Introduction to the production and validation of animal models

Production and Validation of Animal Models

Disease models in wild-type animals

[Mestrado em Neurobiologia (MNe)]

Fani Neto - FMUP – March 2023

FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

Módulo: Production and Validation of Animal Models

Coordenador: Fani Neto

DATE	HOUR			TOPIC	PLACE	LECTURER
13-Mar-23	9:00	10:30	T	Introduction to the production and validation of animal models.	UBEx Library	Fani Neto
13-Mar-23	10:45	11:45	TP	Models of visceral pain: i) Cystitis	UBEx Library	Célia Cruz
13-Mar-23	12:00	13:00	TP	Models of nervous system injury: i) Spinal cord injury	UBEx Library	Célia Cruz
13-Mar-23	14:00	15:00	T	Models of somatic chronic pain	UBEx Library	Fani Neto
13-Mar-23	15:10	16:10	TP	Models of inflammatory and neurodegenerative joint pain: i) Osteoarthritis; ii) Monoarthritis	UBEx Library	Fani Neto / Joana Gomes
13-Mar-23	16:15	16:45	T	Models of visceral inflammation and pain	UBEx Library	António Avelino
14-Mar-23	9:00	10:00	T	Animal models of addition	UBEx Library	Teresa Summavielle
14-Mar-23	10:00	11:00	T	Systemic induced neuropathies: diabetes and cancer treatment	UBEx Library	Isaura Tavares
14-Mar-23	12:00	13:00	T	Zebra fish models in neuroscience research	UBEx Library-ONLINE??	Joana Monteiro
14-Mar-23	14:00	15:30	T	The use of Drosophila in neuroscience research	UBEx Library	César Mendes
15-Mar-23	9:30	11:00	T	Models of degenerative diseases	UBEx Library	Isabel Cardoso
15-Mar-23	11:00	12:00	P	Validation of animal models	UBEx Library	Joana Gomes/

OBJECTIVES

A) Provide theoretical and/or practical knowledge on various ***in vivo*** experimental models currently used in Neuroscience:

i) production: fundaments, applications, practical requirements, advantages, limitations and type of answers they can provide;

ii) question the adequacy of each model to the objective of the study

iii) validation: molecular, biochemical and behavioral approaches

B) Provide the competency:

i) to analyze and plan the best approach to scientific questions in different neuroscience fields by using the most appropriate experimental model;
ii) to design and execute own experiments and understand the work performed by others

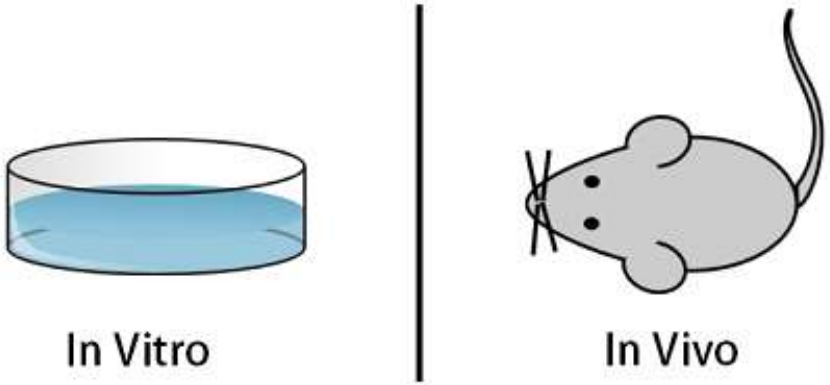
EXPERIMENTAL MODELS

i) In Vitro:

- *Cell culture*

ii) In Vivo:

- *Drug/pathogen-induced*
- *Surgically-induced*
- *Gene manipulation-induced*



WHY USING *IN VIVO* ANIMAL MODELS

"Research on relevant, carefully designed, well-characterized and controlled animal models will remain for a long time an essential step for fundamental discoveries, for testing hypotheses at the organism level and for the validation of human data."

Barré-Sinoussi F, Montagutelli X. Animal models are essential to biological research: issues and perspectives. *Future Sci OA*. 2015;1(4):FSO63. Published 2015 Nov 1. doi:10.4155/fso.15.63

WHY USING *IN VIVO* ANIMAL MODELS

Research studies at multiple levels, in healthy or diseased conditions:

- molecules
- cells
- organs

→ *in vitro* approaches (e.g., cell culture)

- physiological functions and systemic interactions between organs → requires a whole organism

Example:
most hormonal regulations,
dissemination of microorganisms during infectious diseases
influence of the intestinal microorganisms on immune defense
development of brain functions.

→
- ✓ no *in vitro* model currently available to fully recapitulate these interactions;
- ✓ investigations on humans and animals are still necessary

Hypotheses and models can emerge from *in vitro* studies but they must be tested and validated in a whole organism, otherwise they remain speculative. All levels of investigations are required to get a full description and understanding of the mechanisms.

Barré-Sinoussi F, Montagutelli X. Animal models are essential to biological research: issues and perspectives. *Future Sci OA*. 2015;1(4):FSO63. Published 2015 Nov 1. doi:10.4155/fso.15.63

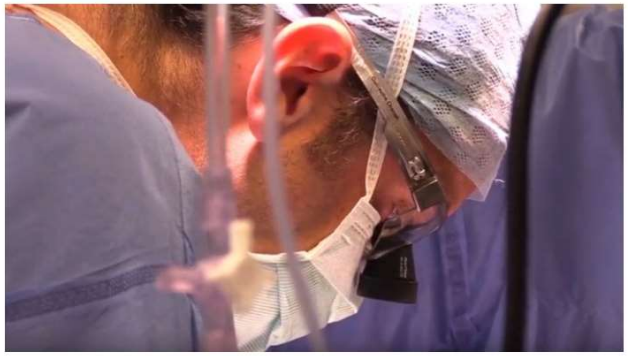
WHY USING *IN VIVO* ANIMAL MODELS

For human health

Human Health Timeline

For animal health

Life Stories



Human diseases and treatments

40 reasons why we need animal research

https://www.understandinganimalresearch.org.uk/why

WHY USING *IN VIVO* ANIMAL MODELS

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HUMAN studies *IN VIVO*;

- ✓ *important ethical issues involved*
- ✓ *mostly restricted to* neuroimaging
- ✓ difficult to get large and reliable samples
- ✓ difficult to get adequate controls (non-diseased humans do not usually want to participate!!)
- ✓ Longitudinal studies: partcipants give up participating in the study during the course of study duration

POST-MORTEM HUMAN tissue:

- ✓ has some limitations:
  - Involves ETHICAL concerns and FAMILY CONSENT;
  - Tissue is most of the times in BAD FIXATION CONDITIONS and/or in BAD conditions for further processing;
  - Not always easy to obtain in adequate number for statistical analysis

WHY USING *IN VIVO* ANIMAL MODELS

ANIMAL MODELS

ADVANTAGES

- ✓ Allows PERFORMING certain studies that are NOT POSSIBLE in HUMAN studies *IN VIVO (allow mostly* neuroimaging): pharmacology, use of biological tissue for expression studies, gene manipulation, drug screening, etc;
- ✓ Allows evaluating CIRCUITS and CELLS BEHAVIOUR in interaction with the whole system(s) instead of studying isolate cell types – particularly important in the nervous system;
- ✓ REPRODUCTIBILITY of the experimental conditions;
- ✓ Possible to use ADEQUATE CONTROLS;

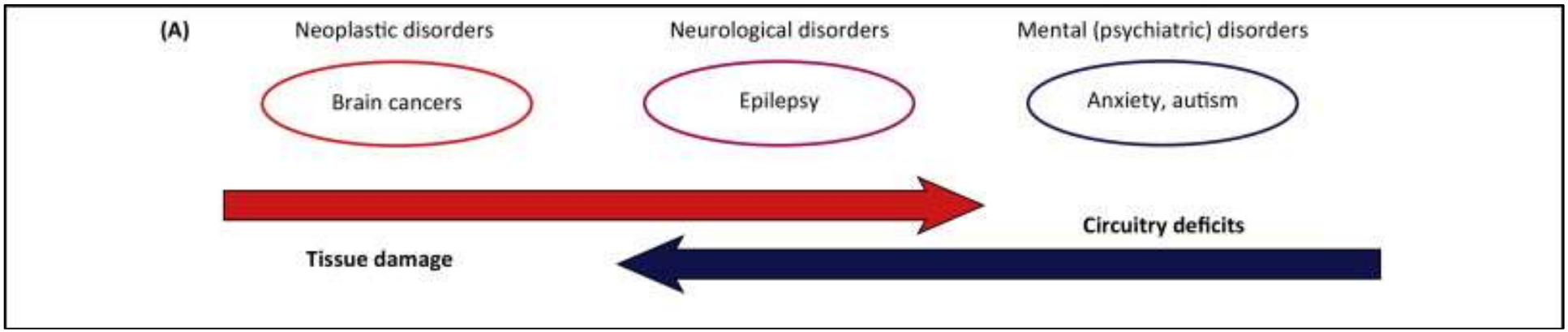
WHY USING *IN VIVO* ANIMAL MODELS

ANIMAL MODELS

DISADVANTAGES

- ✓ SYMPTOMATOLOGY in animals is NOT always equal to that observed in Human
- ✓ Need of VALIDATION:
  - Clinical symptomatology
  - Modelling and evaluating certain HUMAN behaviours: NOT always EASY
  - Testing animal BEHAVIOUR is NOT always EASY, OBJECTIVE and "TRANSLATABLE" into human behaviour
  - Search for MOLECULAR/BIOCHEMICAL markers similar to those observed in post-mortem human tissue (e.g.: amyloid plaques in Alzheimer's Disease models)

IN VIVO ANIMAL MODELS IN NEUROSCIENCES

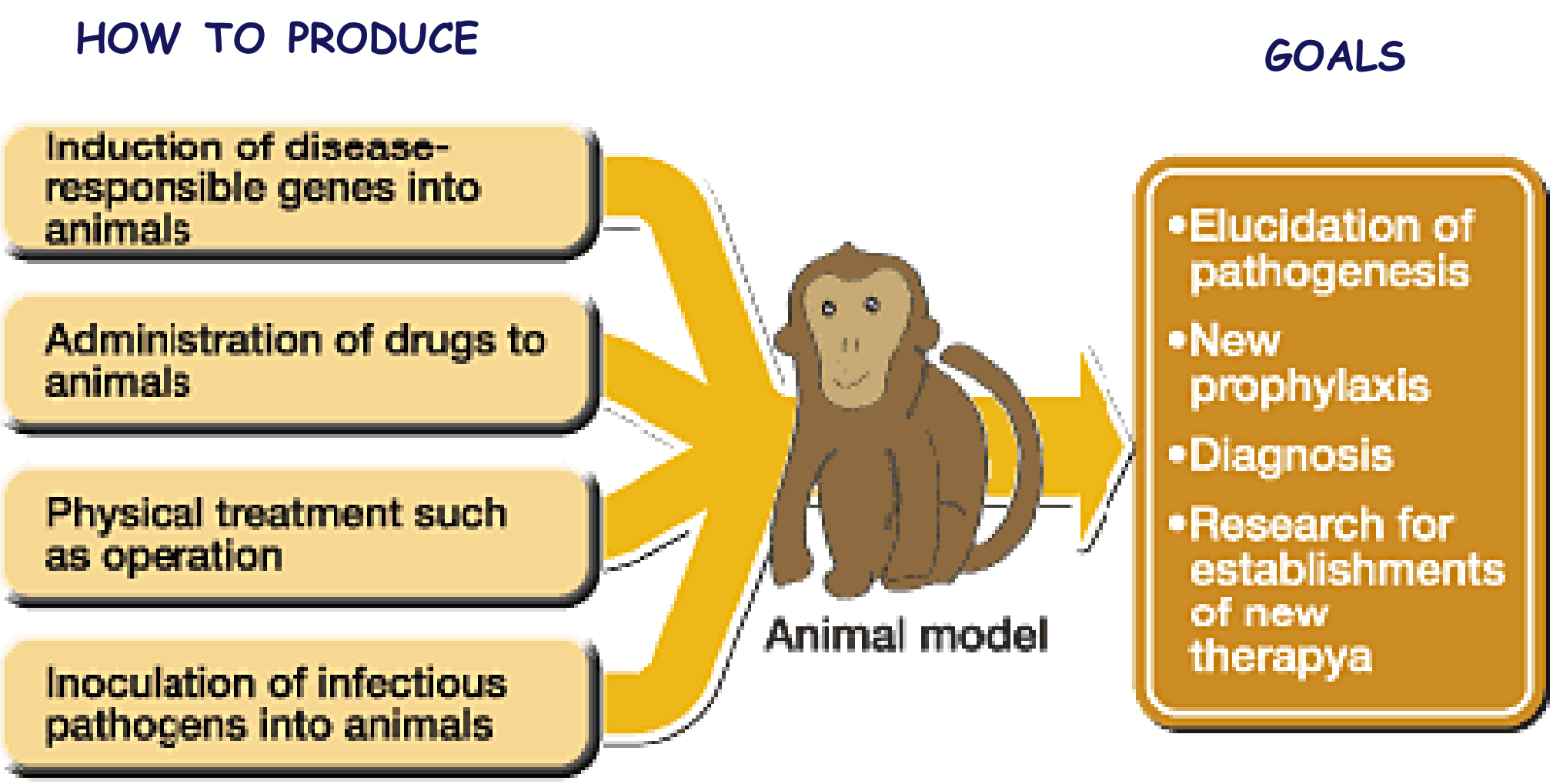


NEURODEGENERATIVE DISORDERS, CHRONIC PAIN

DEPRESSION, OBSESSIVE - COMPULSIVE DISORDER (OCD)

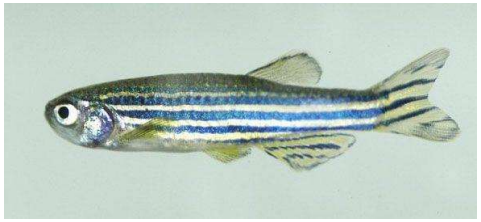
PRODUCTION

IN VIVO ANIMAL MODELS



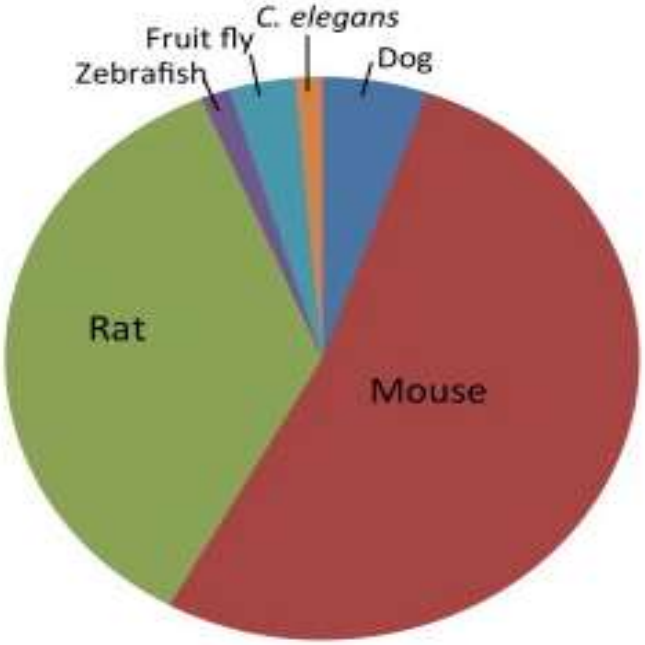
ANIMAL SPECIES

RAT



ZEBRAFISH

DROSOPHILA

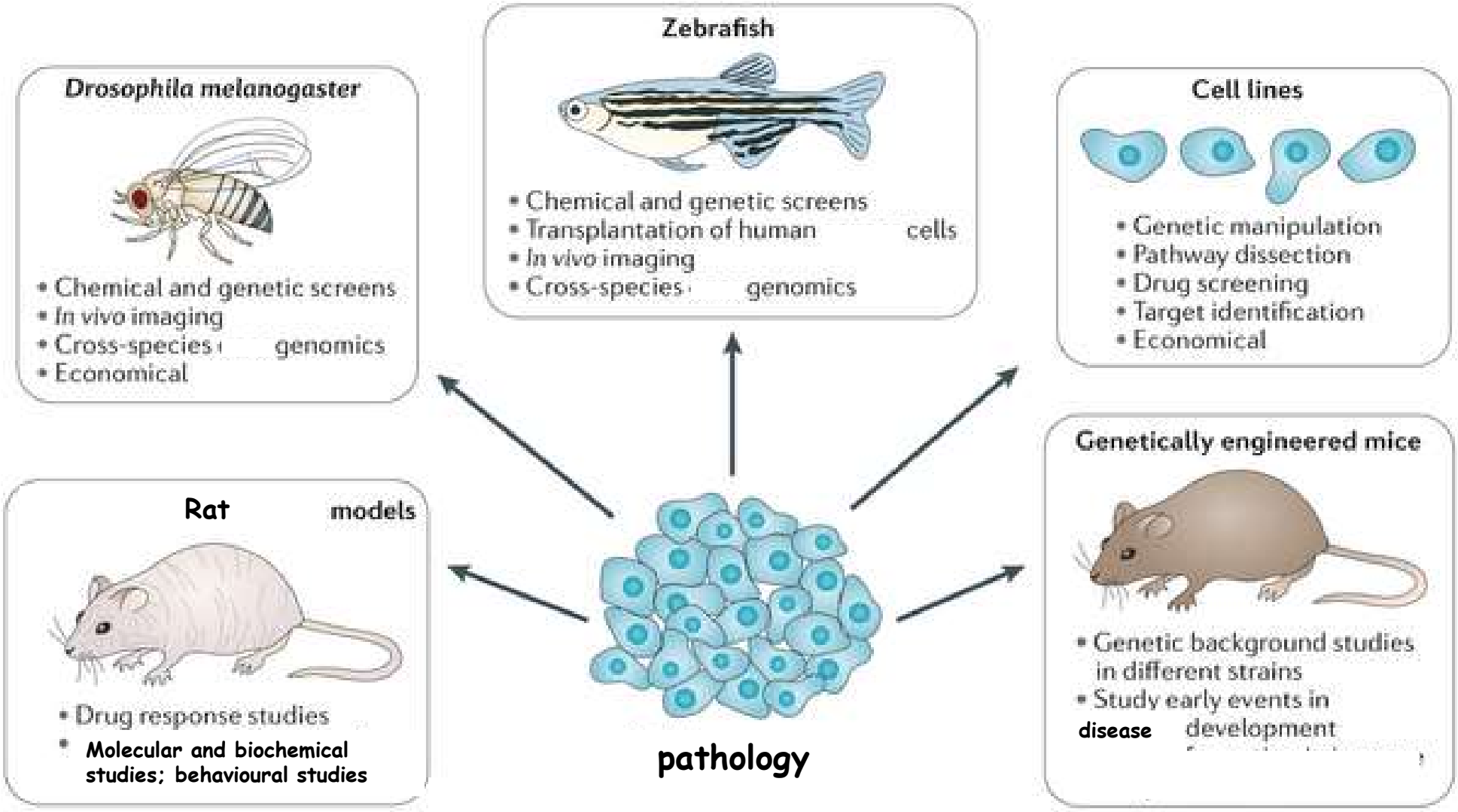


C. ELEGANS

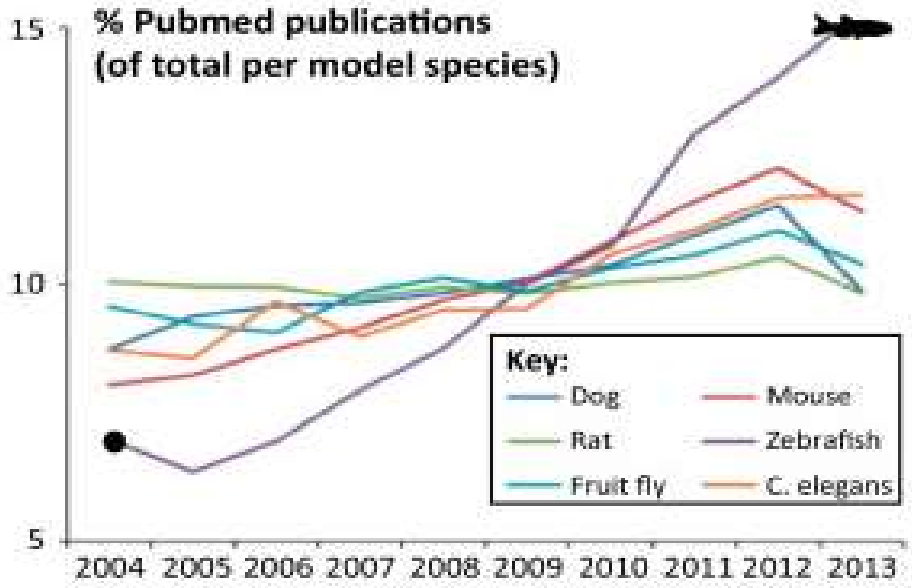
NON-HUMAN PRIMATES: CHIMPANZEE

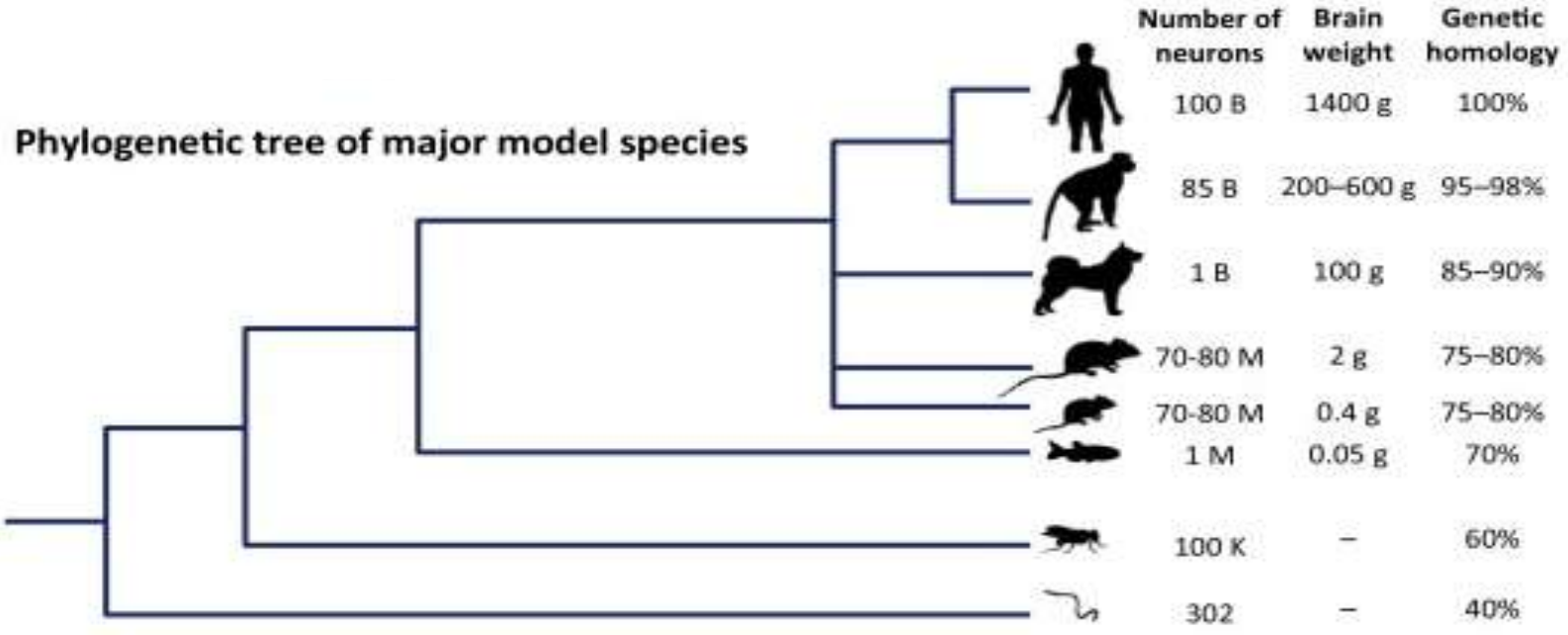
MOUSE

ANIMAL SPECIES



ANIMAL SPECIES





Problems regarding the use of animals for scientific purposes

1. Results obtained on animals are not necessarily confirmed in further human studies:

   - Genetic differences between a given animal species and humans:
     - over 95% of the genes are homologous between mice and humans but there are also differences for example in the members of genes families, in gene redundancies and in the fine regulation of gene-expression level

   - Genetic and physiological variations within each species or between closely related species:
     - Laboratory mice were developed as **inbred strains**, with highly homogeneous genetic composition to increase the reproducibility of results and the statistical power of experiments
     - Experiments in different strains often show distinct data

No single animal model is able to mimic a given human disease which is itself polymorphic between patients, but the differences between strains or species provide unmatched opportunity to understand disease development and differential host response, and to find new cures

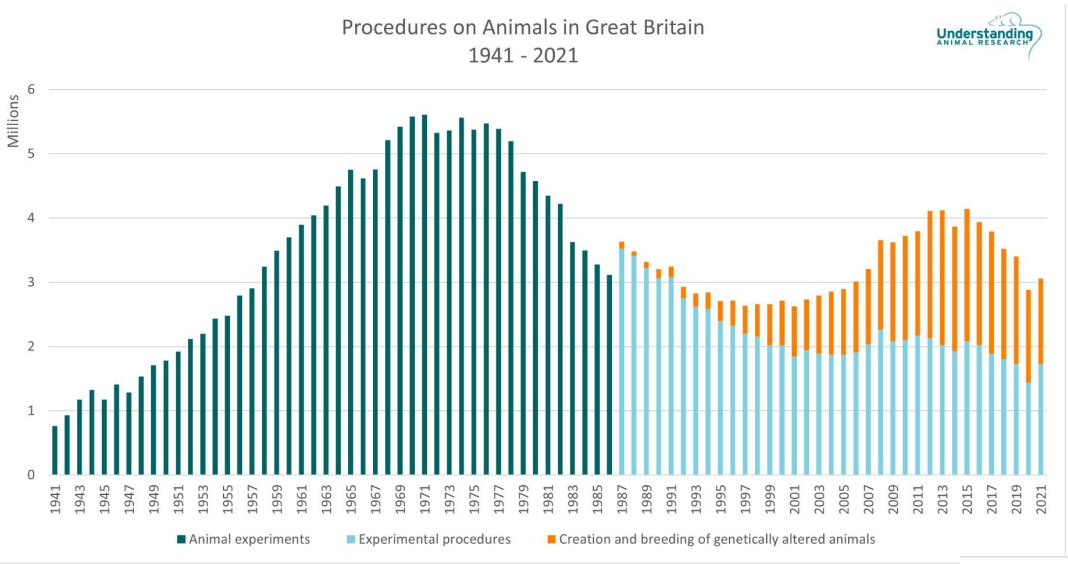
Problems regarding the use of animals for scientific purposes

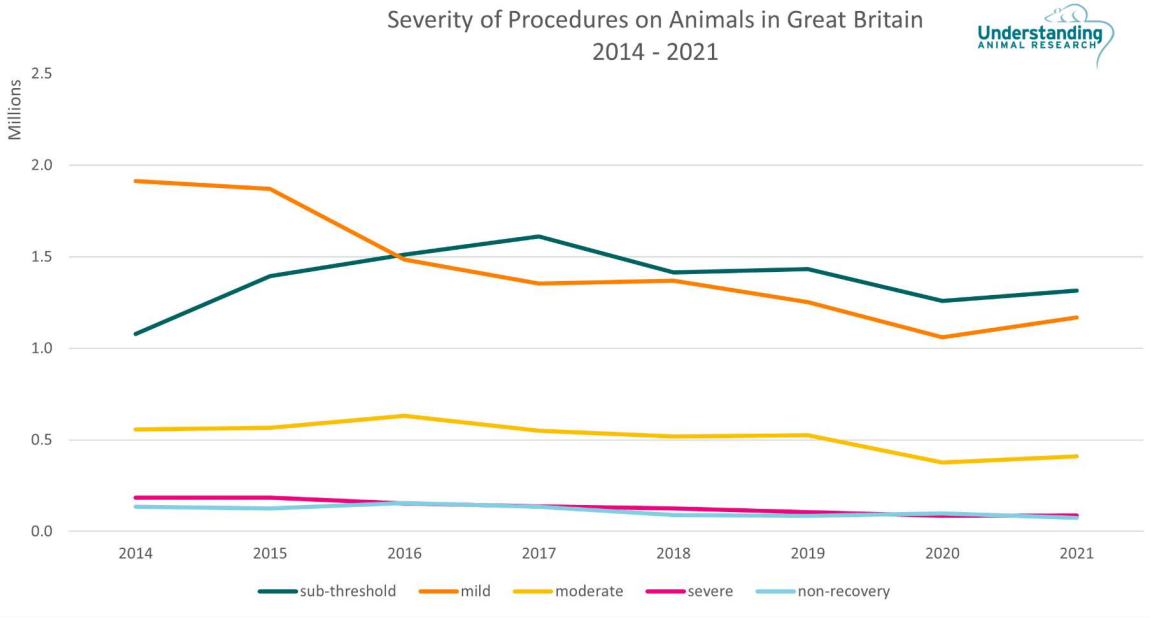
2. Animal protection and welfare (European Directive 2010/63/EU):

Three fundamental principles (the **three Rs**):

- animals must not be used whenever other, non-animal-based, experimental approaches are available, with similar relevance and reliability – **Replacement**
- the number of animals used must be adjusted to the minimum needed to reach a conclusion – **Reduction**
- all provisions must be taken throughout the procedures to minimize any harm inflicted to the animals - **Refinement**

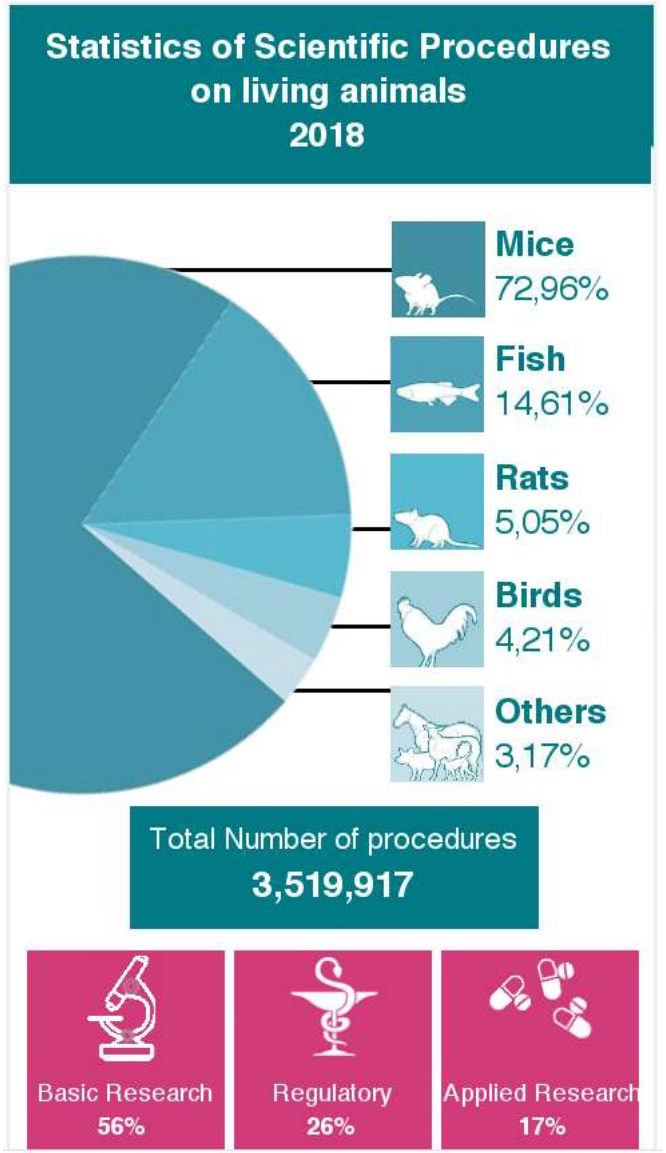
Science in living animals – the numbers (in UK)





https://www.understandinganimalresearch.org.uk/what-is-animal-research/numbers-animals#GB

Science in living animals – the numbers (in UK)



http://www.understandinganimalresearch.org.uk/news/communications-media/animal-research-numbers-in-2018/

Science in living animals – the numbers (in UK)

20 Years of Animal Research in Great Britain (2001 - 2021)

Understanding ANIMAL RESEARCH

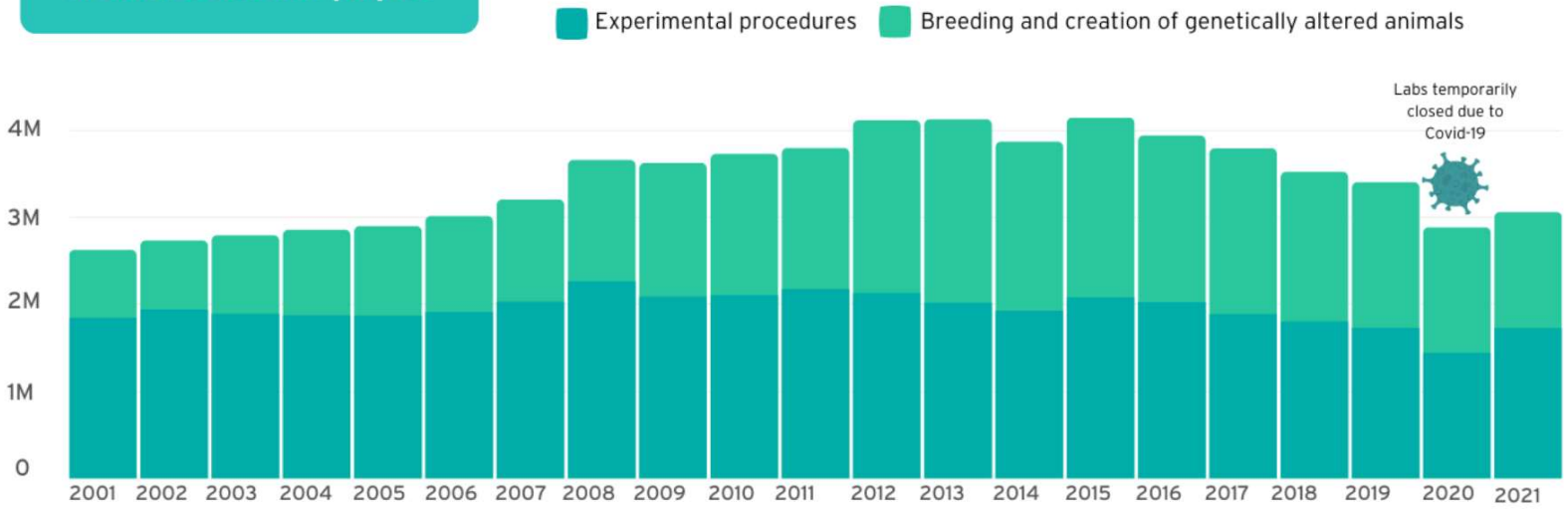
Scientific testing on animals

Organisations that carry out scientific, veterinary and medical research in Great Britain must record the number of procedures that are carried out on live animals each year to comply with the Animals (Scientific Procedures) Act 1986. Animals can only be used in scientific research when there is no viable alternative available.

Types of animal research

Basic | Translational | Regulatory | Environmental | Animals are not used to test cosmetic, household or tobacco products

Procedures and their purpose



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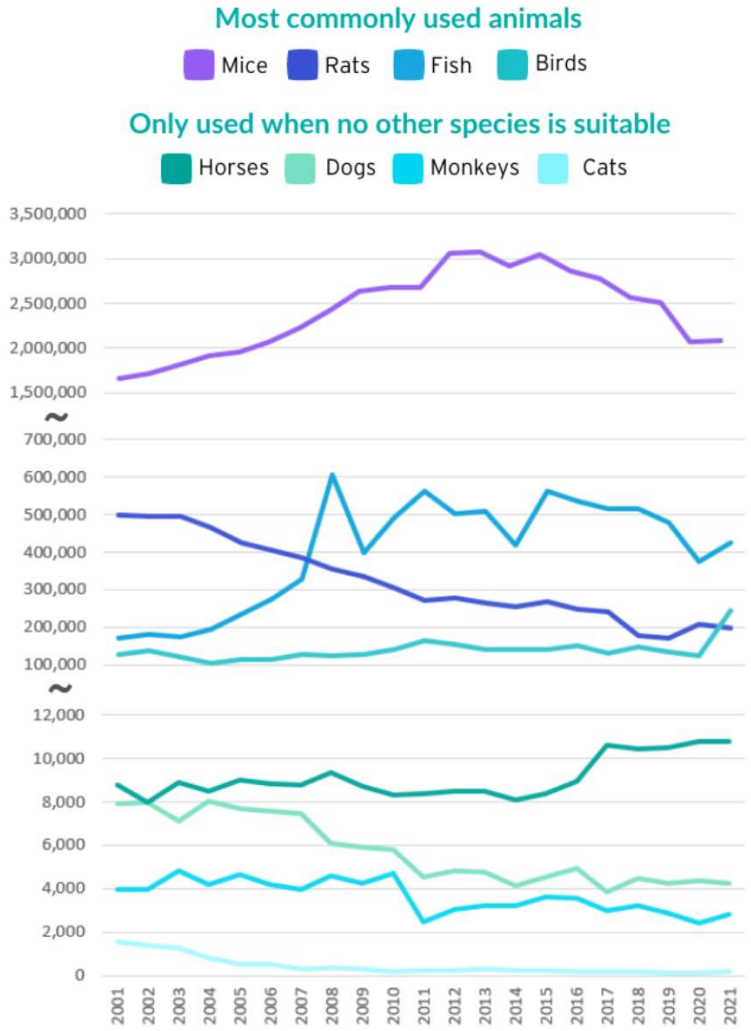
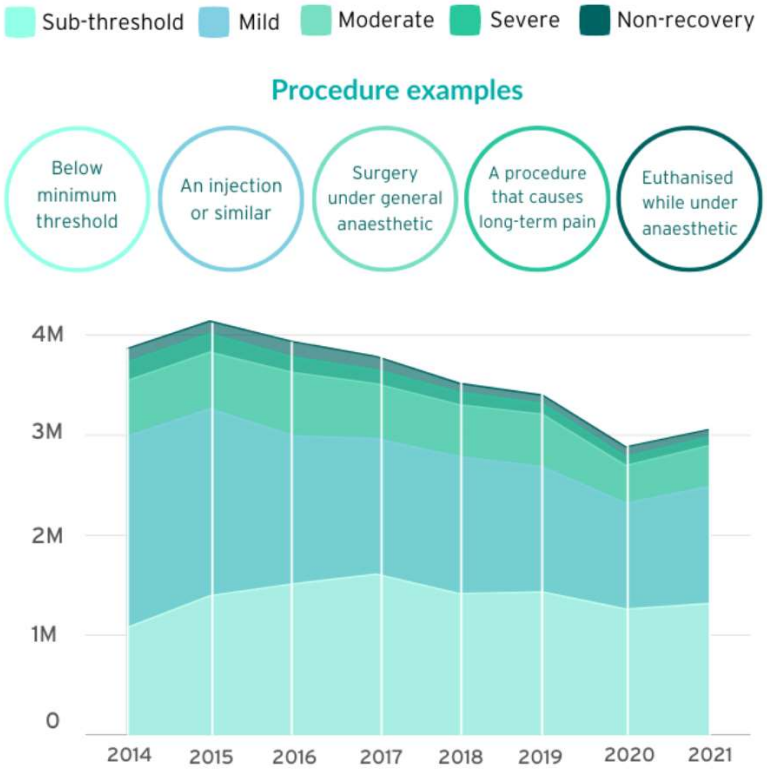
Science in living animals – the numbers (in UK)

Procedures by animal species



Procedures by severity

From 2014, procedures have been categorised by their retrospective severity. Procedures can be as mild as an injection or as severe as an organ transplant.



This data has been sourced from the UK Government Home Office annual report 'Statistics of scientific procedures on living animals, Great Britain' for the years 2001 - 2021.

https://www.understandinganimalresearch.org.uk/what-is-animal-research/numbers-animals#GB

Laboratory Mouse

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Education

Caltech, Oxford, Stanford, Harvard, MIT, Princeton, Cambridge, Imperial, Berkely, Chicago, Yale, ETH Zurich, Columbia, UPenn, John Hopkins, UCL, Cornell, Northwestern, UMichigan, Toronto, Carniege Mellon, Duke, UWashington, UTexas at Austin, GA Tech, Tokyo, Melbourne, Singapore, UBC, Wisconsin-Madison, Edinburgh, McGill, Hong Kong, Santa Barbara, Karolinska Institute, UMinnesota, Manchester ... and just about every other major university, medical school & research institution in the world.

Nobel Prizes

1905 - Transmission and treatment of TB
1906 - Structure of Nervous System
1907 - Role of protozoa in disease
1908 - Immunity to infectious diseases
1928 - Investigations on typhus
1929 - Importance of dietary vitamins
1939 - Discovery of antibacterial agent, Prontosil
1945 - Discovery of penicillin
1951 - Yellow fever vaccine
1952 - Discovery of streptomycin
1954 - Culture of the polio virus
1960 - Understanding of immunity
1970 - Understanding of neurotransmitters
1974 - Structural & functional organisation of cells
1975 - Tumour-viruses and genetics of cells
1977 - Hypothalamic hormones
1984 - Techniques of monoclonal antibody formation
1986 - Nerve growth factor and epidermal growth factor
1990 - Organ transplantation techniques
1992 - Regulatory mechanisms in cells
1996 - Immune-system detection of virus-infected cells
1997 - Discovery and characterisations of prions
1999 - Discovery of signal peptides
2000 - Signal transduction in the nervous system
2004 – Odour receptors and organisation of olfactory systems
2008 - Role of HPV and HIV in causing disease
2010 - Development of in vitro fertilization
2011 - Discoveries around innate and adaptive immunity
2012 - Reprogramming mature cells to pluripotent ones

CV of a Lifesaver

Overview

- Involved in around 75% of research
- Short life-span and fast reproductive rate means mice are suitable for studying disease across whole life cycle
- 98% of genes have comparable genes in humans
- Similar reproductive and nervous systems and suffer many of the same diseases as humans including cancer diabetes and anxiety
- Can be genetically modified to include human genes in enhance biological relevance
- Can act as an avatar for a human cancer to allow drug therapies to be trialled safely

Research Areas

Alzheimer's disease, anaesthetics, AIDS & HIV, anticoagulants, antidepressants, asthma, blindness, bone and joint disease, brain injury, breast cancer, cardiac arrest, cystic fibrosis, deafness/hearing loss, Down's sndrome, drugs for high blood pressure, transplant rejection, Hepatitis B, C & E, Huntington's disease, influenza, leukaemia, malaria, motor neurone disease, multiple sclerosis, muscular dystrophy, Parkinson's disease, prostate cancer, schistomiasis, spinal cord injury, stroke, testicular cancer, tuberculosis,

Contact

www.understandinganimalresearch.org.uk
www.animalresearch.info
www.amprogress.org
www.speakingofresearch.com

VALIDATION

VALIDITY OF ANIMAL MODELS

DEFINITION:

„(..) the agreement between a test score or measure and the quality it is believed to measure." (Kaplan RM, Saccuzzo DP: Psychological testing. Principles, applications, and issues. Pacific Grove: Brooks/Cole Publishing Company; 1997)

- ✓ One validates, not an animal model, but the interpretation of the data arising from this model.
- ✓ No animal model can be valid in all situations, for all purposes. Validity is restricted to a specific use of the model.

OBJECTIVE OF VALIDATION:

- ✓ To increase the probability that the results in the model predict similar results in humans
- ✓ To improve the confidence in a model, i.e. to evaluate its plausibility and consistency

VALIDITY OF ANIMAL MODELS

CRITERIA FOR VALIDATION

✓ **Willner, 1991** (Behavioural models in Psychopharmacology. Paul Willner. Cambridge University Press, Cambridge, 1991)

Three criteria:

1) *Face validity:* does the model phenotype recapitulates the clinical manifestations of the disease of interest?
2) *Construct validity:* do experimental manipulations produce mechanisms of pathogenesis similar to those observed in the disease of interest? - This criterion may be more difficult to achieve since the first criterion can be achieved without the pathological/molecular mechanism being similar
3) *Predictive validity:* is the model able to predict pathological consequences of manipulations which are known to exacerbate or mitigate the physiological conditions in the disease of interest (eg, response to therapeutic drugs) - our *ultimate goal*

VALIDITY OF ANIMAL MODELS

CRITERIA FOR VALIDATION

✓ **van der Staay et al., 2009** (F Josef van der Staay, Saskia S Arndt and Rebecca E Nordquist; Evaluation of animal models of neurobehavioral disorders, Behavioral and Brain Functions 2009, 5:11 doi:10.1186/1744-9081-5-11)

Five criteria:

1) Reliability and replicability (internal validity): **NEW**

- **reliability** indicates how consistent an assessment/ testing device/method is;
- **replicability or reproducibility** is the degree of accordance between the results of the same experiment performed independently in the same or different laboratories

Internal validity: **High reliability and replicability**

Reflects the quality of the experimental evaluation of the animal model:

i) how well a study was performed
ii) how strictly putative confounding variables were controlled
iii) how confident one can be that the changes observed in the dependent variable(s) are caused by experimentally manipulating the independent variable(s), and not by confounding factors

VALIDITY OF ANIMAL MODELS

1) Reliability and replicability (internal validity) - NEW

Confounding factors

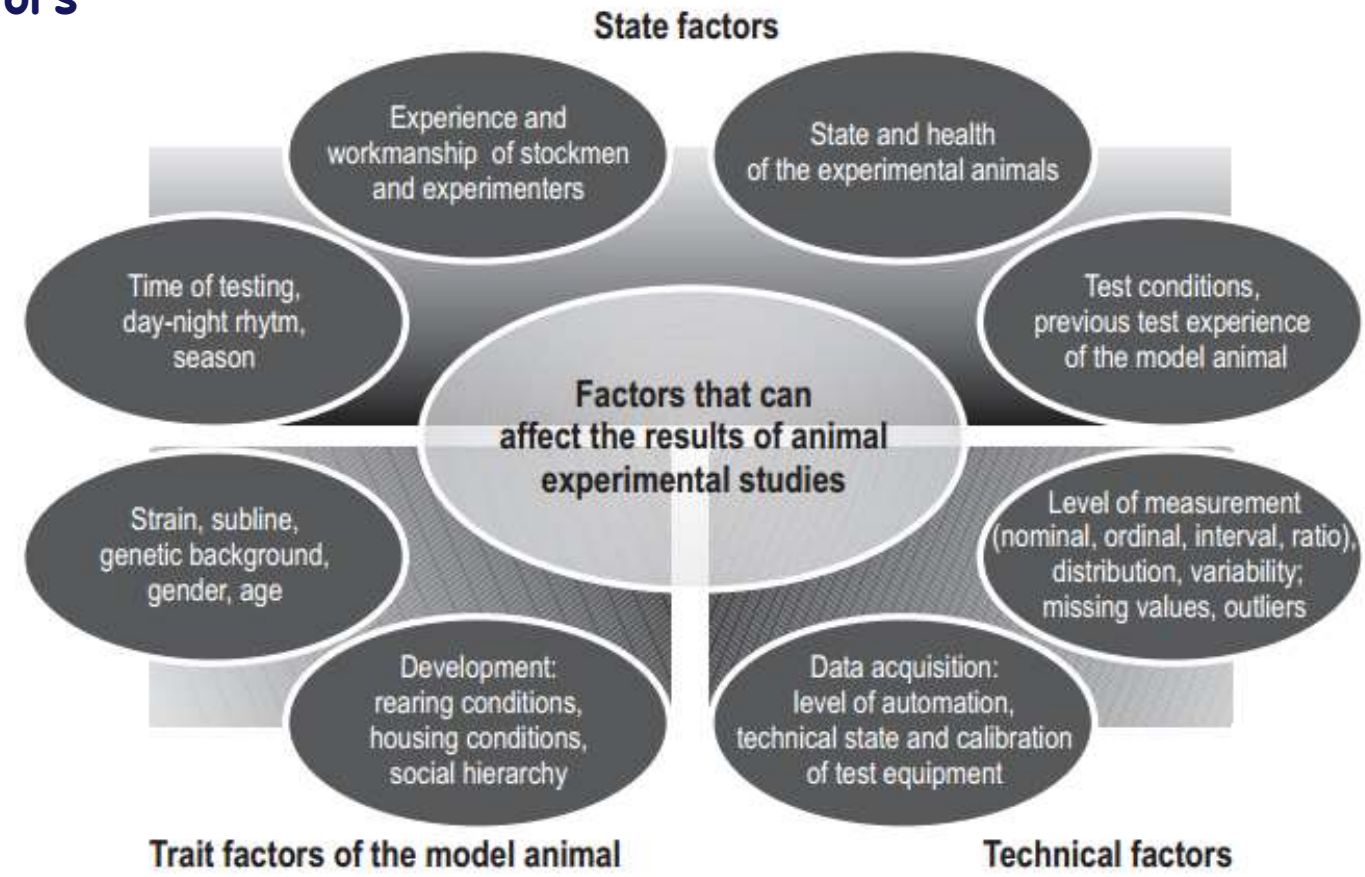

Figure 3
Factors affecting the results of animal experimental studies. In order to increase internal validity, care must be taken to identify, control and/or eliminate confounding factors (after [83]).

VALIDITY OF ANIMAL MODELS

CRITERIA FOR VALIDATION

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2) Face validity: is the degree of descriptive similarity between, for example, the behavioural dysfunction seen in an animal model and in the human affected by a particular neurobehavioral disorder.

- ✓ proposed to constitute a major or even the most important criterion for model evaluation
- ✓ a too strong emphasis on face validity may be an obstacle for developing animal models using phylogenetically lower animal species as the similarity of symptoms is generally higher in species that are phylogenetically closer to humans

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3) Predictive validity: allows extrapolation of the effect of a particular experimental manipulation from one species to other species, including humans, and from one condition (e.g. the laboratory) to the other (e.g. the 'Real World'), or from one testing time point to another;

In psychopharmacology: refers to the ability of a drug screening or an animal model to correctly identify the efficacy of a putative therapeutic

VALIDITY OF ANIMAL MODELS

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4) Construct validity: do experimental manipulations produce mechanisms of pathogenesis similar to those observed in the disease of interest? - This criterion may be more difficult to achieve since the face validity can be achieved without the molecular mechanism being similar or knowing it

- ✓ Measures the degree of similarity between the mechanisms underlying the behaviour in the model and that underlying the behaviour in the condition that is being modelled.

- ✓ Is a theory-driven, experimental substantiation of the behavioural, pathophysiological, and/or neuronal components of the model

VALIDITY OF ANIMAL MODELS

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5) External validity (generalizability): NEW

the extent to which the results obtained using a particular animal model can be generalized/applied to and across populations (and eventually, species) and environments, or "the extent to which experimental findings make us better able to predict real-world behavior"

VALIDITY OF ANIMAL MODELS

5) External validity (generalizability):

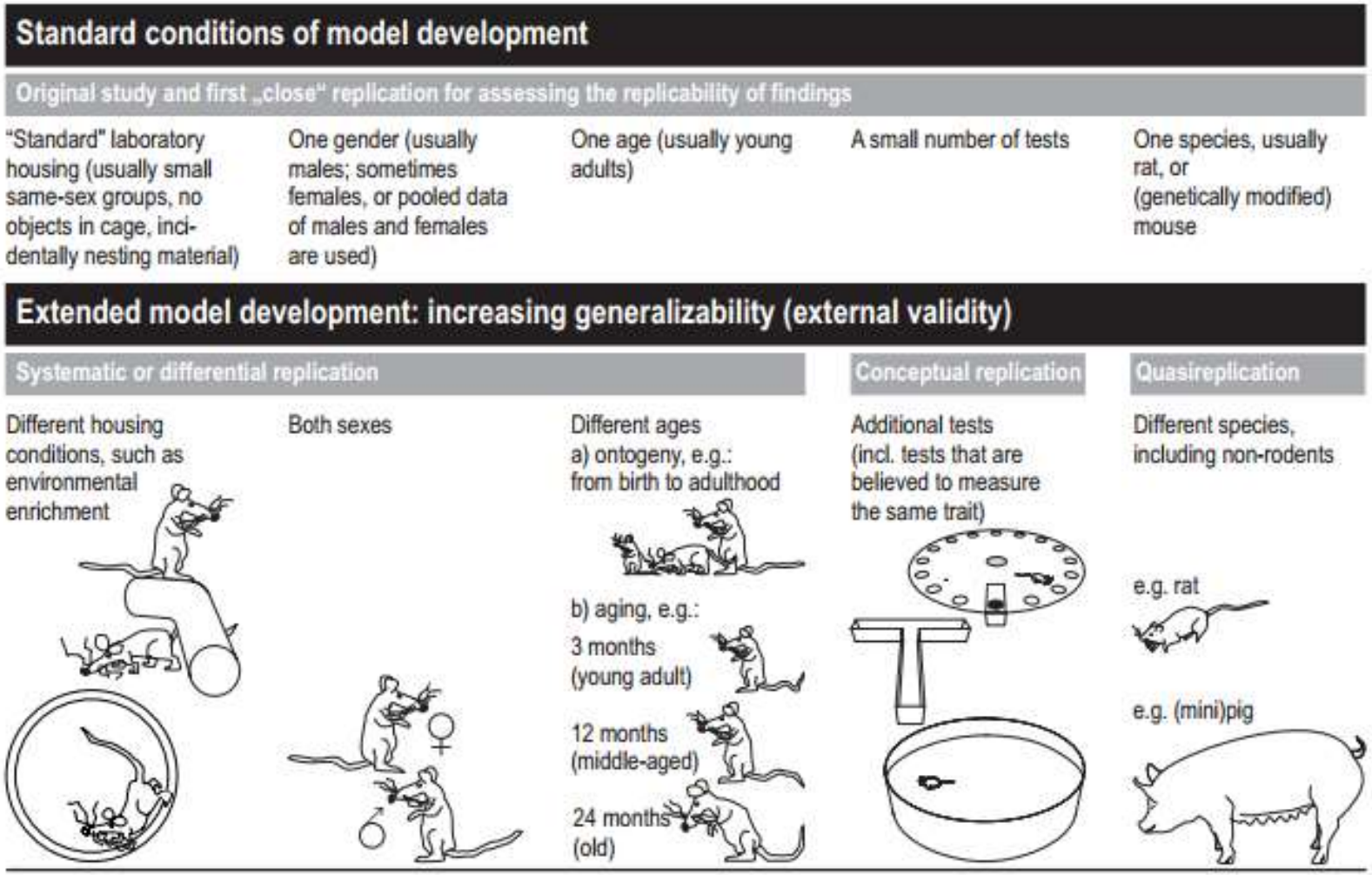

Figure 2
Increasing the generalizability (or external validity) of a model. This can be achieved by assessing the effects of rearing and housing conditions (first column) through partial, systematic, and conceptual replications (see Fig. 1). Gender effects (second column), ontogenetic and aging effects (third column) should be an integral part of the model building process. In addition, the battery of tests for assessing the dependent variables (see Table 1, Part B, second and third column) should be extended and should include tests that are believed to measures the same trait/construct (fourth column; e.g. the Barnes maze [78], the T-maze [80], and the Morris maze [79] may be used to assess spatial working memory performance). Quasireplications are not part of the model building process, but may be used for assessing the generalizability across species.

CHOICE OF SPECIES/ANIMAL MODEL

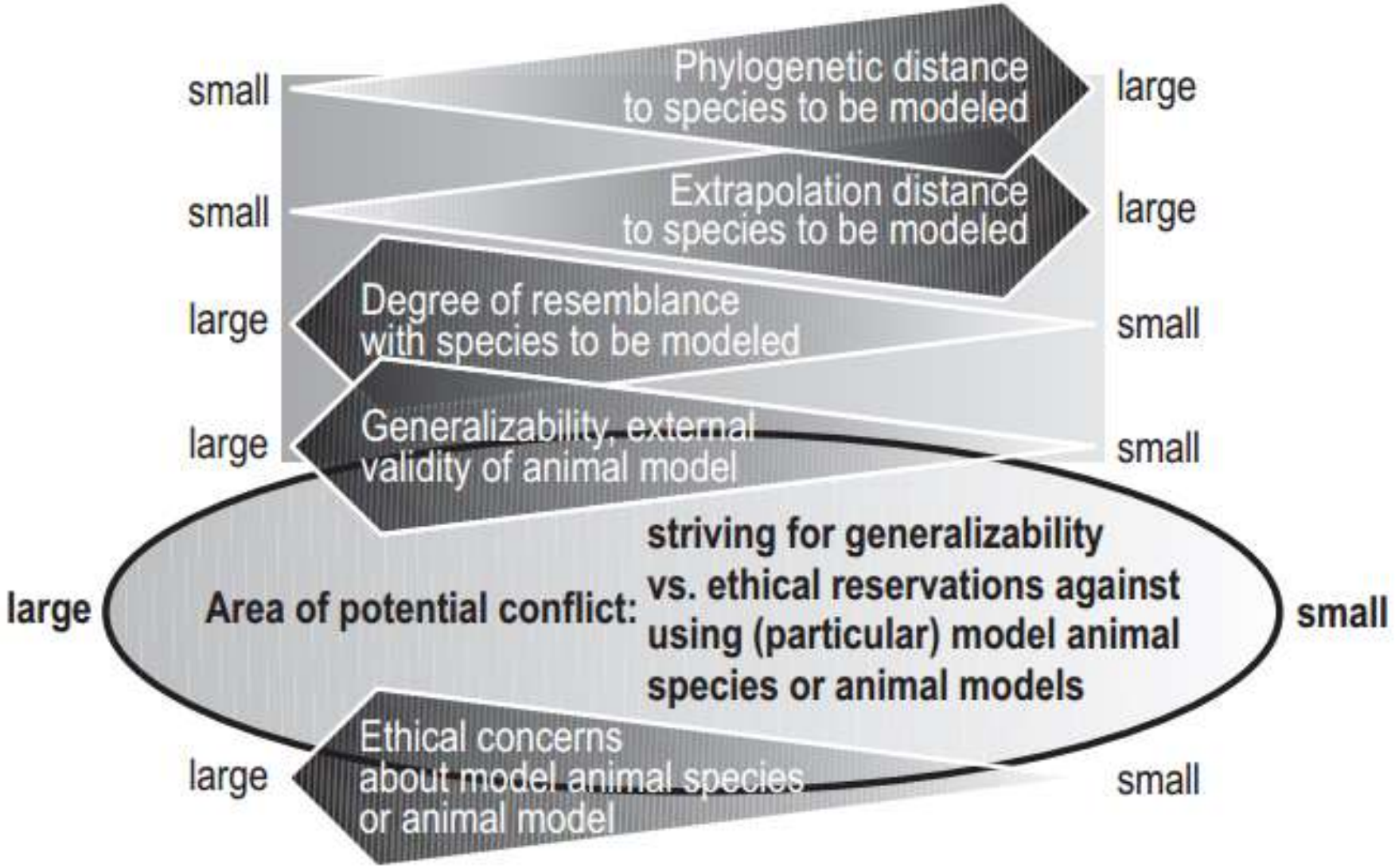

Figure 4
Area of potential conflict between the choice of a specific model animal species/animal model and the expected degree of generalizability of the results obtained and the ethical reservations against using a particular model animal species/animal model.

MODEL EVALUATION

STEPS:

- ✓ **Relevance** of the model
- ✓ **Ethical concerns**: the degree of discomfort shown by the model animal as consequence of the experimental manipulations is acceptable, considering the expected gain of knowledge
- ✓ **Internal validity**: data obtained in the model are reliable and replicable
- ✓ **Face validity**
- ✓ **Predictive validity**
- ✓ **Construct validity**: satisfy criteria developed by basic and clinical experts
- ✓ **Generalizability/external validity**: validity across different housing conditions and laboratories, across different behavioural tests, etc

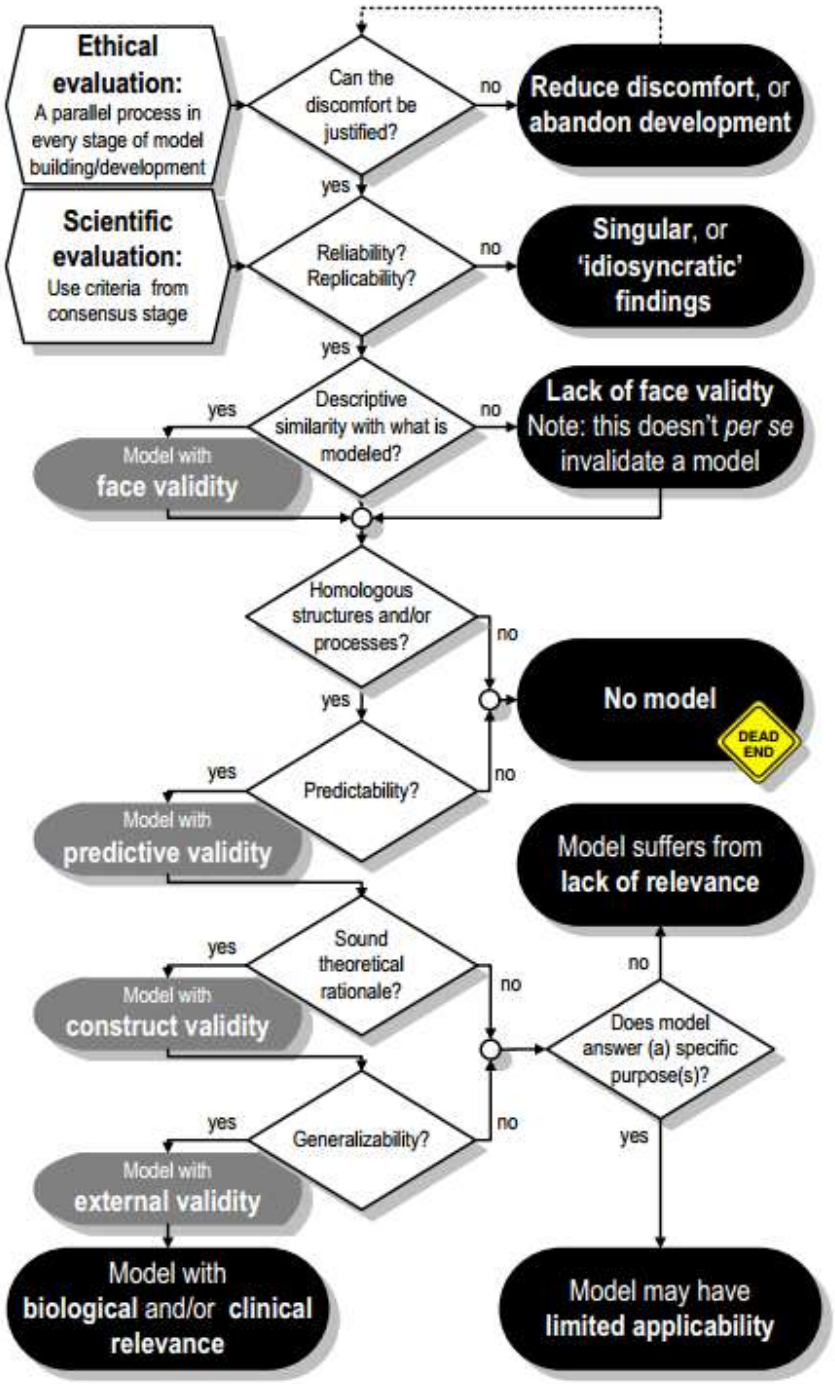

Figure 6
Evaluation of an animal model using ethical and scientific evaluation criteria.

RECOMMENDED LITERATURE & WEB PAGES

- F Josef van der Staay, Saskia S Arndt and Rebecca E Nordquist; Evaluation of animal models of neurobehavioral disorders, Behavioral and Brain Functions 2009, 5:11 doi:10.1186/1744-9081-5-11

- Barré-Sinoussi F, Montagutelli X. Animal models are essential to biological research: issues and perspectives. Future Sci OA. 2015;1(4):FSO63. Published 2015 Nov 1. doi:10.4155/fso.15.63

- Ericsson AC, Crim MJ, Franklin CL. A brief history of animal modeling. Mo Med. 2013;110(3):201–205.

- https://speakingofresearch.com/facts/the-animal-model/animal-research-by-species/

- http://www.understandinganimalresearch.org.uk