoculate (i.c) 10 mice of the same mouse line. This is repeated until the strain is considered stable, defined as no significant change in the incubation period of disease between consecutive sub-passages, after which the strain is characterised by sub-passage into C57BL/6, VM and C57BL/6xVM lines. The RIII line is not generally used for full strain characterisation.

B: Bioassay protocol using transgenic mice.

According to traditional mouse bioassay methodology, TSE strains can be defined by the relative incubation period of disease in standard mouse lines and the pattern of disease-specific vacuolation in CNS tissue following haematoxylin and eosin staining (Bruce 2003). Indeed using the bioassay, classical BSE was shown to be caused by a single strain of agent with a phenotype that was retained following experimental transmission through a variety of intermediates species (Fraser et al. 1992; Bruce et al. 1994). This methodology was also the basis for concluding that variant CJD was caused by the BSE agent (Bruce et al. 1997). In contrast classical scrapie isolates have presented with several distinct phenotypes on transmission to mice indicating the presence of more than one strain in the natural host (Bruce et al. 1997, 2002; Beck et al. 2010).

Using a semi-quantitative approach, the degree of TSE specific vacuolation in predetermined neuroanatomical areas can be scored and plotted graphically to produce 'lesion profiles' which are specific to a given host adapted TSE strain (Fraser and Dickinson 1968). For a lesion profile to be reliable for a given inoculum, it must be constructed as the mean profile of at least five clinically and histopathologically positive mice (Bruce et al. 2004).

6 Current and future research

Using the wild-type bioassay, over 20 classical mouse-adapted scrapie strains have been characterised based on the parameters of incubation period and lesion profile. However, many of these represent experimentally generated strains and their relevance, or indeed prevalence in the national ovine flock remains questionable.

A key focus of our TSE research is in the range of strains that may exist in the field and whether or not they may be zoonotic. The bioassay method can be readily employed to strain type natural TSE cases. Indeed we are currently concluding the largest strain typing study to date of field cases of classical scrapie in the UK, a study which will enable us to hypothesise the diversity and prevalence of classical scrapie strains in the UK national flock. However, it is also known that a proportion of field isolates do not transmit readily or indeed at all to wild-type mice (see below).

It is essential that mouse bioassay methodology constantly evolves to ensure that it is 'fit for purpose' and able to identify newly emerging strains. Indeed three new TSE strains have been identified relatively recently. In sheep an 'atypical' form of scrapie termed 'Nor98', owing to its discovery in Norwegian sheep in 1998 (Benestad et al. 2003), was identified in sheep with PrP genotypes that are generally resistant to classical scrapie (Le Dur et al. 2005). In cattle, two atypical forms of BSE have been reported termed H- type and L-type in reference to a specific aspect of their molecular phenotype (molecular mass of low molecular weight PrP isoforms on Western blot) relative to that of 'classical' BSE (Casalone et al. 2004; Terry et al. 2007).

A disadvantage to using the traditional wild-type bioassay is **the influence of the** 'species' or 'transmission' barrier (Kimberlin et al. 1987). Indeed some classical scrapie isolates are found to transmit to mice but only with long incubation periods and low attack rates whilst some cases fail to transmit at all despite the presence of PrP^{Sc}, on many occasions at high levels, in the CNS tissue (Bruce et al.

2002; Beck et al. 2010). The factors which govern lack of transmission in these cases are not known but may involve specific strain(s), ovine genotype(s), agent titre, the influence of mouse genome outside of the PrP gene, environmental factors or even a competition or blocking effect between two or more co-existing strains. The lack of transmission to wild-type mice may be a feature of new strains with unknown properties and pathogenicity. These isolates may be highly adapted to sheep but unable to cross some/all transmission barriers.

To circumvent the transmission barrier, transgenic mouse lines which express the PrP gene of different natural hosts can now be employed (Scott et al. 1999; Laude et al. 2002; Castilla et al. 2004, 2005; Kupfer et al. 2007) (Figure 1B). Indeed certain strains of TSE i.e. atypical scrapie will only transmit to transgenic mouse lines (Griffiths et al. 2010). It is worth noting the caveat that, somewhat counter-intuitively, this does not necessarily follow the predilection that natural scrapie strains show for certain ovine host PrP genotypes. The use of transgenic mice with the same genotype as the donor inoculum is advantageous from an ethical stance. Ultimately fewer mice need to be inoculated as attack rates are generally higher and the incubation period of disease is also reduced (Thackray et al. 2008). Transgenic bioassay is arguably more effective at isolating strain(s) that are representative of the donor inoculum (Thackray et al. 2011a) and can theoretically mimic certain elements of transmission studies between naturally susceptible species (Padilla et al. 2011). Indeed human transgenic models are increasingly being used to indicate the possible zoonotic potential of TSE strains.

However, should policy decisions be based on unvalidated models, where interpretation of results can be contentious? For example experiments have found that classical BSE does not transmit to transgenic mice expressing the cervid PrP gene (MM Simmons, unpublished data), whereas it does transmit experimentally to red deer (Dagleish et al. 2008). However, if BSE is first passaged through deer and tissue of infected animals is used to inoculate cervid transgenic mice, they then succumb to disease (MM Simmons, unpublished data). Therefore it cannot be assumed that transgenic mice will always fully model transmission barriers.

We have recently found that several classical scrapie isolates which either did not transmit to wild-type mice, or that propagated poorly, could transmit and undergo characterisation in ovine transgenic mice (Thackray et al. 2011b). In turn there is evidence to suggest that the phenotype of the agent isolated in cases that transmit poorly to wild-type mice may differ to those that transmit well (Thackray et al. 2011b). We are therefore currently characterising the isolated agent from 'non-

transmitter' cases in ovine transgenic mice. This is important as it will enhance our knowledge of strain diversity in classical scrapie and the parameters which influence inter-species transmission.

Since traditional mouse bioassay methodology dictates that strain characterisation requires several sub-passages through mice, the process can still take several years and a single bioassay can use at least 70 mice. As such it is our aim to characterise strain specific markers at earlier passages with the aim of reducing the time and number of mice required to perform strain characterisation of a given isolate. In this respect lesion profile and incubation period may be less reliable parameters as they represent mean values of a group of observations. Also in serial passages a single animal is used as a donor, the disease phenotype of which will likely propagate resulting in selection of strains. Two of our recent studies have highlighted that during first passage (donor – mouse) more than one strain may be isolated from a single source and therefore identification of strains on an individual animal basis during primary isolation may better reflect the true repertoire of agents in the host (Beck et al. 2010, 2011). This has required the characterisation of additional phenotypic parameters using alternative methodologies; immunohistochemistry (IHC) (Beck et al. 2010) and paraffin embedded tissue (PET) blot (Schulz-Schaeffer et al. 2000; Lezmi et al. 2006), to detect the specific type(s) of PrP^{Sc} deposition associated with different strains and to map their neuroanatomic distribution in individual mice. Since these methods are currently being applied to two large classical scrapie strain characterisation studies we aim to collect enough data to determine whether strain identification using alternative methodologies in conjunction with the traditional approach can occur earlier than when using the traditional approach only.

PrP^{Sc} mapping using IHC is a method that can also be used to identify and distinguish TSE strains in the original host (González et al. 2003; Simmons et al. 2007; Spiropoulos et al. 2007; Moore et al. 2008; Stack et al. 2011) but it can be difficult to make complex comparisons of PrP^{Sc} patterns objectively. To address this, a bioinformatics approach is currently under development at the AHVLA to enable TSE sources to be distinguished based on PrP^{Sc} deposition patterns with reduced subjectivity. This approach will use a statistical package/algorithm for the analysis/comparison of whole brain pathology data across large numbers of animals. Currently this involves mapping the PrP^{Sc} deposition patterns of classical and atypical forms of BSE and scrapie in cattle and sheep brains to construct the algorithm and as proof of principle. A benefit of this model will be that it is widely applicable to data generated from other animals, i.e. mice. Indeed by extending

the bioinformatics approach to the same isolates obtained following bioassay in mice, our aim will be to determine whether the pathological characteristics of isolates following mouse adaptation can in fact be identified in the original ruminant host and to identify strain-specific markers, should they exist, in mice and ruminants. Ideally this may ultimately permit discrimination of individual strains in the original host including co-infection with two (or more) discrete strains, without recourse to bioassay in laboratory models.

Current international policy regarding TSEs has ensured that the animal disease is well controlled, and measures are in place to protect the human food chain, so it could be argued that further research on these diseases is becoming harder to justify. However, parallels are being drawn increasingly between prion disorders and other protein-misfolding diseases particularly linked to age-related neurodegeneration. Naturally occurring large animal models of such diseases therefore have a key part to play in helping to understand this whole area of neurobiology, with enormous relevance to human health.

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References

Andréoletti O, Orge L, Benestad SL, Beringue V, Litaise C, Simon S, Le Dur A, Laude H, Simmons H, Lugan S, Corbière F, Costes P, Morel N, Schelcher F, Lacroux C (2011) Atypical/Nor98 scrapie infectivity in sheep peripheral tissues. PLoS Pathog 7(2):e1001285

Beck KE, Sallis RE, Lockey R, Simmons MM, Spiropoulos J (2010) Ovine PrP genotype is linked with lesion profile and immunohistochemistry patterns after primary transmission of classical scrapie to wild-type mice. J Neuropathol Exp Neurol 69:483–497

Beck KE, Sallis RE, Lockey R, Vickery CM, Béringue V, Laude H, Holder TM, Thorne L, Terry LA, Tout A, Jayasena D, Griffiths PC, Cawthraw S, Ellis R, Balkema-Buschmann A, Groschup MH, Simmons MM, Spiropoulos J (2011) Use of murine bioassay to resolve ovine transmissible spongiform encephalopathy cases showing a bovine spongiform encephalopathy molecular profile. Brain Pathology: doi: 10.1111/j.1750-3639.2011.00526.x. [Epub ahead of print]

Benestad SL, Sarradin P, Thu B, Schönheit J, Tranulis MA, Bratberg B (2003) Cases of scrapie with unusual features in Norway and designation of a new type, Nor98. Vet Rec 153(7):202–208

Brown P, Bradley R (1998) 1755 and all that: a historical primer of transmissible spongiform encephalopathy. BMJ 317:1688–1692

Bolton DC, McKinley MP, Prusiner SB (1982) Identification of a protein that purifies with the scrapie prion. Science 218(4579):1309–1311

Bruce ME (1993) Scrapie strain variation and mutation. British Medical Bulletin 49:822–838

Bruce ME (2003) TSE strain variation: An investigation into prion disease diversity. Br Med Bull 66:99–108

Bruce ME, McConnell I, Fraser H, Dickinson AG (1991) The disease characteristics of different strains of scrapie in Sinc congenic mouse lines: implications for the nature of the agent and host control of pathogenesis. J Gen Virol 72 (Pt 3):595–603

Bruce M, Chree A, McConnell I, Foster J, Pearson G, Fraser H (1994) Transmission of bovine spongiform encephalopathy and scrapie to mice: strain variation and the species barrier. Phil Trans R Soc Lon B 343:405–411

Bruce ME, Will RG, Ironside JW, McConnell I, Drummond D, Suttie A, McCardle L, Chree A, Hope J, Birkett C, Cousens S, Fraser H, Bostock CJ (1997) Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. Nature 389(6650):498–501

Bruce ME, Boyle A, Cousens S, McConnell I, Foster J, Goldmann W, Fraser H (2002) Strain characterisation of natural sheep scrapie and comparison with BSE. J Gen Virol 83:695–704

Bruce ME, Boyle A, McConnell I (2004) TSE Strain Typing in Mice. In: Lehmann S, Grassi J (eds) Techniques in Prion Research. Birkhäuser Verlag, Basel, pp 132–146

Casalone C, Zanusso G, Acutis P, Ferrari S, Capucci L, Tagliavini F, Monaco S, Caramelli M (2004) Identification of a second bovine amyloidotic spongiform encephalopathy: Molecular similarities with Creutzfeldt-Jacob disease. PNAS 101:3065–3070

Castilla J, Gutiérrez-Adán A, Brun A, Doyle D, Pintado B, Ramírez MA, Salguero FJ, Parra B, Segundo FD, Sánchez-Vizcaíno JM, Rogers M, Torres JM. (2004) Subclinical bovine spongiform encephalopathy infection in transgenic mice expressing porcine prion protein. J Neurosci 24(21):5063–5069

Castilla J, Brun A, Díaz-San Segundo F, Salguero FJ, Gutiérrez-Adán A, Pintado B, Ramírez MA, del Riego L, Torres JM (2005) Vertical transmission of bovine spongiform encephalopathy prions evaluated in a transgenic mouse model. J Virol 79(13):8665–8668

Chandler RL (1961) Encephalopathy in mice produced with scrapie brain material. Lancet i:378–379

Collinge J (2001) Prion diseases of humans and animals: their causes and molecular basis. Annu Rev Neurosci 24:519–550

Collinge J, Clarke AR (2007) A general model of prion strains and their pathogenicity. Science 318:930–936

Cuillé J, Chelle PL (1936) La maladie dite "tremblante" du mouton; est-elle inoculable? C R Acad Sci Paris 203:1552–1554

Cuillé J, Chelle PL (1938) La maladie dite "tremblante" du mouton est bien inoculable. C R Acad Sci Paris 206:78–79

Cunningham AA, Kirkwood JK, Dawson M, Spencer YI, Green RB, Wells GA (2004) Bovine spongiform encephalopathy infectivity in greater kudu (Tragelaphus strepsiceros). Emerg Infect Dis 10(6):1044–1049

Dagleish MP, Martin S, Steele P, Finlayson J, Sisó S, Hamilton S, Chianini F, Reid HW, González L, Jeffrey M (2008) Experimental transmission of bovine spongiform encephalopathy to European red deer (Cervus elaphus elaphus). BMC Vet Res 4:17

Dickinson AG, Outram GW (1988) Genetic aspects of unconventional virus infections: the basis of the virino hypothesis. Ciba Found Symp 135:63–83

Fraser H, Dickinson AG (1968) The sequential development of the brain lesion of scrapie in three strains of mice. J Comp Pathol 78(3):301–311

Fraser H, Bruce ME, Chree A, McConnell I, Wells GA (1992) Transmission of bovine spongiform encephalopathy and scrapie to mice. J Gen Virol 73:1891–1897

Gajdusek DC, Zigas V (1957) Degenerative disease of the central nervous system in New Guinea: epidemic occurrence of "kuru" in the native population. N Engl J Med 257:974–978

González L, Martin S, Jeffrey M (2003) Distict profiles of PrPd immunoreactivity in the brain of scrapie- and BSE-infected sheep: implications for differential cell targeting and PrP processing. J Gen Virol 84:1339–1350

Griffiths PC, Spiropoulos J, Lockey R, Tout AC, Jayasena D, Plater JM, Chave A, Green RB, Simonini S, Thorne L, Dexter I, Balkema-Buschmann A, Groschup MH, Béringue V, Le Dur A, Laude H, Hope J. (2010) Characterization of atypical scrapie cases from Great Britain in transgenic ovine PrP mice. J Gen Virol 91:2132–2138

Hadlow WJ (1959) Scrapie and kuru. Lancet ii:289–290

Kimberlin RH, Cole S, Walker CA. (1987) Temporary and permanent modifications to a single strain of mouse scrapie on transmission to rats and hamsters. J Gen Virol 68:1875–1881

Klatzo I, Gajdusek DC, Zigas V (1959) Pathology of kuru. Lab Invest 8:799–847

Kupfer L, Eiden M, Buschmann A, Groschup MH (2007) Amino acid sequence and prion strain specific effects on the in vitro and in vivo convertibility of ovine/murine and bovine/murine prion protein chimeras. Biochim Biophys Acta 1772:704–713

Laude H, Vilette D, Le DA, Archer F, Soulier S, Besnard N et al (2002) New in vivo and ex vivo models for the experimental study of sheep scrapie: development and perspectives. C R Biol 325:49–57

Le Dur A, Beringue V, Andreoletti O, Reine F, Lai TL, Baron T et al (2005) A newly identified type of scrapie agent can naturally infect sheep with resistant PrP genotypes. Proc Natl Acad Sci USA 102:16031–16036

Lezmi S, Bencsik A, Baron T (2006) PET-blot analysis contributes to BSE strain recognition in C57Bl/6 mice. J Histochem Cytochem 54:1087–1094

McCutcheon S, Alejo Blanco AR, Houston EF, de Wolf C, Tan BC, Smith A, Groschup MH, Hunter N, Hornsey VS, Macgregor IR, Prowse CV, Turner M, Manson JC (2011) All Clinically-Relevant Blood Components Transmit Prion Disease following a Single Blood Transfusion: A Sheep Model of vCJD. PLoS One 6(8):e23169

Moore SJ, Simmons M, Chaplin M, Spiropoulos J (2008) Neuroanatomical distribution of abnormal prion protein in naturally occurring atypical scrapie cases in Great Britain. Acta Neuropathol 116(5):547–559

Oesch B, Westaway D, Wälchli M, McKinley MP, Kent SB, Aebersold R, Barry RA, Tempst P, Teplow DB, Hood LE, et al. (1985) A cellular gene encodes scrapie PrP 27-30 protein. Cell 40(4):735–746

Padilla D, Béringue V, Espinosa JC, Andreoletti O, Jaumain E, Reine F, Herzog L, Gutierrez-Adan A, Pintado B, Laude H, Torres JM (2011) Sheep and goat BSE propagate more efficiently than cattle BSE in human PrP transgenic mice. PLoS Pathog 7: e1001319

Pan K-M, Baldwin M, Nguyen J, Gasset M, Serban A, Groth D, Mehlhorn I, Huang Z, Fletterick RJ, Cohen FE, Prusiner SB (1993) Conversion of α-helices into β-sheets features in the formation of the scrapie prion proteins. Proc Natl Acad Sci USA 90:10962–10966

Prusiner SB (1982) Novel proteinaceous infectious particles cause scrapie. Science 216:136–144

Prusiner SB (1992) Natural and experimental prion diseases of humans and animals. Curr Opin Neurobiol 2(5):638–647

Schulz-Schaeffer WJ, Tschöke S, Kranefuss N, Dröse W, Hause-Reitner D, Giese A, Groschup MH, Kretzschmar HA (2000) The paraffin-embedded tissue blot detects PrPSc early in the incubation time in prion diseases. American J Pathol 156:51–56

Scott MR, Will R, Ironside J, Nguyen HO, Tremblay P, DeArmond SJ et al (1999) Compelling transgenetic evidence for transmission of bovine spongiform encephalopathy prions to humans. Proc Natl Acad Sci USA 96:15137–15142

Simmons MM, Konold T, Simmons HA, Spencer YI, Lockey R, Spiropoulos J, Everitt S, Clifford D. (2007) Experimental transmission of atypical scrapie to sheep. BMC Vet Res 3:20

Simmons MM, Spiropoulos J, Hawkins SAC, Bellworthy SJ, Tongue SC (2008) Approaches to investigating transmission of spongiform encephalopathies in domestic animals using BSE as an example. Vet Res 39:34

Simmons MM, Moore SJ, Konold T, Thurston L, Terry LA, Thorne L, Lockey R, Vickery C, Hawkins SA, Chaplin MJ, Spiropoulos J (2011) Experimental oral transmission of atypical scrapie to sheep. Emerg Infect Dis 17(5):848–854

Spiropoulos J, Casalone C, Caramelli M, Simmons MM (2007) Immunohistochemistry for PrPSc in natural scrapie reveals patterns which are associated with the PrP genotype. Neuropathol Appl Neurobiol 33(4):398–409

Stack MJ, Moore SJ, Davis A, Webb PR, Bradshaw JM, Lee YH, Chaplin M, Focosi-Snyman R, Thurston L, Spencer YI, Hawkins SA, Arnold ME, Simmons MM, Wells GA (2011) Bovine spongiform encephalopathy: investigation of phenotypic variation among passive surveillance cases. J Comp Pathol 144(4):277–288

Terry LA, Jenkins R, Thorne L, Everest SJ, Chaplin MJ, Davis LA, Stack MJ (2007) First case of H-type bovine spongiform encephalopathy identified in Great Britain. Vet Rec 160:873–875

Thackray AM, Hopkins L, Spiropoulos J, Bujdoso R (2008) Molecular and transmission characteristics of primary-passaged ovine scrapie isolates in conventional and ovine PrP transgenic mice. J Virol 82:11197–11207

Thackray AM, Hopkins L, Lockey R, Spiropoulos J, Bujdoso R (2011a) Emergence of multiple prion strains from single isolates of ovine scrapie. J Gen Virol 92(Pt 6):1482–1491

Thackray AM, Hopkins L, Lockey R, Spiropoulos J, Bujdoso R (2011b) Propagation of ovine prions from “poor” transmitter scrapie isolates in ovine PrP transgenic mice. Exp Mol Pathol 19;92(1):167–174

Wells GA, Scott AC, Johnson CT, Gunning RF, Hancock RD, Jeffrey M, Dawson M, Bradley R (1987) A novel progressive spongiform encephalopathy in cattle. Vet Rec 121(18):419–420

Westaway D, Goodman PA, Mirenda CA, McKinley MP, Carlson GA, Prusiner SB (1987) Distinct prion proteins in short and long scrapie incubation period mice. Cell 51(4):651–662

Wilesmith JW, Wells GAH, Cranwell MP, Ryan JBM (1988) Bovine spongiform encephalopathy: Epidemiological studies. Vet Rec 123:638–644

Wyatt JM, Pearson GR, Smerdon TN, Gruffydd-Jones TJ, Wells GAH, Wilesmith JW (1991) Naturally occurring scrapie-like encephalopathy in five domestic cats. Vet Rec 129:233–236

The use of the rabbit as an animal model for clinical electroencephalogram studies applied to depth of anaesthesia research: ethical considerations[1]

Luis Antunes and Aura Silva

1 Introduction

Despite the long history of anaesthesia, the correct assessment of depth of anaesthesia (DoA) is still one area for which anaesthesiologists do not have adequate solutions. Relatively new electroencephalogram analysis based monitors have been introduced in human medicine. Such equipments may titrate anaesthetics at the brain level and reduce the risk of maintaining patients at too deep or too light levels of anaesthesia. There are no similar equipments available for veterinary medicine. In laboratory animal sciences in particular, precise control and knowledge of the animal's anaesthetic state should be maintained in order to reduce variability between animals, and to subsequently increase the statistical power of the study. Furthermore, variation and stress responses during anaesthesia are a welfare problem with implications in the quality of recorded data.

Animal models may be used to compare the performance of the existing electroencephalogram analysis based monitors, as they provide controlled conditions with minimized variability and high quality electroencephalographic recordings. We have previously shown (Silva et al. 2010) the potential of open source electroencephalogram analysis indexes to reflect changes in anaesthesia depth measured from intracranial recordings in laboratory conditions. However, it is essential to understand how they behave in non-invasive and clinical conditions. To accomplish this, a rabbit model was developed for comparison of the performance of these indexes and to validate the rabbit as a potential animal model to study the electroencephalogram. Ethical consideration when studying DoA and using this animal model are discussed.

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1 The practical example is based on work previously published in the British Journal of Anaesthesia, 2011, 106(4):540–547, by permission of Oxford University Press, and is the result of experiments developed in collaboration with David Ferreira, Carlos Venâncio and Almir Souza. The same material is part of Aura Silva's PhD (2011) thesis developed at Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal. Material from the introduction section is part from Luis Antunes' Ph.D. thesis (University Newcastle upon-Tyne, UK, 2001).

2 Ethical justification

Rabbits compared with other species have several advantages for studies in anaesthesia. Rabbits are gentle animals and if care is taken they are not upset by the experience because they become accustomed to handling. This is very important in anaesthesia studies because it allows the collection of baseline data before the induction of anaesthesia. Anaesthesia is often directly induced in smaller species, whereas other animals need to be sedated before handling. Furthermore, of the many breeds of the domesticated European rabbit, the albino New Zealand White is the most common breed used in biomedical research. The visibility of the peripheral vasculature in albino rabbits is advantageous to research because it allows easy venous access. This is essential for fluids and drugs administration during anaesthesia and to collect monitoring data or blood samples. The same tasks are much more difficult with smaller species, where researchers may end up using more animals. The small/medium size of rabbits makes them easy to house and transport.

Despite the long history of anaesthesia, the correct assessment of anaesthesia depth is still one area for which anaesthesiologists do not have adequate solutions. There are two main reasons for monitoring DoA: firstly, to avoid patient awareness under general balanced anaesthesia with neuromuscular blocking agents (NMBAs) and secondly, to adjust each anaesthetic to the requirements of the patient (Antunes, 2001). No conclusive clinical signs exist which can serve as a basis for rational administration of drugs to achieve unconsciousness (Stanski 1994). Before the introduction of NMBAs into anaesthetic practice, patient movement granted a clear indication of DoA. But, when NMBAs are administered, the possibility of patient responses to inadequate anaesthesia is no longer available and reliance has been placed on indirect measures, which are not always effective. Due to reports of patients being aware during surgery, a substantial effort has been made in the last decades to develop a reliable system to evaluate DoA.

The number of cases of awareness in humans raises questions about the occurrence of similar situations in animals. Studies of learning and memory in animals during anaesthesia are limited and produce divergent conclusions. Classically, DoA assessment in animals, as in humans, is based on purposeful movement in response to a noxious stimulus, palpebral reflex and on jaw tone. As already stated these responses are absent when NMBAs are used in animals. Furthermore, there is a lack of information regarding which reflex response needs to be lost in a particular species to ensure adequate anaesthesia for a particular procedure (Whelan 1996). These factors may

contribute substantially to situations of awareness in animals in research laboratories and in veterinary clinical practice.

The importance of the appropriate and ethical use of NMBAs in animals involved in scientific investigations is documented in reports and guidelines by different organisations. An editorial by Drummond and co-authors from *Anesthesiology* pointed out several reasons why the use of NMBAs in animals must be avoided. He emphasised reasons for rejection of papers submitted to that journal as a result of inappropriate use of these drugs (Drummond et al. 1996).

Situations of awareness in animals could be more frequent in research projects where recordings of brain activity are required and animals are therefore subjected to combined anaesthesia with NMBA. These research groups have a specific interest in maintaining a more active brain, and the dose of hypnotic agents used is frequently reduced to achieve this. This, however, results in an increased risk of awareness. The fact that researchers are not aware of this possible phenomenon and the use of obsolete anaesthetic techniques without proper advice does only increase such possibilities. Detrimental effects on the welfare of the animals' and on research results will happen if periods of inadequate anaesthesia remain undetected and untreated.

Intraoperative awareness in laboratory animals will increase the variability of data collected under such circumstances. A variety of stress related responses will develop and this may affect the quality of the data collected. Thus, precise control or knowledge of the animals' anaesthetic state must be maintained to accurately interpret the results of experiments. Several studies carried out in research animals under general anaesthesia also demand that a constant and reproducible DoA is maintained throughout the procedure. For example, the level of arousal can dramatically influence neural processing of sensory stimuli. In addition the application of the "Three Rs" rule (reduction, replacement and refinement, Russell and Burch 1959) is being severely disregarded. Precise control and knowledge of the animal's anaesthetic state must be maintained to reduce variability between animals, and subsequently increase the statistical power of the study. If the opposite happens more animals will be used unnecessarily.

During the past 20–30 years, the advances that have been made in medical engineering have allowed a tremendous improvement in anaesthesia, especially in monitoring standards. However, this evolution has not been paralleled by Central Nervous System (CNS) monitoring techniques. Yet, this is the system that one needs to monitor since it is the primary site of action for the anaesthetics. In the last 15 years a tremendous effort has been made in the development of new tools to monitor the CNS

activity. The introduction of the Bispectral index (BIS) of the electroencephalogram (EEG) is an example of such improvements. These data complement the measurement of responses of the autonomic nervous system, which do not adequately reflect DoA if used alone, resulting either in awareness or in maintenance of patients at unnecessarily deep planes of anaesthesia, thereby increasing the risk of the procedures.

When NMBAs are used the anaesthetist is dependent on the commonly used haemodynamic measurements, which are not appropriate gauges of anaesthetic depth in either humans or animals (Whelan 1996). Changes in blood pressure (P), heart rate (R), sweating (S) and tear production (T) abbreviated to the PRST score; the minimum alveolar concentration and constant plasma concentration; the lower oesophageal contraction; the isolated forearm technique; the spontaneous surface electromyography¸ respiratory sinus arrhythmia index, heart rate variability and the EEG are some examples of techniques used and investigated for monitoring DoA. The value of the EEG in assessing DoA has been questioned in humans and other species due to its poor correlation with the commonly accepted clinical signs of DoA, e.g. haemodynamic responsiveness and purposeful movement in response to surgical stimuli. The interest in the EEG was recently increased by the introduction of new technological and mathematical developments, which are now easily available (e.g. new computational solutions). Techniques based on power spectral analysis, frequency ratios, relative band powers, burst suppression rate (BS/BSR), the BIS, entropy based indexes, Index of Consciousness (IoC) and auditory evoked responses (AEP) are now used to quantify and interpret the raw EEG.

AEP are generated as an average of EEG activity following repetitive stimulus presentation. Dose-dependent reductions in AEP peak amplitudes and increased latencies are accepted as useful indices of DoA for some anaesthetics (e.g. volatile anaesthetics and propofol) (Antunes et al. 2003a, b).

The BIS quantifies the power spectrum, inter-frequency phase information, phase coupling between EEG frequency components and how much the EEG is isoelectric or burst suppressed (Rampil 1998). This information is related in a proprietary algorithm which is optimised using a patient database to correlate with the level of hypnosis or sedation, defined by a sedation score that ranges from 100 (awake) to 0 (isoelectric EEG) (Glass et al. 1997). This algorithm was empirically developed by relating the EEGs from hundreds of anaesthetised human patients to certain clinical end-points such as response to verbal, tactile and noxious stimuli (Rampil 1998).

New monitors, such has spectral entropy, narcotrend, cerebral state index and IOC, also based on EEG analysis, were introduced later and are now clinically used. The

spectral parameters and the burst suppression ratio are frequency-domain and time-domain analysis methods, respectively, which do not take into account the nonlinear characteristics of the EEG. However, the brain behaves as a nonlinear system and it is reasonable to apply nonlinear systems analysis methods to extract information from the EEG, such as entropy-based concepts.

BIS has been validated across several anaesthetics and sedatives including isoflurane, propofol and midazolam (Glass et al. 1997). In contrast, N_2O, ketamine, fentanyl and some levels of sevoflurane showed no correlation with BIS (Barr et al. 2000). These results have not decreased BIS and new EEG based monitors popularity, which are now routinely used in the clinical practice without substantial basic understanding of the anaesthesia induced EEG changes by the anaesthesiologist.

Our group has showed that the regular use of BIS results in lighter DoA, reflected in average higher BIS values maintained during anaesthesia procedures in humans (Nunes et al. 2005). By indicating the exact requirements for anaesthesia, BIS may reduce moments of deep anaesthesia which could be harmful in certain patient populations, influencing the postoperative outcome (Monk et al. 2005). These observations are complemented with evidence that BIS based drug titration decreases the anaesthetic doses used, with more rapid and improved recovery from anaesthesia in outpatients (Bauer et al. 2004; White et al. 2004). The use of BIS has no effect on surgical stress response (Bauer et al. 2004) and detects awareness during anaesthesia (Myles et al. 2004). Studies measuring the performance of DoA indicators showed that BIS is the best predictor of movement and transitions between conscious/unconscious (Glass et al. 1997; Struys et al. 2003).

The application of BIS in animals is questionable since its algorithm is based on data collected in humans. Recently March and Muir reviewed the use of BIS in animal. The interspecies differences in the EEG features associated with different hypnotic and amnesic states may affect the correlation between BIS value and DoA (March and Muir 2005). This possibility is supported by a later study, which suggest that clinical BIS endpoints used in humans may not be used in cats due to the fact that BIS in this species varied between 5 to 32 with 1.5 to 0.8 MAC isoflurane (Lamont et al. 2005).

We have studied EEG parameters in rats as DoA monitoring tools. Results pointed the difficulties to use such parameters as simple DoA predictors (Antunes et al. 2003a). However, we demonstrated the potential to use EEG derived techniques as a pharmacodynamic/pharmacokinetic research tool and the possibility that these techniques may be useful for measuring the effects of drugs upon the CNS (Antunes et al. 2003b). Later studies also in rats performed with intracranial recordings explored more com-

putationally advanced indexes. The approximate entropy, the index of consciousness, the spectral edge frequency and the permutation entropy were estimated using epochs of eight seconds. A correction factor for burst suppression was applied to the spectral edge frequency and to the permutation entropy. The burst suppression corrected permutation entropy showed the highest correlation with the end-tidal isoflurane concentration (Silva et al. 2010). However, these results were not confirmed in subsequent research in rabbits using extracranial recordings (EEG) (Silva et al. 2011a, b) which may reflect the high sensitivity of the EEG indexes to external artefacts which are present in EEG data.

As Prys-Roberts stated in 1987, the search for a single method to measure DoA resembles that for the Philosopher's Stone (Prys-Roberts 1987). Such a remark is justified by the fact that if the anaesthetic action results from a combination of different effects used to achieve variable objectives of anaesthesia, a single index could not be used to express DoA. Several authors have addressed the same issue, and recommended that the search for a reliable index of DoA should be changed into a search for independent indexes for the different components of anaesthesia (Kissin 1993; Glass et al. 1997; Stanski 1994). In this way, one would have different monitors to evaluate loss of consciousness, motor responsesiveness or hemodynamic and sympathetic responses to noxious stimulation.

Analgesia is an important part of adequate anaesthesia and direct measures for adequacy of analgesia do not exist. Nociception indexes have been proposed (Anemon-I and RSA-index, Fathom®), but have not given proper solutions. It seems probable that it will be difficult to base the analgesia measure on a single physiological parameter and the strategy should pass by a multiparameter hybrid model. Data extracted from the electrocardiography and the photoplethysmography may be used to build a nociception index (Seitsonen et al. 2005). This information may be incorporated in a multiparametric index analysis for DoA.

3 A practical example from research with the rabbit as a model

Background: Research for the development of indexes of consciousness is mainly made in humans during anesthesia for depth of anesthesia assessment. The Bispectal index, index of consciousness, permutation and approximate entropy, as well as the classic median and spectral edge frequency 95% are examples of indexes developed. In order to find the best parameter, it is essential to compare the performance

of the existing parameters. Animal models may give important insights at this level, as they provide controlled conditions with minimized variability and high quality electroencephalographic recordings. We have previously shown the potential of the presently studied indexes to reflect differences from intracranial recordings in laboratory conditions. However, it is essential to understand how they behave in non-invasive and clinical conditions. In this study, a rabbit model under fentanyl and isoflurane anesthesia was used to compare the performance of these indexes and to validate the rabbit as potential animal model to study the electroencephalogram.

Methods: EEG recordings were obtained from six New Zealand White male rabbits. Animals received fentanyl for premedication, followed by induction with propofol and maintenance with isoflurane (Figure 1). Depth of anesthesia was evaluated according to a clinical scale from 1 (awake) to 4 (surgical anesthesia) (Table 1). The correlation between EEG derived indexes with the clinical scale of anesthesia was analyzed using prediction probability (Pk). Repeated measures analysis of variance or its non-parametric equivalent were used to analyze the indexes' values at the study times and to compare their variability (for more information see Silva et al. 2011b).

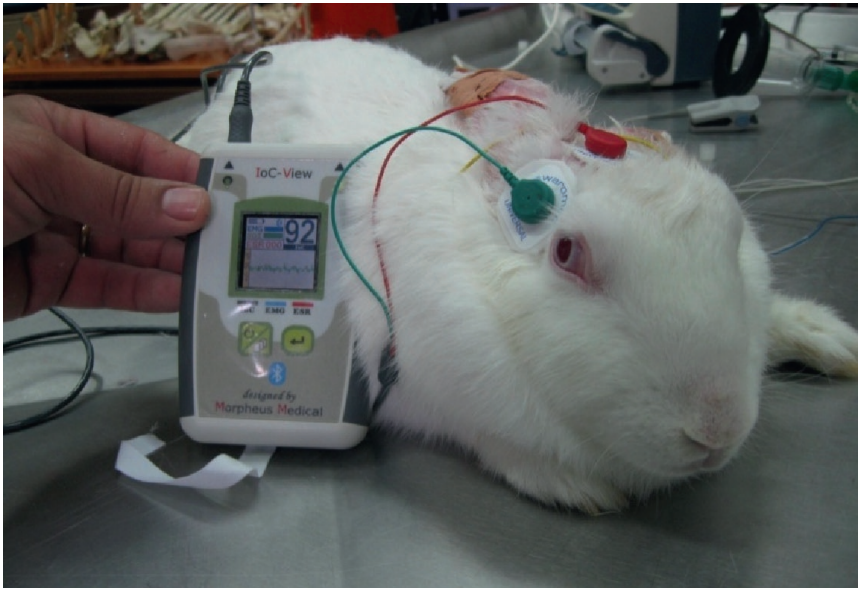

Figure 1: Gel-coated silver-silver chloride electrodes applied on the rabbit's head during recording of the electroencephalogram with the IoC-View monitor.

Table 1: Definition of the anaesthetic states based on clinical signs. The main clinical signs of each state are referred to and attributed to a figure on a numerical scale.

Anaesthetic state	Clinical signs observed	Numerical Scale
Awake	Fully awake and alert	1
Drowsy	Awake, but still drowsy and responsive to any stimulation	2
Sedated	Decreased mobility but still responsive to stimuli	3
Surgical anaesthesia	Absence of consciousness, high muscular relaxation with the eyeball fixed ventromedially. Absence of nociceptive response and palpebral reflex but corneal reflex present.	4

Results: The rabbits showed good electroencephalogram traces. The animals' behavior allowed to record EEG during the awake phase and following induction of anaesthesia. All animals showed EEG recordings with a similar pattern, a shift from high frequency and low voltage waves to low frequency and high amplitude waves after induction of anesthesia. None of the animals showed burst suppression[2] patterns after induction with propofol, but four had burst suppression patterns during the surgery period. Permutation entropy, median and spectral edge frequency 95% showed a tendency to increase when burst suppression was present. This turned useful to apply a correction for BS in these indexes. Overall the index of consciousness showed the best mean prediction probability value followed by burst suppression corrected permutation entropy (Figure 2). Both parameters also showed less variability than the others. For more information about results from this study see Silva et al. (2011b) from which we have taken some material here by permission of Oxford University Press.

Conclusions: This study showed that the index of consciousness and permutation entropy may be promising indexes for DoA monitoring. The permutation entropy may benefit from the application of a burst suppression correction, at deeper stages of anaesthesia. Interestingly this study also showed that the rabbit is large enough for EEG dermal electrodes and has the advantage of having a thin muscular layer between the skin and the skull resulting in little electromyographic artefacts in extracranial recordings which may replace the need for invasive models using intracranial electrodes and with direct translation to clinical research. In this study the rabbit showed good properties to be used as a translational research animal model for the validation of clinical indexes.

In general, rabbits require more space for housing compared with smaller rodents, which may increase expenses, and rabbits are often regarded as pets. However, the

2 Burst suppression represents an abnormal cortical activity, where 95% of cortical cells are silent during suppression.

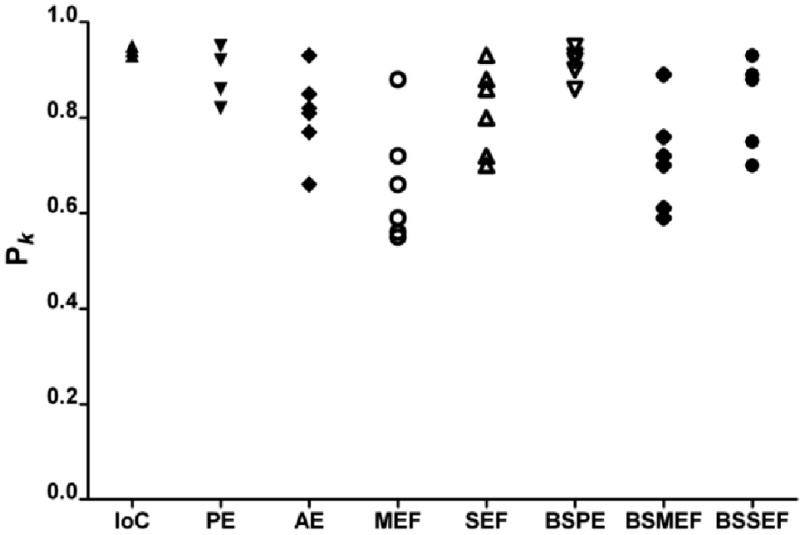

Figure 2: Prediction probabilities (Pk) for the studied indexes: index of consciousness (IoC), permutation entropy (PE), approximate entropy (AE), median edge frequency (MEF), spectral edge frequency 95% (SEF), PE corrected for burst suppression ratio (BSPE), MEF corrected for burst suppression ratio (BSMEF), SEF corrected for burst suppression ratio (BSSEF). Each animal Pk value is shown by a symbol (N=6) (Silva et al. **(2011b) by permission of Oxford University Press).**

use of rabbits may be justified because biological samples are easy to obtain, and access to veins and volume are typically not a problem. In mice and rats this is frequently a difficulty, which may lead to the use of more animals, to data variability, and to violation of the 3Rs principles. Furthermore, rabbits are easy to handle, and, if needed, multiple blood samples can be collected. On the other hand, when compared with mice and rats, there are less genetically different rabbit strains available, biomedical research done with this species is much less frequent and the amount of published data is relatively spread. These facts may limit the use of the rabbit as a more common animal model.

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References

Antunes LM (2001) The use of the electroencephalogram and auditory evoked potentials to assess the depth of anaesthesia and effects of anaesthetic agents in the laboratory rat (Rattus norvegicus). PhD thesis University of Newcastle, UK

Antunes LM, Roughan JV, Flecknell PA (2003) Effects of different propofol infusion rates on EEG activity and AEP responses in rats. J Vet Pharmacol Ther 26:369–376

Barr G, Anderson RE, Samuelsson S, Owall A, Jakobsson JG (2000) Fentanyl and midazolam anaesthesia for coronary bypass surgery: a clinical study of bispectral electroencephalogram analysis, drug concentrations and recall. Brit J Anaesth 84:749–752

Bauer M, Wilhelm W, Kraemer T, Kreuer S, Brandt A, Adams HA, Hoff G, Larsen R (2004) Impact of bispectral index monitoring on stress response and propofol consumption in patients undergoing coronary artery bypass surgery. Anesthesiology 101:1096–1104

Drummond JC, Todd MM, Saidman LJ (1996) Use of neuromuscular blocking drugs in scientific investigations involving animal subjects. The benefit of the doubt goes to the animal. Anesthesiology 85:697–699

Glass PS, Bloom M, Kearse L, Rosow C, Sebel P, Manberg P (1997) Bispectral analysis measures sedation and memory effects of propofol, midazolam, isoflurane, and alfentanil in healthy volunteers. Anesthesiology 86:836–847

Kissin I (1993) General anesthetic action: an obsolete notion? Anesth Analg 76:215–218

Lamont LA, Greene SA, Grimm KA, Tranquilli WJ (2005) Relationship of feline bispectral index to multiples of isoflurane minimum alveolar concentration. Comp Med 55:269–274

Locher S, Stadler KS, Boehlen T, Bouillon T, Leibundgut D, Schumacher PM, Wymann R, Zbinden AM (2004) A new closed-loop control system for isoflurane using bispectral index outperforms manual control. Anesthesiology 101:591–602

Mahfouf M, Nunes CS, Linkens DA, Peacock JE (2005) Modelling and multivariable control in anaesthesia using neural-fuzzy paradigms: Part II- closed-loop control of simultaneous administration of propofol and remifentanil. Artif Intell Med 35:207–213

March PA, Muir WW (2005) Bispectral analysis of the electroencephalogram: a review of its development and use in anesthesia. Vet Anaesth Analg 32:241–255

Monk TG, Saini V, Weldon BC, Sigl JC (2005) Anesthetic management and one-year mortality after noncardiac surgery. Anesth Analg 100:4–10

Myles PS, Leslie K, McNeil J, Forbes A, Chan MT (2004) Bispectral index monitoring to prevent awareness during anaesthesia: the B-Aware randomised controlled trial. Lancet 363:1757–1763

Nunes CS, Ferreira DA, Antunes L, Amorim P (2005) Regular Clinical Use Bispectral Index Monitoring May Result in Lighter Depth of Anesthesia as Reflected in Average Higher Bispectral Index Values. Anesthesiology 103:1320–1321

Nunes CS, Mahfouf M, Linkens DA, Peacock JE (2005) Modelling and multivariable control in anaesthesia using neural-fuzzy paradigms: Part I- classification of depth of anaesthesia and development of a patient model. Artif Intell Med 35:195–206

Prys-Roberts C (1987) Anaesthesia: a practical or impractical construct? Brit J Anaesth 59:1341–1345

Rampil I (1998) A primer for the EEG signal processing in anesthesia. Anesthesiology 89:980 –1002

Russell WMS, Burch RL (1959) The principles of humane experimental technique. Methuen & Co. LtD, London

Seitsonen ER, Korhonen IK, van Gils MJ, Huiku M, Lotjonen JM, Korttila KT, Yli-Hankala AM (2005) EEG spectral entropy, heart rate, photoplethysmography and motor responses to skin incision during sevoflurane anaesthesia. Acta Anaesthesiol Scand 49:284–292

Silva, A (2011) Electroencephalogram-derived indexes for precise control of animal anaesthesia. PhD thesis at Universidade de Trás-os-Montes e Alto Douro

Silva A, Cardoso-Cruz H, Silva F, Galhardo V, Antunes L. (2010) Comparison of anesthetic depth indexes based on thalamocortical local field potentials in rats. Anesthesiology 112:355–363

Silva A, Campos S, Monteiro J, Venâncio C, Costa B, Guedes de Pinho P, Antunes L (2011a) Performance of Anesthetic Depth Indexes in Rabbits under Propofol Anesthesia: Prediction Probabilities and Concentration-effect Relations. Anesthesiology 115:303–314

Silva A, Ferreira DA, Venâncio C, Souza AP, Antunes LM (2011b) Performance of electroencephalogram-derived parameters in prediction of depth of anaesthesia in a rabbit model. Br J Anaesth 106(4):540–547

Stanski DR (1994) Monitoring depth of anesthesia, In: Miller, RD (ed) Anesthesia. Churchill Livingstone, New York, pp 1127–1159

Struys M, Vereecke H, Moerman A, Jensen E, Verhaeghen D, De Neve N, Dumortier F, Mortier E (2003) Ability of the bispectral index, autoregressive modelling with exogenous input-derived auditory eveoked potentials and predicted propofol concentration to measure patient responsiveness during anesthesia with propofol and remifentanil. Anesthesiology 99:802–812

Struys MM, De Smet T, Versichelen LF, Van De Velde S, Van den Broecke R, Mortier EP (2001) Comparison of closed-loop controlled administration of propofol using Bispectral Index as the controlled variable versus “standard practice” controlled administration. Anesthesiology 95:6–17

Whelan G (1996) The assessment of depth of anaesthesia and the effects of anaesthesia in the rat (Rattus norvegicus). PhD. Newcastle University, Newcastle upon Tyne

White PF, Ma H, Tang J, Wender RH, Sloninsky A, Kariger R (2004) Does the use of electroencephalographic bispectral index or auditory evoked potential index monitoring facilitate recovery after desflurane anesthesia in the ambulatory setting? Anesthesiology 100:811–817

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