

Use of Experimental Models in Neuroscience: Applications and Potential

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Background

- ❖ Why use animals?
- ❖ Animal Rights
- ❖ Legislation covering animal experiments
- ❖ Numbers and types of animals used in experimental research
- ❖ Animal models in neuroscience
- ❖ Sources of information

Please stand up

- Sit down if you have ever:
 - had an organ transplant
 - had general anaesthetic
 - used an asthma inhaler
 - taken pills for hay fever
 - taken antibiotics
 - had a vaccination
 - taken headache tablets



What do they all have in common?

animals

Why use animals?

Use of animals in research

Animals are widely used in experiments

- Understanding normal physiology
- Understanding gene function
- Animal models of human disease
- Research into animal disease
- Safety testing and toxicology

There are a range of views on whether this is acceptable

Worldwide requirements to do animal studies before licensing human medicines

- ADME, PK
- Safety pharmacology
- Toxicology (2 species)
 - Acute
 - Chronic
- Teratogenicity
- Carcinogenicity

Animal trials are not always predictive

BBC NEWS

Six taken ill after drug trials

Six men remain in intensive care after being taken ill during a clinical drugs trial in north-west London.

The healthy volunteers were testing an anti-inflammatory drug at a research unit based at Northwick Park Hospital when they suffered a reaction.

Relatives are with the patients, who suffered multiple organ failure. Two men are said to be critically ill.

An investigation has begun at the unit, run by Parexel, which said it followed recommended guidelines in its trial.

The men were being paid to take part in the early stages of a trial for the drug to treat conditions such as rheumatoid arthritis and leukaemia until they were taken ill on Monday within hours of taking it.

Eight volunteers were involved, but two were given a placebo at the unit which is on Northwick Park Hospital's grounds but is run independently.



- Cats and aspirin
- Guinea pigs and penicillin
- Failure to spot effects of thalidomide

The Thalidomide Story



Thalidomide – the sleeping pill

- *synthesised 1953;
prescribed for a "safe, sound
sleep"*



- *drug was also found to cure
morning sickness in pregnant
women*

The Thalidomide Story

- *teratogenic - severe congenital malformation or foetal deaths*
- *harmed developing foetus only if taken in the first trimester*
- *intake, severity and type of resulting malformations follow the recognised sequence of organ development*
- *detectable in rabbit reproduction studies*

	RAT	RABBIT	MONKEY	HUMAN
Blastocyst formation	3-5	2.6-6	4-9	4-6
Implantation	5-6	6	9	6-7
Organogenesis	6-17	6-18	20-45	21-56
Primitive streak	9	6.5	18-20	16-18
Neural plate	9.5	—	19-21	18-20
First somite	10	—	—	20-21
First branchial arch	10	—	—	20
First heartbeat	10.2	—	—	22
10 Somites	10-11	9	23-24	25-26
Upper limb buds	10.5	10.5	25-26	29-30
Lower limb buds	11.2	11	26-27	31-32
Testes differentiation	14.5	20	—	43
Heart septation	15.5	—	—	46-47
Palate closure	16-17	19-20	45-47	56-58
Urethral groove closed in male	—	—	—	90
Length of gestation	21-22	31-34	166	267

*Developmental ages are days of gestation.

SOURCE: Adapted from Shepard (1992, 1998), with permission.

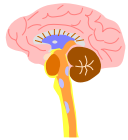
- *Estimations of ca. 10,000 children affected*

Led to stricter guidelines for medicine safety, efficacy and registration



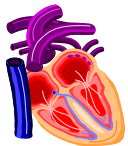
"phocomelia" →

What is often measured?



Central nervous system

In vivo rodent Irwin or Functional Observational Battery (FOB): Motor activity, behavioural changes, coordination, sensory/motor reflex responses, body temperature (e.g. using FOB).



Cardiovascular system

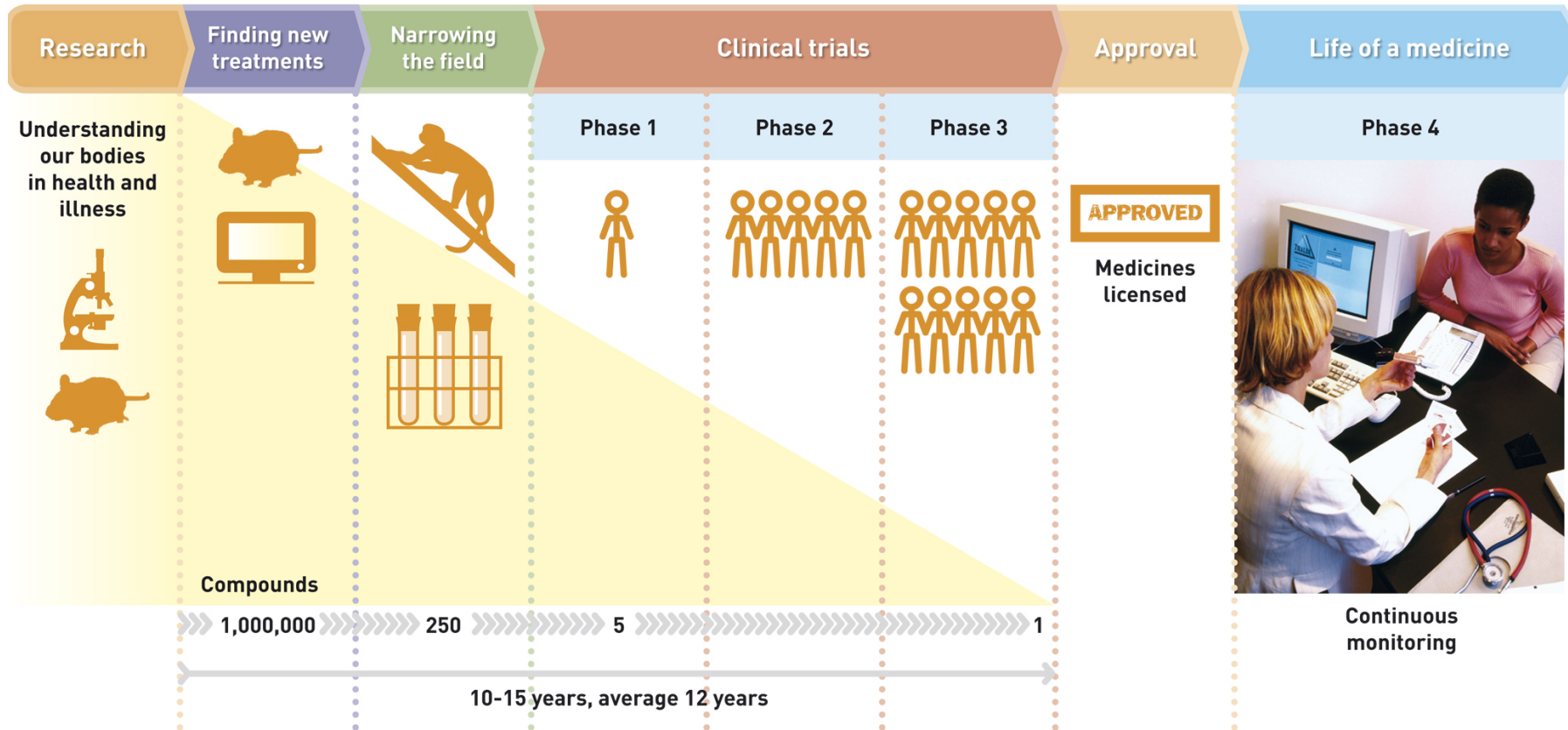
In vitro hERG (IKr) study and In vivo non-rodent (usually telemetry): Blood pressure, heart rate, ECG; In vivo, in vitro and/or ex vivo methods incl. methods for repolarization and conductance abnormalities should also be considered.



Respiratory system

In vivo rodent generally using plethysmography: Respiratory rate and tidal volume or hemoglobin oxygen saturation. Clinical observation of animals is generally not adequate.

The making of a Medicine



Animals Rights

What are human rights?

- **Human rights** refer to the "basic rights and freedoms to which all humans are entitled."
- Examples of human rights include civil and political rights, such as the right to life and liberty, freedom of expression, and equality before the law; and economic, social and cultural rights, including the right to participate in culture, the right to food, the right to work, and the right to education.

Basic animal rights



"The question is not, can they reason? nor, can they talk? but, can they suffer?"

Jeremy Bentham 1789 *Introduction to the Principles of Morals & Legislation*

South Cloisters of the main building of UCL

Extended animal rights

Animal rights means that animals deserve certain kinds of consideration—consideration of what is in their own best interests regardless of whether they are cute, useful to humans, or an endangered species and regardless of whether any human cares about them at all (just as a mentally-challenged human has rights even if he or she is not cute or useful or even if everyone dislikes him or her). It means recognizing that animals are not ours to use—for food, clothing, entertainment, or experimentation.

Clash of perspective

Outcomes:

benefits can outweigh harms

Intrinsic concerns:

animals are not means to ends



Range of views

*Absolute
Dominion*

RDS

UFAW

RSPCA

*ALF
BUAV
NAVS*

Speciesist

“Troubled middle”

Abolitionist

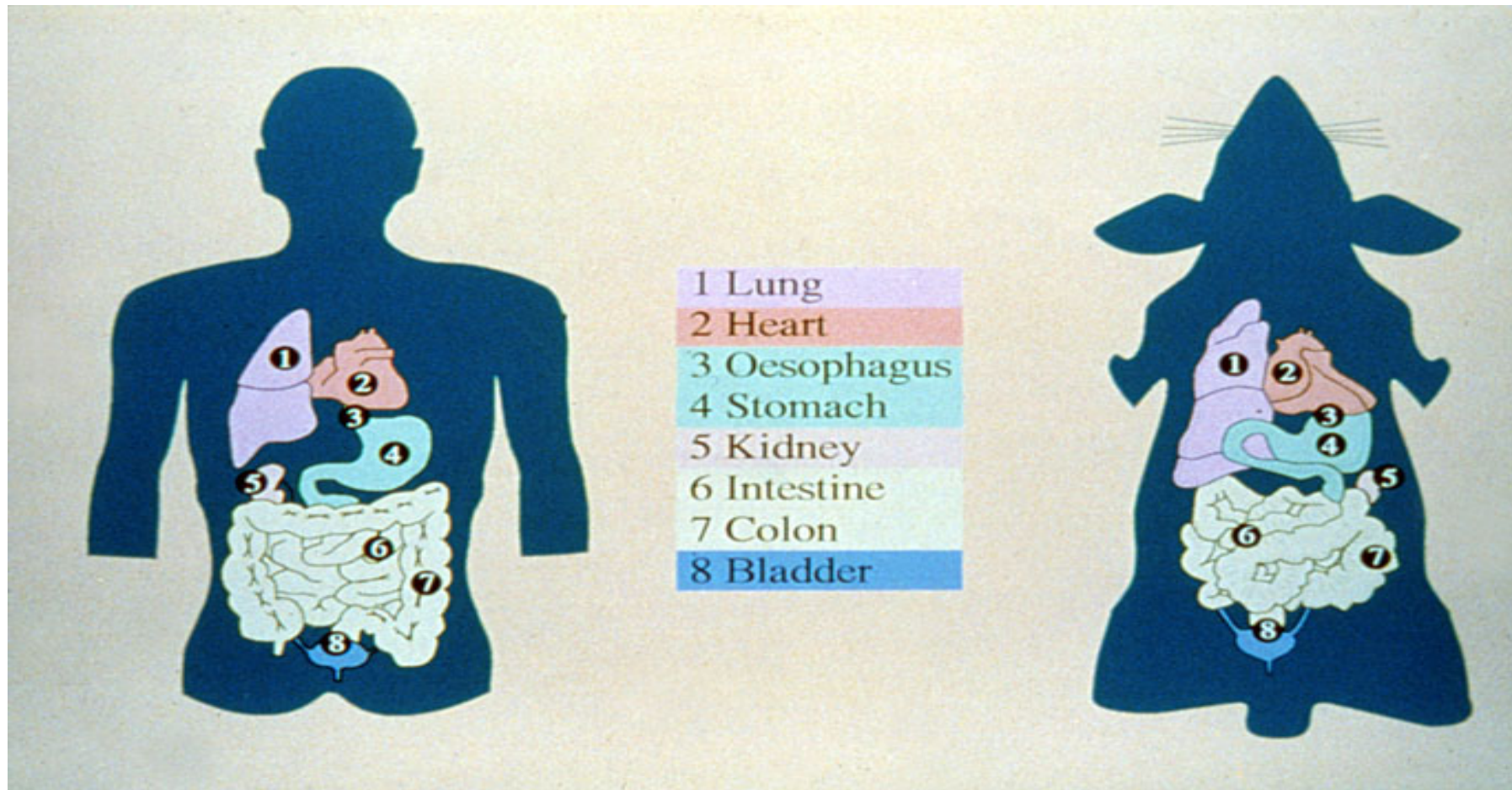
But aren't animals and people different?

There are important differences,
that is why all medicines are
triallyed on humans too.

But ...



More similarities than differences



The 3Rs

Replacement

Do not use an animal if there is an alternative.

Reduction

Use the smallest number of animals possible.

Refinement

Do everything you can to make the lives of research animals as painless, pleasant and stress-free as possible.



*The National Centre for the
3Rs*

www.nc3rs.org.uk

Legislation covering animal experiments

Legislation

- Cruelty to Animals Act (1876)
- All animal research in the UK is regulated by the Animal (Scientific Procedures) Act (ASPA), 1986.
- All research using animals must be authorised by the Home Office.
- All animal facilities are regularly inspected, as often as once a month and usually without warning.
- Inspectors must be qualified vets or doctors.



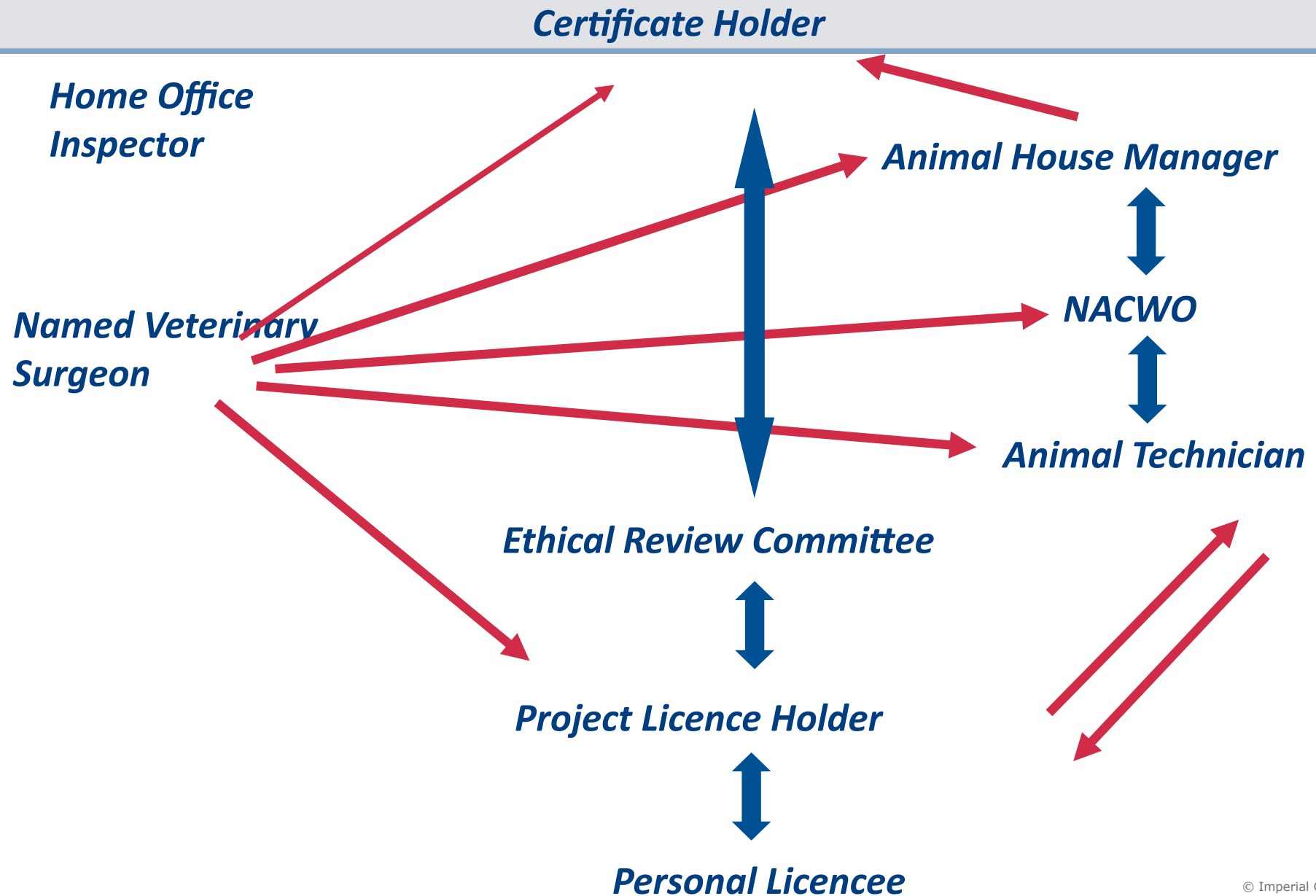
Animal (Scientific Procedure) Act 1986

All animal research requires three separate licences:

- an animal facility licence
- an individual project licence
- a personal licence to show you have the skills to care for animals properly.

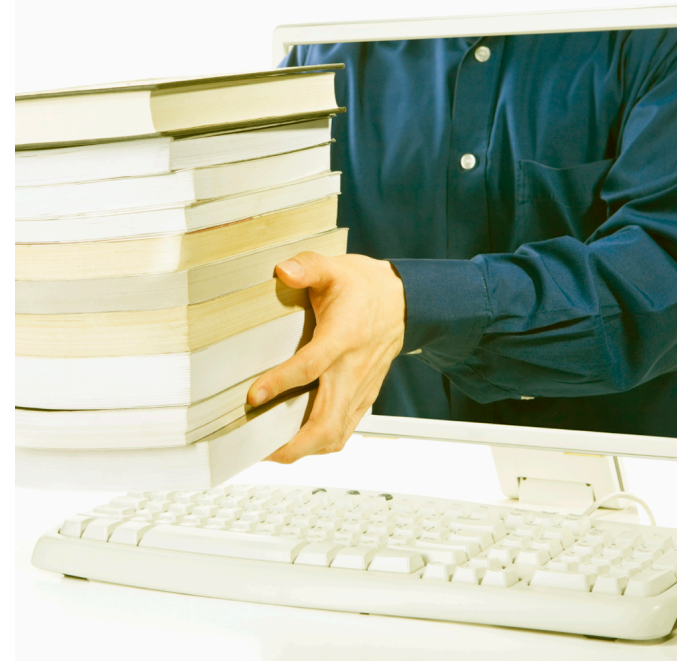


No research can be given a licence if there is a practical alternative to animal research.



Ethical Review

- Before you can apply for a licence, you have to hold an 'ethical review'. The review looks at:
 - whether the research is justified
 - whether similar work is being done elsewhere
 - whether there are alternatives to using animals or ways to reduce the number of animals to be used.



Ethical Review

The ethical review involves, as a minimum:

- the Named Veterinary Surgeon
- the Named Animal Care and Welfare Officer (NACWO)
- a representative of the project and personal licence holders



Non-scientists and community representatives are also encouraged to take part.

Comparison with other countries

- UK has the only complete national scheme for controlling all animal experiments
- Involves local institutional review as first step in licensing process
- In most other countries most if not all of the control is at the Institutional level (IACUC)
- EU attempting to harmonise European legislation – Directive 86/609

Numbers and types of animals used in experiments

Number of research animals used in UK

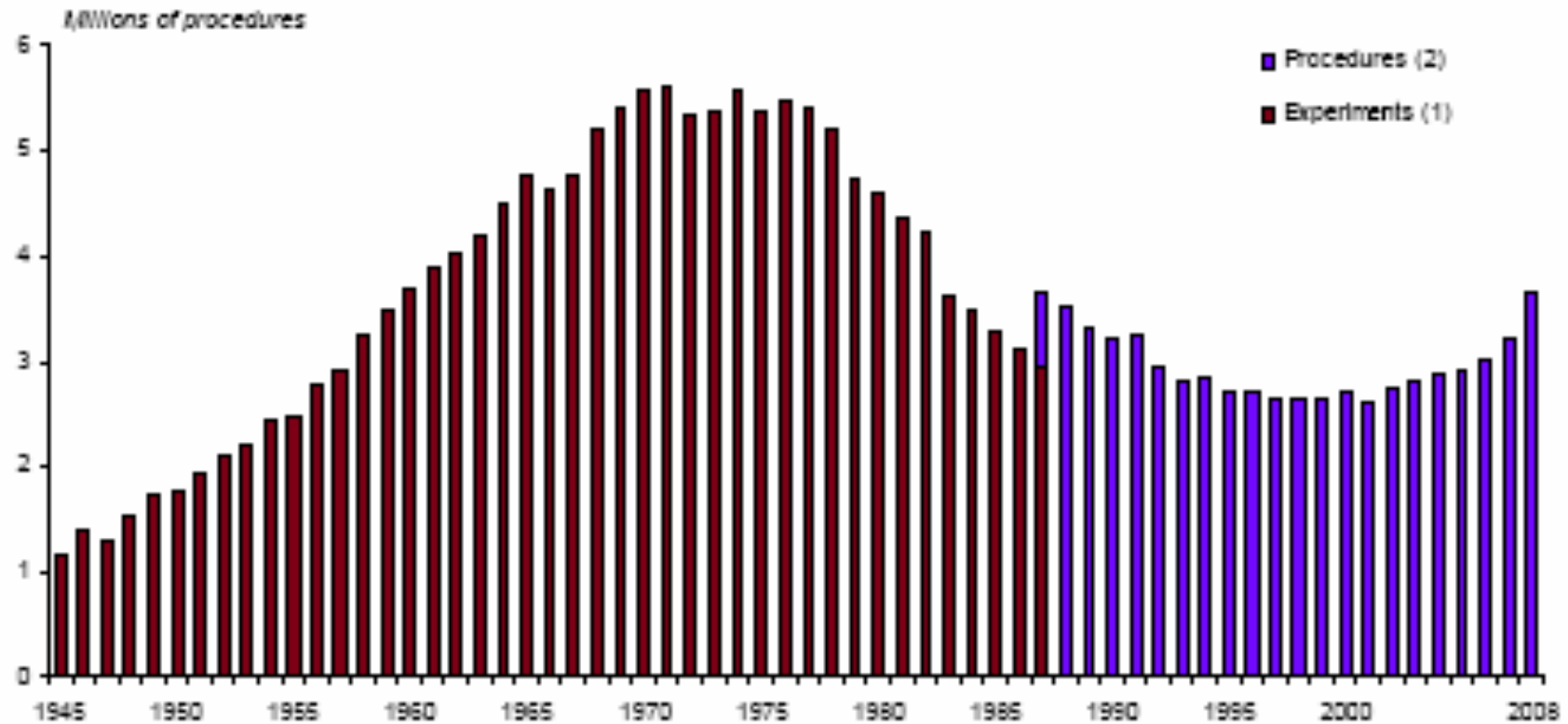
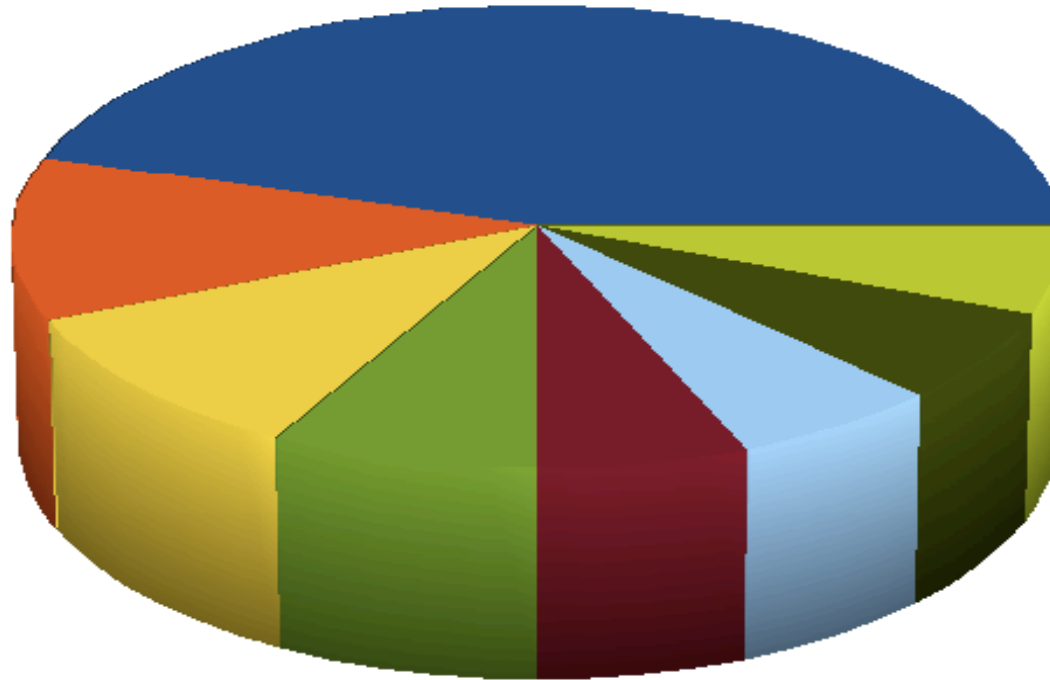


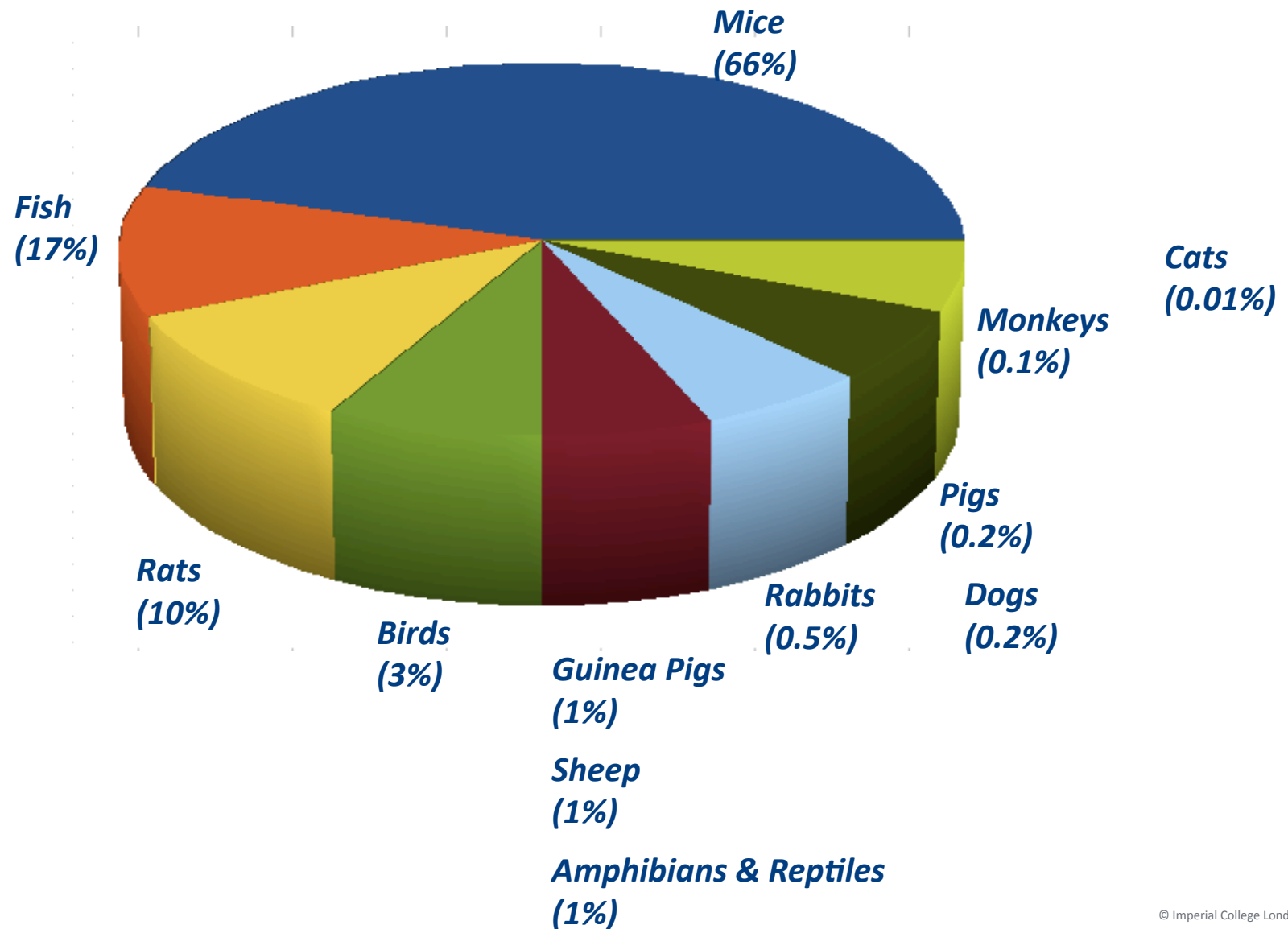
Figure 1: Experiments or procedures commenced each year, 1945-2008(1)

HO A(sp)A statistics 2008

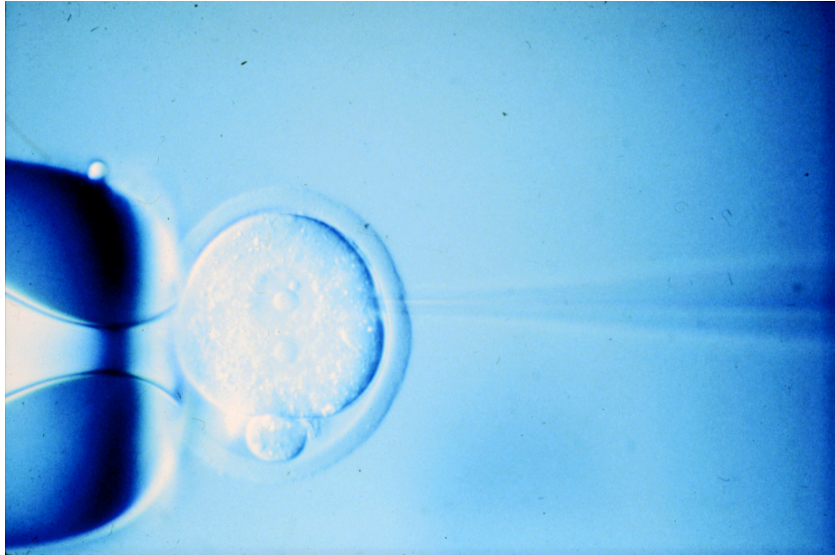
Types of animals used: Pie Chart



Types of animals used: Pie Chart (Answers)



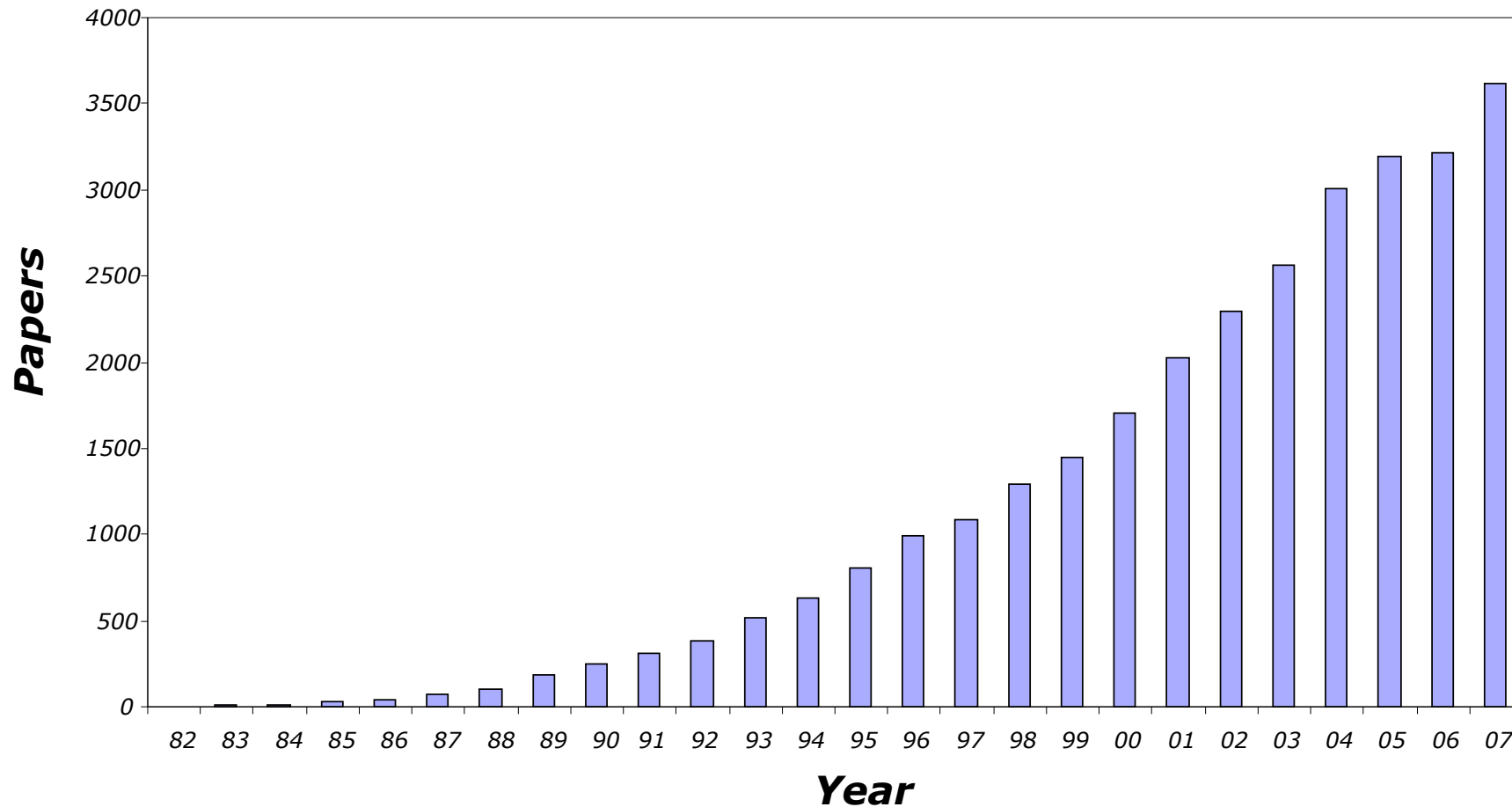
Genetic modification of animals



- *Manipulation of gene function by:
Microinjection transgenics
Gene targeting using embryonic stem cells*

Use of transgenic mice

Papers on transgenic mice from PubMed



Breeding of genetically altered animals

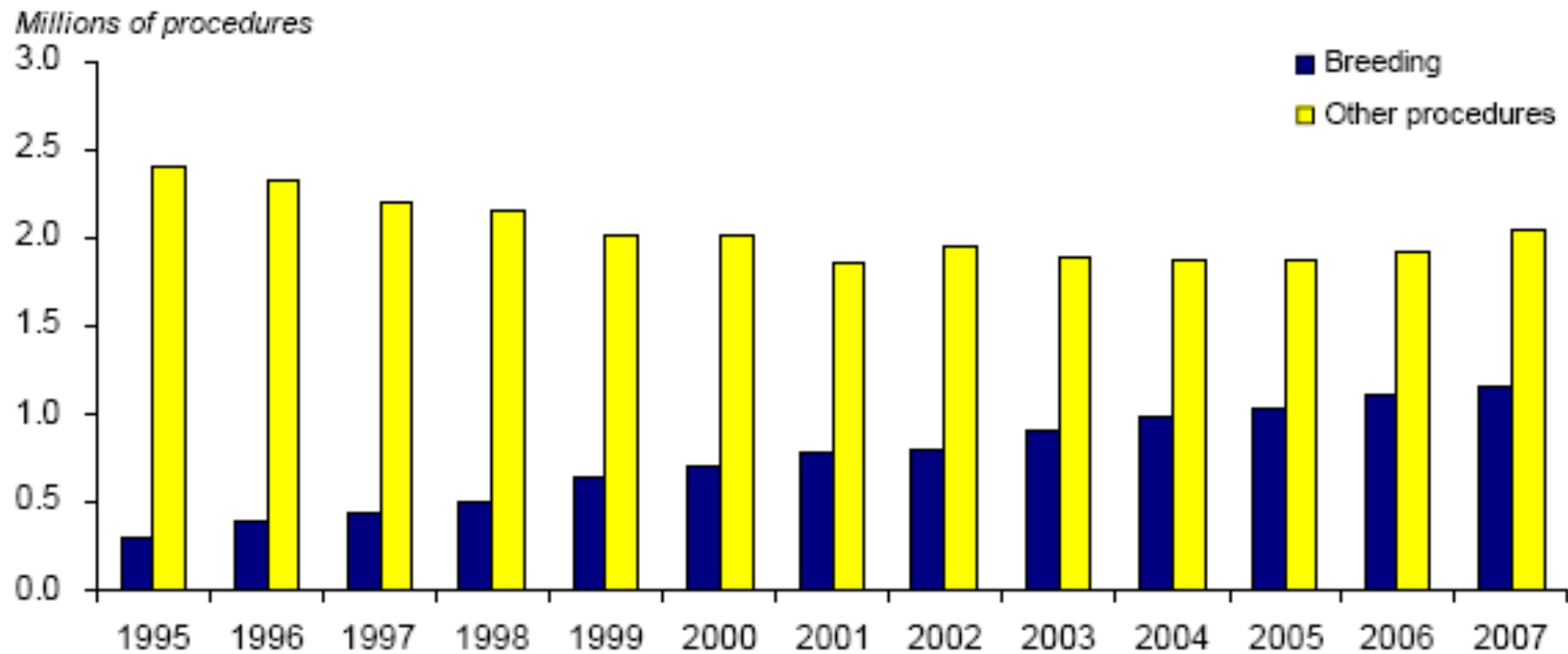


Figure 5: Comparison of breeding with all other procedures, 1995-2007

Who uses animals?

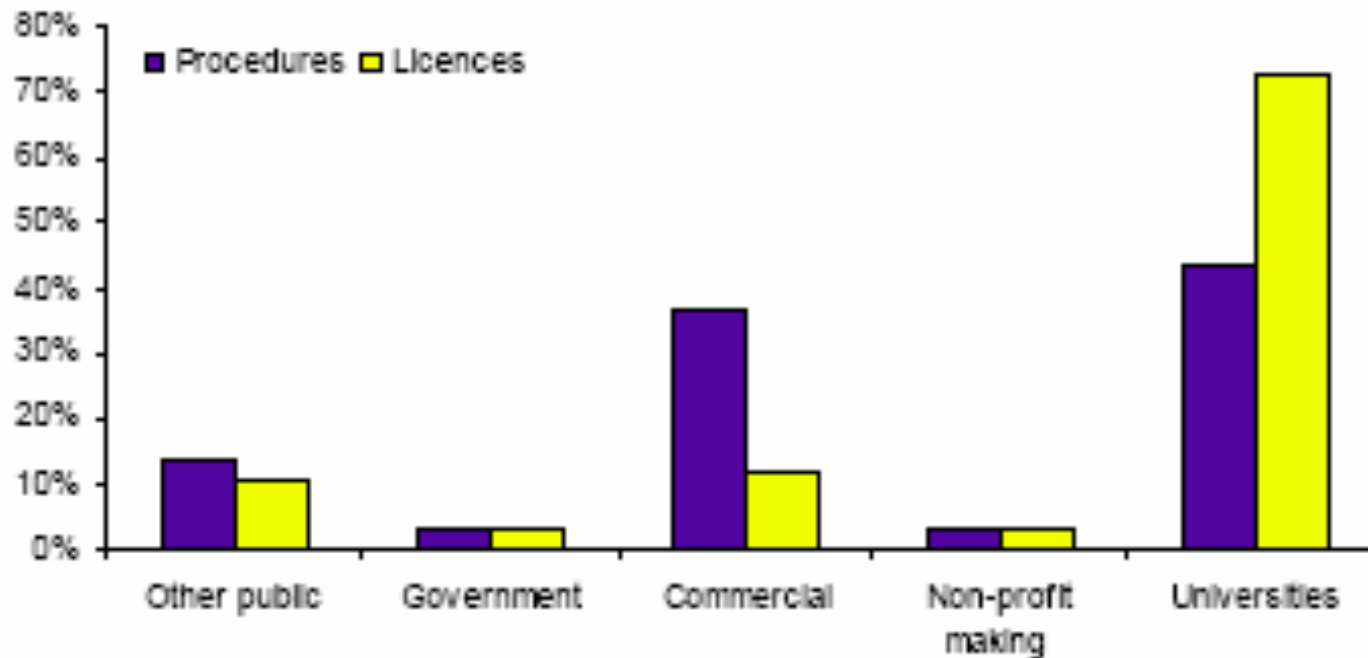


Figure 9 Project licence holders and procedures, by type of designated place

International estimates of animals used in research

Many countries do not provide comprehensive statistics.

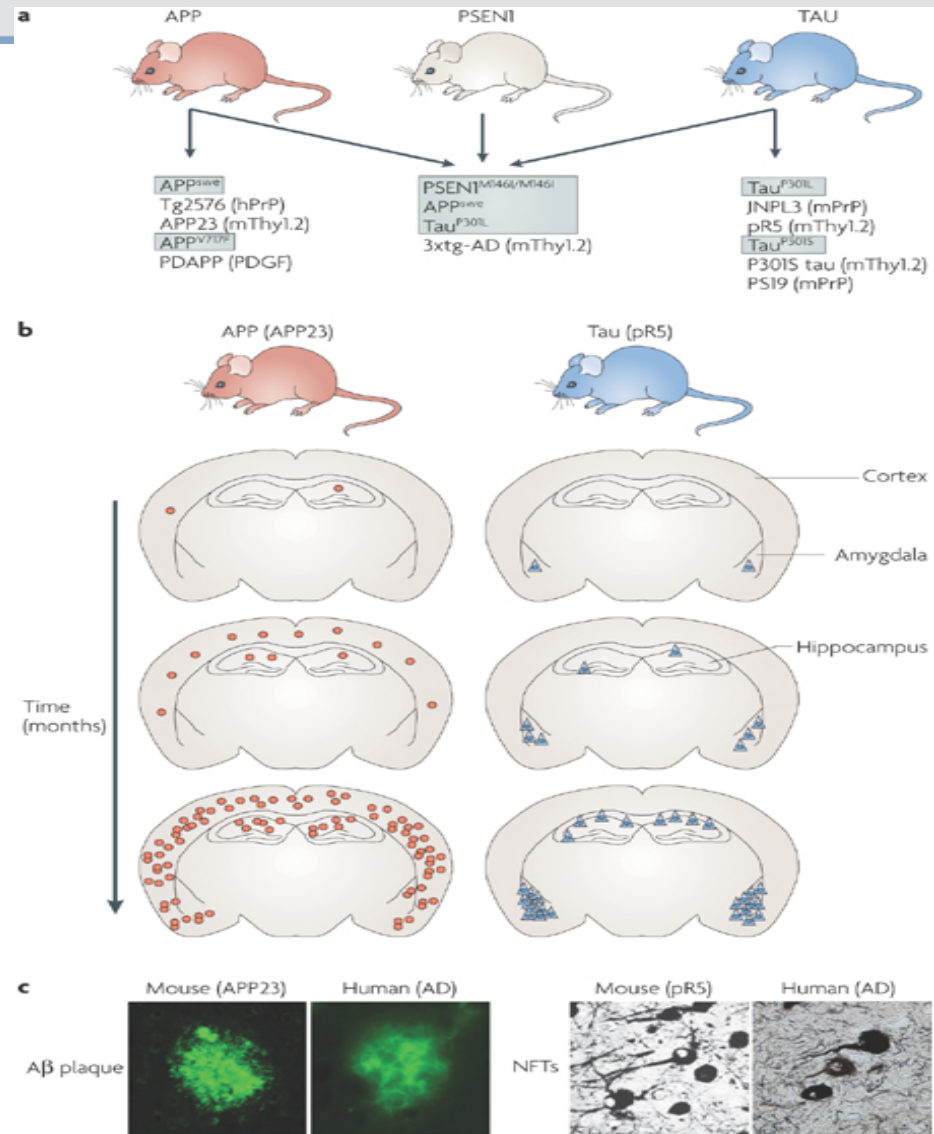
The major centres for research:

- USA (about 15 million procedures)
- EU (about 11 million procedures)
- Japan (about 5 million procedures)
- Canada (2 million), Switzerland (less than 1 million) and Australia (less than 1 million).

Can estimate that other countries might carry out 10 million animal procedures every year.

The total worldwide maximum is in the order of 50 million animal procedures per year.

Popular in vivo models in neuroscience: AD



Gotz *et al*, 2008

Popular in vivo models in neuroscience: MS

Experimental autoimmune encephalomyelitis (EAE):

- Induced by immunization of mice and rats, primarily, with myelin antigens including myelin oligodendrocyte glycoprotein (MOG), myelin basic protein (MBP), and proteolipid protein (PLP)

- Several MS features are recapitulated by EAE, including paralysis, weight loss, demyelination, and inflammation in the CNS.

Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD):

Popular in vivo models in neuroscience: MS

Chemical injury; such as cuprizone, lysolecithin and ethidium bromide

- result in focal demyelination in the white matter.
- Used for studying the mechanism of demyelination/remyelination (as remyelination is initiated upon termination of the chemical injury reagent)
- devoid of a massive leukocyte infiltration

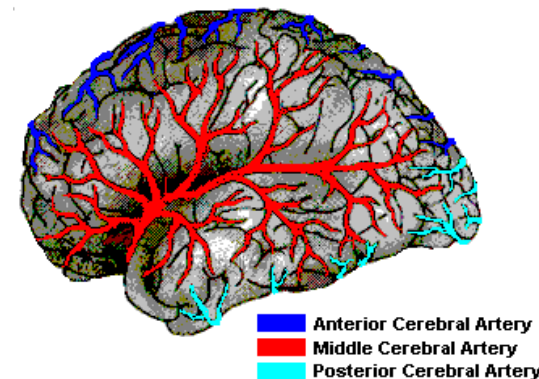
Popular in vivo models in neuroscience: PD

MODEL	BEHAVIOURAL SYMPTOMS	USES OF MODEL	DISADVANTAGES
6OHDA	Rotational behaviour after unilateral injection	Screen therapies that may improve PD	ICV injection, little synuclein involvement
	Motor impairments in primates	Screen therapies that may improve PD	ICV injection, little synuclein involvement
MPTP	Less obvious motor impairments in rodents	Screen therapies that may improve PD	Non progressive model of cell death
ROTENONE	Decreased Motor impairments in rodents	Test neuroprotective compounds	Inc. morbidity and mortality
PARAQUAT	No clear motor deficits	Test neuroprotective strategies	Not extensively teste
ALPHA-SYNUCLEIN	Severe motor deficits in A53T model, less in the A30T model	Study the role of synuclein aggregaton in PD	Generally no DA neuron death
LRRK2	Few behavioural deficits seen in drosophila mutation models	Stidy the role of LRRK2 mutations related to PD	General lack of degeneration

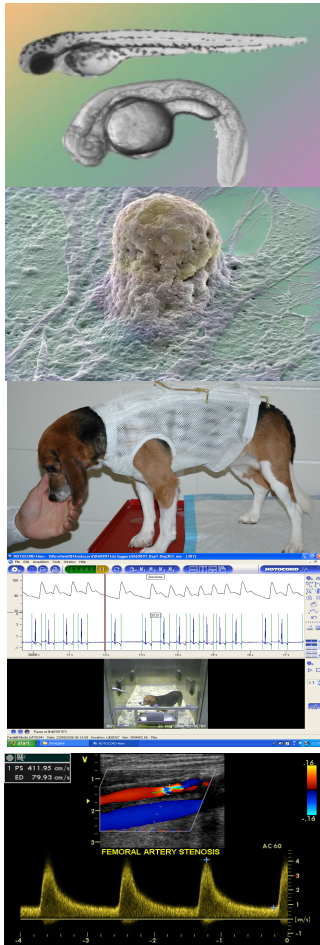
Blesa, 2012

Popular in vivo models in neuroscience: Stroke

- Endothelin-1 -induced constriction of arteries and veins
- Embolic middle cerebral artery occlusion
- Endovascular filament middle cerebral artery occlusion
- Permanent transcranial middle cerebral artery occlusion
- Transient transcranial middle cerebral artery occlusion
- Cerebrocortical photothrombosis



Future Considerations



- Increased involvement of safety pharmacology in early drug discovery to help reduce attrition for safety pharmacology reasons e.g. zebrafish
- Humanised safety screens e.g. stem cells
- Provision of repeat dose safety pharmacology data by inclusion of endpoints in repeat dose toxicology studies e.g. Holter-type ECG
- Simultaneous assessment of multiple organ systems
- Non-invasive imaging of organ function and correlation with pathological changes e.g. echo

Something to think about....

Mike Robins and that he has a severe case of Parkinson's Disease

The drugs to treat it only work for a short time.

Research on the brain, using monkeys, could lead to treatments that will transform lives.



Sources of Information

Home Office Animal statistics

Statistics of Scientific Procedures on Living Animals. GREAT BRITAIN 2008 Published
by HMSO

<http://www.homeoffice.gov.uk/rds/pdfs09/spanimals08.pdf>

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Javier Blesa,^{1,2} Sudarshan Phani,^{1,2} Vernice Jackson-Lewis,^{1,2,3} and Serge Przedborski^{1,2,3} Classic and New Animal Models of Parkinson's Disease. Journal of Biomedicine and Biotechnology. 2012

Zhen Gao and Stella E. Tsirka Animal Models of MS Reveal Multiple Roles of Microglia in Disease Pathogenesis. Neurology Research International. 2011.

Pros and Antis: websites

<http://www.understandinganimalresearch.org.uk/>

<http://www.rspca.org.uk/> (click on science group and then research animals)

<http://www.drhadwentrust.org.uk/>

<http://www.animalliberationfront.com/>

<http://www.buav.org/>

See also articles in Wikipedia