Introduction to neurophenotyping research

(Part 1)

1st ISBS Summer School

St. Petersburg, Russia

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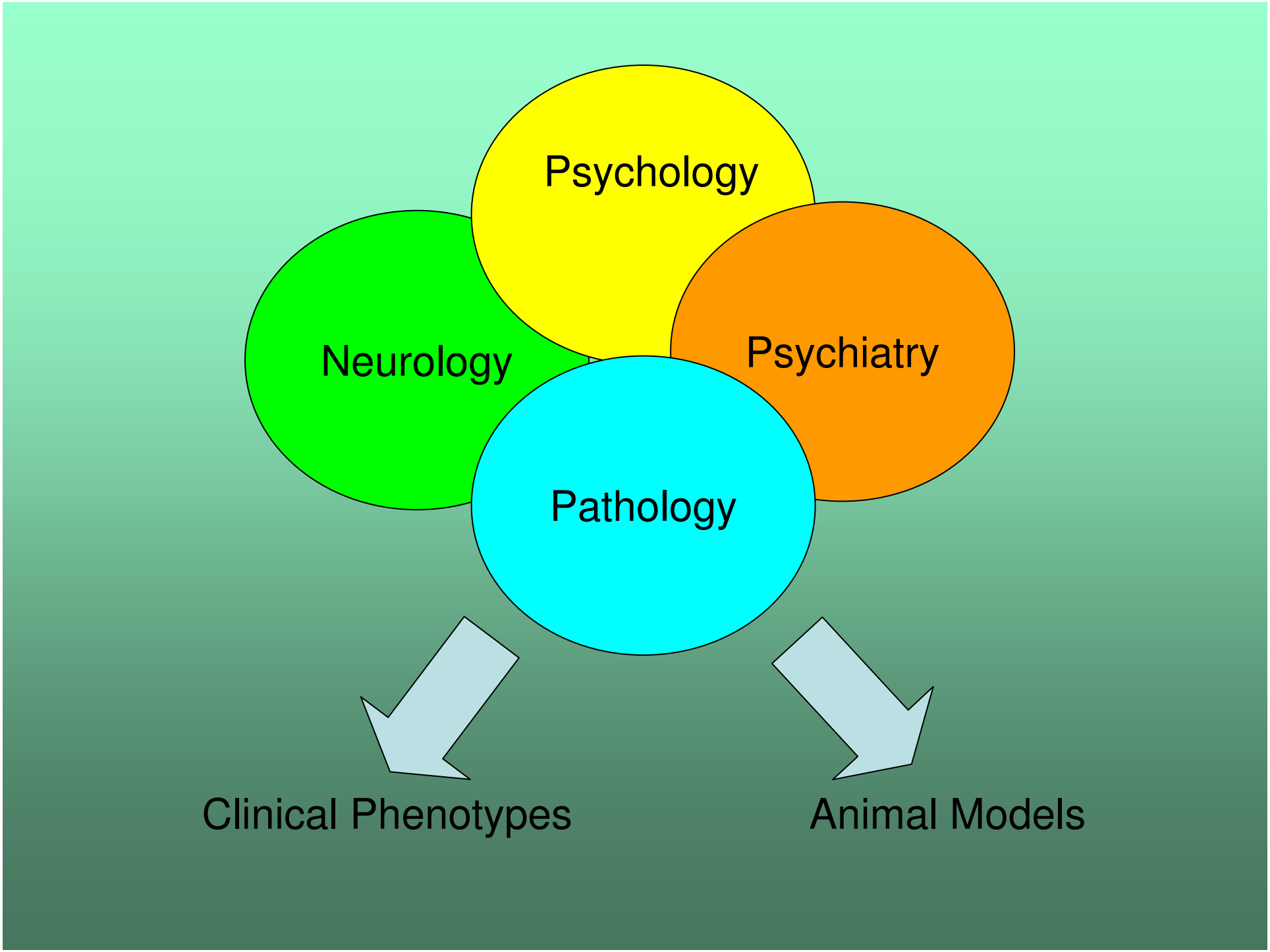
Animal modeling for brain disorders

How?

- Correctly define the brain disorder to model
- Use components of complex behavioral traits
- Recognize the translational similarities to human disorders
- Correctly interpret animal behavioral responses


Psychology
Neurology
Psychiatry
Pathology
Clinical Phenotypes
Animal Models

Behavioral domains for analysis

- Circadian behavior and sleep
- Fear, anxiety, and emotionality
- Social interactions and aggression
- Reproductive and parental behaviors
- Learning and memory, and attention
- Sensorimotor gating
- Motor and exploratory behavior
- Feeding behavior

NIMH, 2000

Testing Considerations

- When?
- Where?
- What subjects?
- What test frequency?
- Which test?
- Which test battery?
- Who will test?
- How to measure?

Subject considerations

- Species
- Age
- Sex differences
- Strain, substrain
- Other genetic factors (mutants, transgenics)
- Previous experience (test battery)
- Environment (rearing, enrichment)

Hierarchical strategy for behavioral phenotyping

I. General health and observation profile

II. Motor functions
Sensory functions
Food and water intake
Circadian rhythms

III. Other domains:
Social and emotional behavior
Learning and memory

Voikar, 2006

Considerations for test batteries

1. The source of the animals
2. The health of the animals
3. Physical housing conditions
   - Cage size
   - Animals per cage
   - Light/dark cycle
   - Temperature
   - Humidity

Brown et al., 2000

Considerations for test batteries

4. Food, water and medications provided
    - Brand and amount of food
    - Tap, distilled, or specially-treated water
    - Medications
5. Litter size and sex composition
6. Social experiences from weaning to adulthood
    - Isolation vs. social rearing

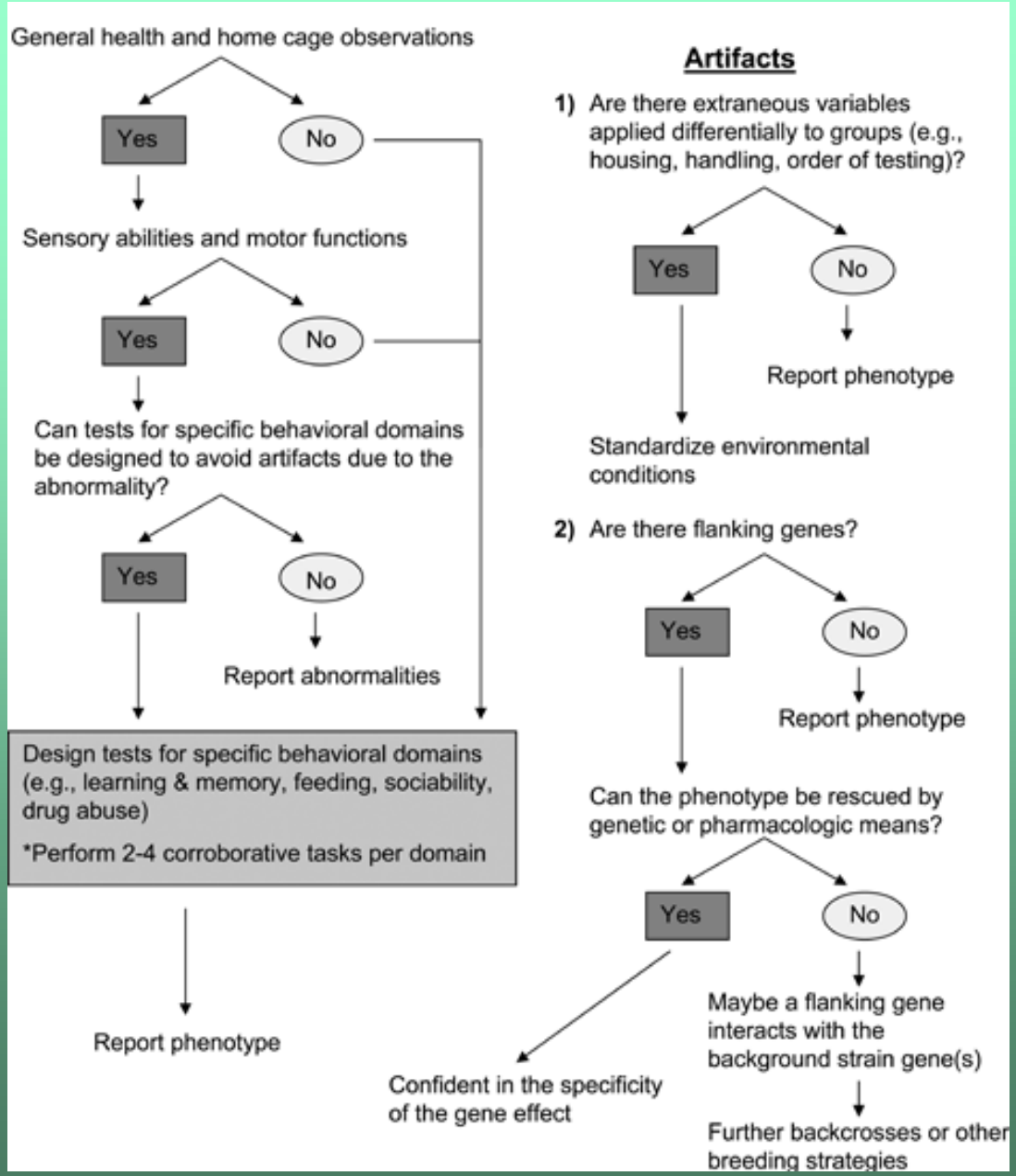
Brown et al., 2000

Designing behavioral studies

1. What control strains should be used?
2. At what age should mice be tested?
3. Should both males and females be tested?
4. What time of the day: night cycle should mice be tested?
5. How many tests should be given to each animal?
6. How many subjects per group should be tested?
7. How many different test paradigms should be used?
8. How should mice be handled before and during testing?
9. What apparatus should be used for each test?
10. What is the testing room environment?

Brown et al., 2000

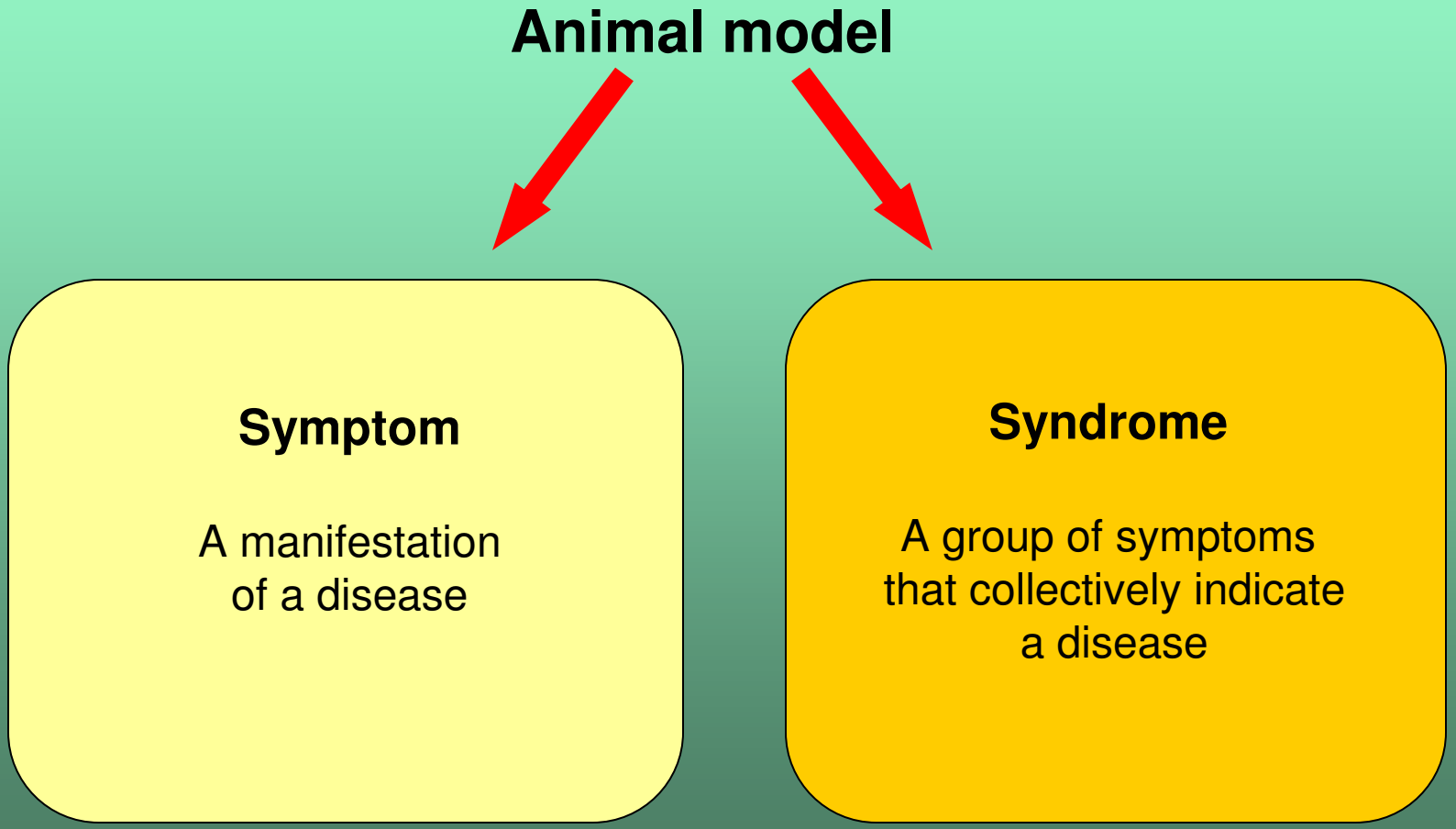
Behavioral Phenotyping Strategy



Is your new mutant mouse different from its littermate control?

Bailey et al., 2006

Symptoms or syndromes?



Endophenotypes

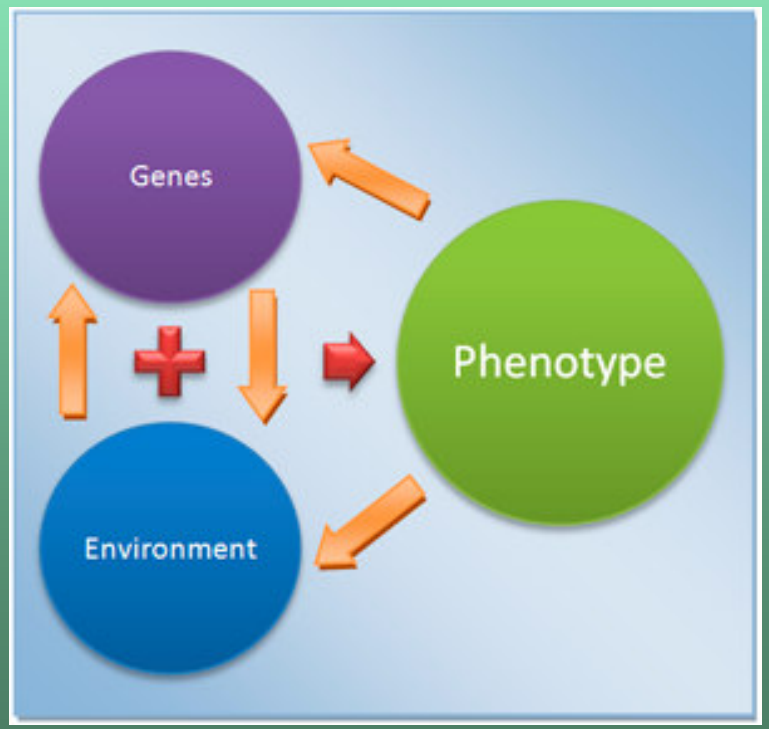
- The endophenotype concept deconstructs complex neuropsychiatric diseases into endophenotypes
- Objective, quantifiable, and inheritable traits that serve as biological (anatomical, developmental, electrophysiological, metabolic, sensory or psychological/cognitive) markers of a brain disorder
- Present regardless of whether a specific disorder is active
- Can be found in non-affected relatives of the patient at a higher rate than the general population

Gould & Gottesman, 2006

Endophenotypes

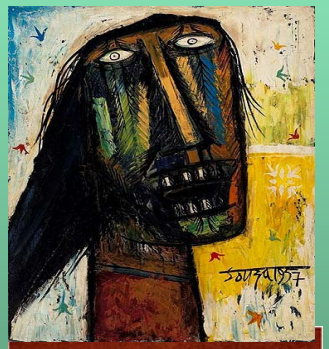
- The term is analogous to the “intermediate phenotype”, often used to describe a quantitative trait that is between the genes and the disorder
- Researchers could focus instead on endophenotypic domains more specifically, to discover novel genes/alleles related to a disorder and elucidate pathogenetic mechanisms by modeling causal pathways
- Endophenotypes may have a simpler genetics

From genotype to phenotype



Endophenotypes (biological markers)

Brain disorder

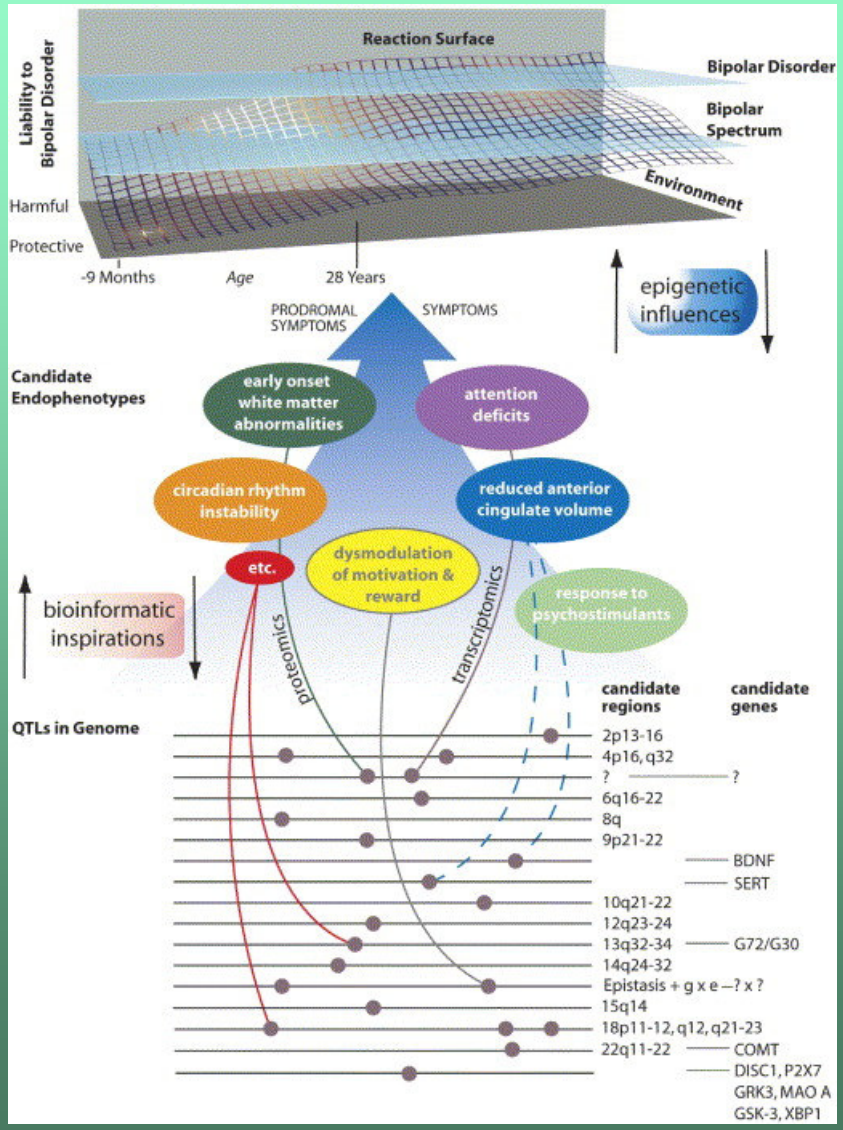


Normal brain

Endophenotypes

- A heuristic model showing underlying bipolar disorders gene susceptibility loci and implicated genes, modulated by environmental, epigenetic, and stochastic events

- Along this continuum between genes and distal phenotype lie putative bipolar endophenotypes, the identification of which will be useful for studies of the underlying neurobiology and genetics of bipolar disorders



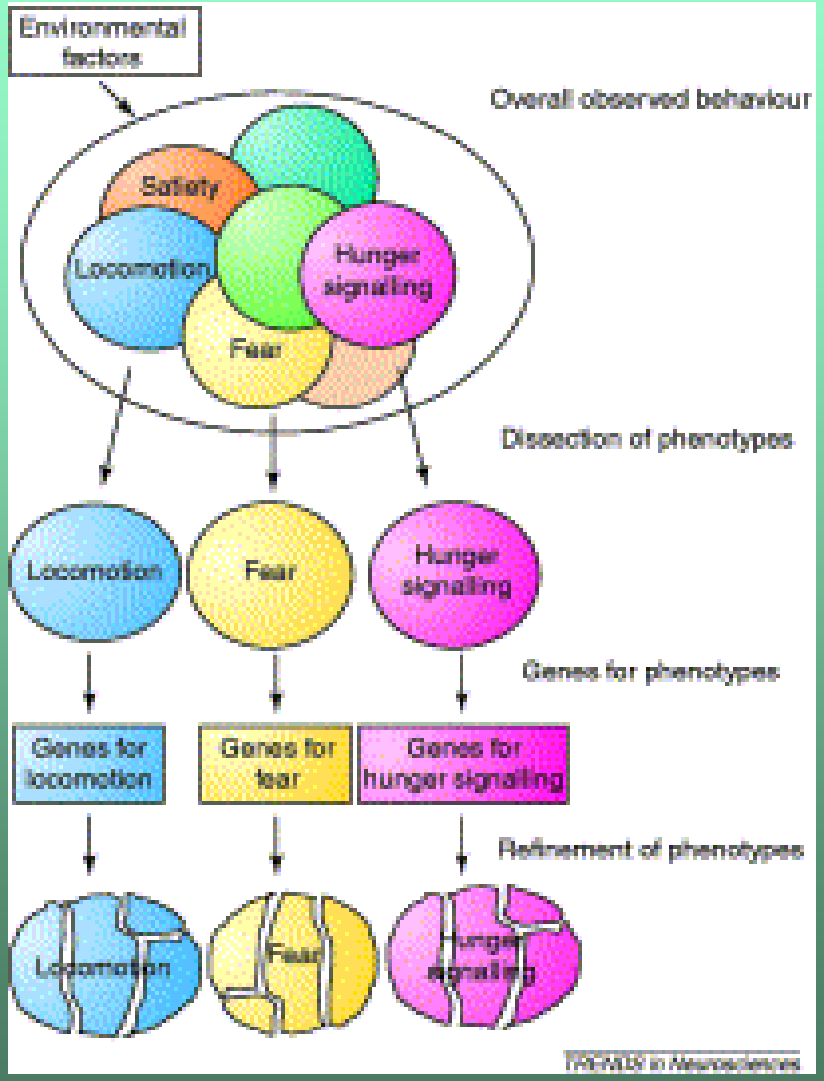
Hasler et al., 2006

Methods: animal “challenge”

- Manipulations inducing psychological stress (novelty, predator exposure)
- Drugs (anxiogenics, pro-depressants)
- Environmental stimuli (pre-, neo-, post-natal stress or drugs)
- Pathogens (bacterial or viral infection) cytokine-induced sickness behaviors
- Genetic installation (gene delivery)
- Inducible mutation

Domain-oriented research

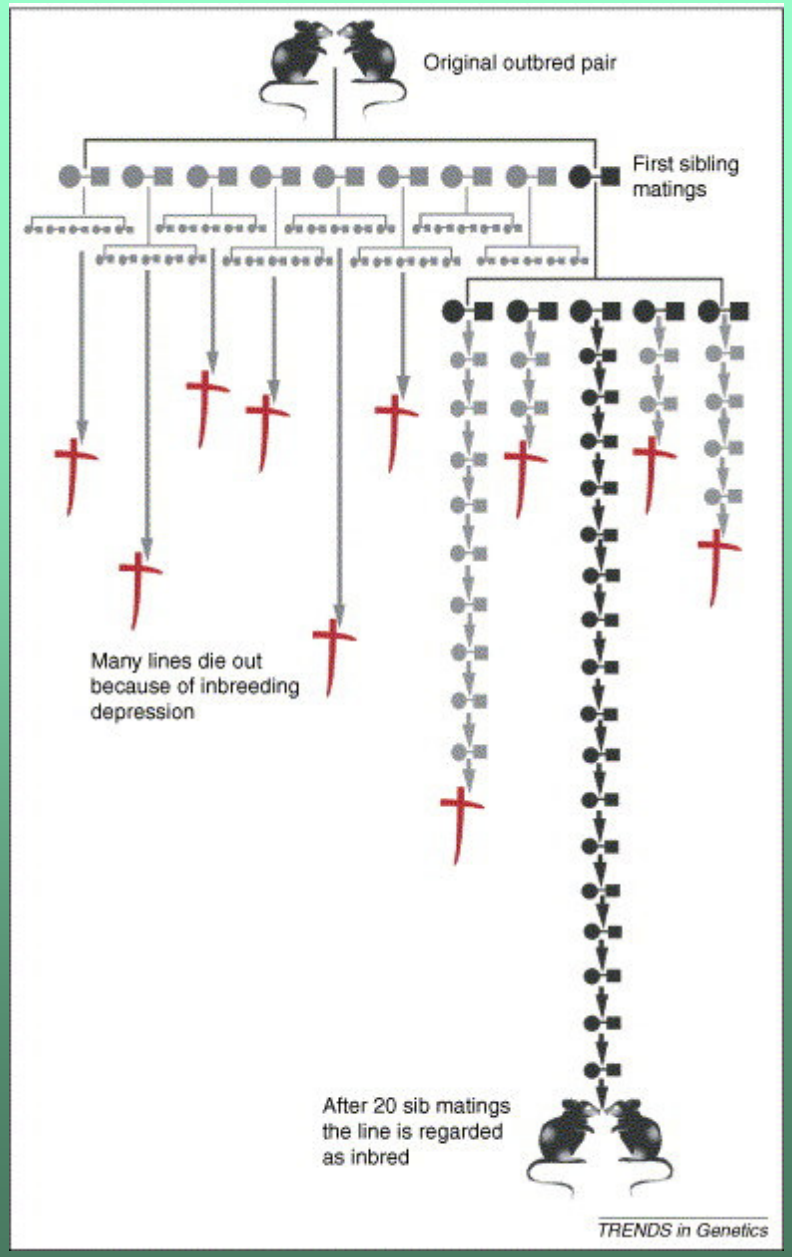
- Determination of gene–behavioral phenotype relationships first requires dissection of the overall observed behavior into behavioral phenotypes
- The expression of the overall observed behavior is likely to result from a complex gene–environment interaction



Kas and Ree, 2004

Glossary/breeding terms

- Inbreeding – the genetic state of mice that have been sibling mated for at least 20 generations
- Intercross – When mating pairs are siblings selected from the F1 generation
- Incross – when the two parents are derived from the same inbred strain
- Backcross – when one parent is heterozygous for one allele and the other is homozygous for either allele



- Inbred strains are developed by continual sib mating, which ‘forces’ homozygosity and a subsequent loss of genetic variability within the developing strain

- After 20 inbred generations, any surviving line is 98.7% homozygous at all loci and is regarded as an inbred strain

Taft et al., 2006

Glossary

Quantitative trait locus (QTL):

- A sections of DNA that importantly contributes to a phenotype of a continuous character, some of which are involved in the pathogenesis of neuropsychiatric diseases
- QTL can also be affected by environmental factors. QTL mapping has become an effective technique for identifying regions of a genome that contain genes pertinent to a clinically relevant trait
- These locations are often found by screening genetic animal models of disorders (and other complex traits), and can be successfully used for cross-species genetic analyses

Glossary

Susceptibility and candidate genes:

- Susceptibility genes affect the causes of a certain neuropsychiatric disorder
- They have been found for some disorders including autism, anxiety and schizophrenia
- Candidate genes are the genes suspected to play a role in the pathogenesis (based on QTL, linkage, association or family studies, genomics analyses, or genetic animal models) but not conclusively identified as contributing to the cause of the disorder

Glossary

Genetic polymorphism:

- The situation when two or more versions of a gene exist in the same population
- To be considered a polymorphism, each discrete allele must occur at a rate that cannot be accounted for by mutation alone (an allelic frequency rate of $\geq$1% is used for this determination)
- Polymorphisms of some brain genes are particularly strongly implicated in neuropsychiatric disorders. In addition, several different polymorphisms of the same gene may have combined effects on the expression of specific brain disorders

Glossary

Genetic animal models:

- Include inbred, selectively bred strains and genetically-altered (mutant or transgenic) animals that are used to mimic neuropsychiatric disorders based on their genetic traits
- These models are available in an ever-increasing range of phenotypes and offer a wealth of information for researchers investigating candidate genes as well as the molecular mechanisms and circuits of brain pathogenesis.

Strain nomenclature

- Ever-increasing production of new strains each year
- This high number brings complexity to mouse nomenclature

Example: mouse 129 sub-strains

- Same strain, very high variability in behavioral and physiological indeices among sub-strains (Simpson et al., 1997)

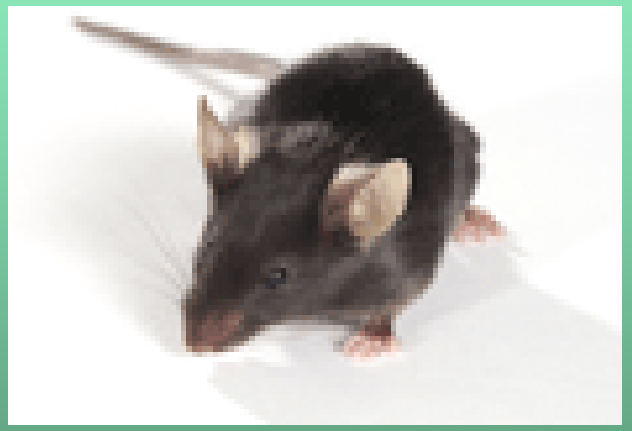
Vendor:

- Same substrains vary markedly if purchased from different vendors

Mouse nomenclature

- C57BL/6 mice are a frequently background strain. At some point, little attention was given to interstrain differences

- Different strains from different breeders:
  - C57BL/6N for National Institute of Health
  - C57BL/6N for The Jackson Laboratory



Researchdiets.com

Wotjak, 2003

Why is strain nomenclature important?

- Substrains should have an additional acronym to indicate the breeding stock
  - e.g. C57BL/6JOlaHsd for Olac-Harlan-Sprague Dawley
- Recent reports show a chromosomal deletion in C57BL/6JOlaHsd mice.
- This mutation ablates the alpha-synuclein locus, which encodes a presynaptic telencaphalic protein
- This protein has been implicated in the aetiology of Parkinson and Alzheimer diseases

Wotjak, 2003

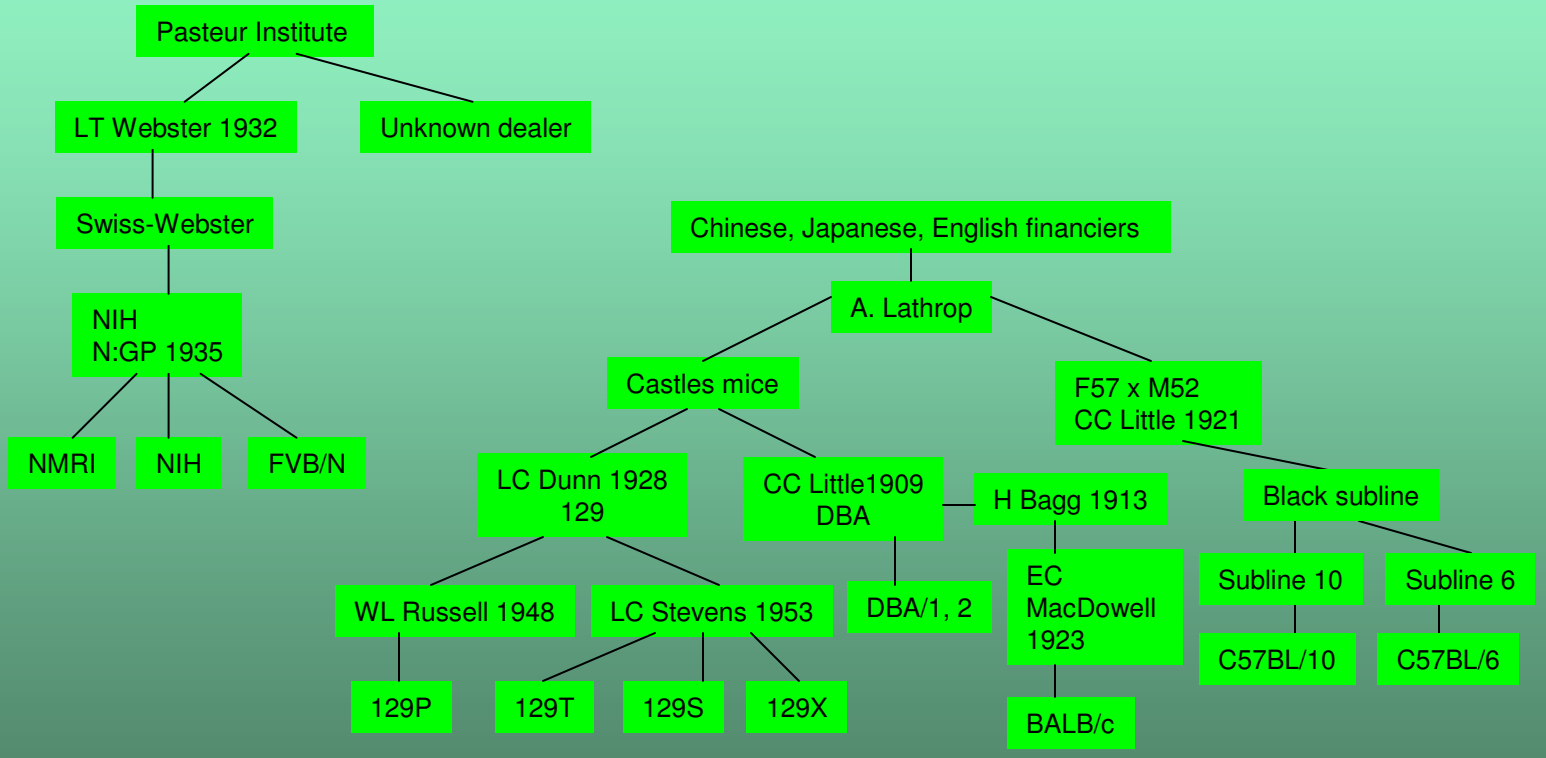
Background strains

Some potential effects:

- Blindness (rd1 gene) – FVB, NON, BUB, C3H, CBA, SJL, SWR
- Hearing/vestibular defects – A/J, DBA, C57BR & L, some 129, C57BL/6J

Pletnikov, 2006

Origins of some mice inbred strains



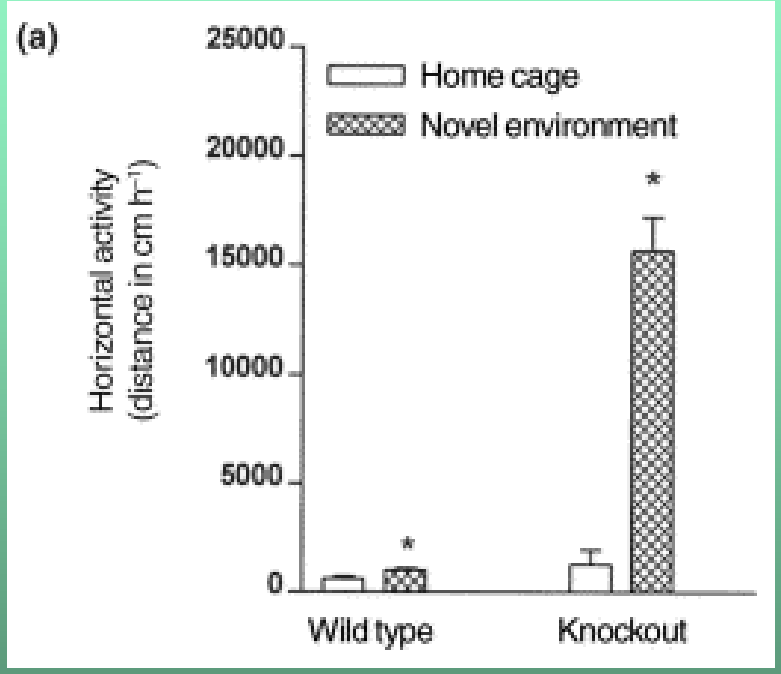
Beck et al., 2000

Recommendations

1) Adhere to the nomenclature rules so that results obtained with a new mouse strain can be related to a newly described mutation *a posteriori*
2) Include specific information on the animals that were used in the title or keyword list
3) Scientific community should press commercial animal suppliers to provide new info on the strains they supply
4) Creation of a database of citations of papers using a given inbred strain

Specht and Schoepfer, 2001

Home cage vs. novel environment



Brief assessments of behavior (e.g. for 1 h) are attractive with respect to the screening capacity, they can yield discrepancies with longer assessments of behavioral testing (e.g. 24 h)

Kas and Van Ree, 2004

Housing and caging considerations

Caging system	Illumination	Contact persons	Protection wear
Cage distributor	Light/dark (LD) pattern	Direct animal - contact	Gloves
Cage ID	Light on	No direct animal - contact	Mask
Cage material	Light off	Human presence (time)	Face - shield
Cage size	Light - intensity (light phase)	Handling	Protection - suite
Lid distributor	Light color (light phase)	– with hands	Hood
Lid material	Light - intensity (dark phase)	– with tweezers	Laboratory - coat
Lid ID	Light color (dark phase)	– with net	Laboratory - shoes
Raised lid	Disturbance LD pattern	– with transfer box	
Filter top	Light - source	Certificated caretakers	
Filter top distributor		Allergic persons	
Enrichment		Perfume users	
Bedding material		Smokers	

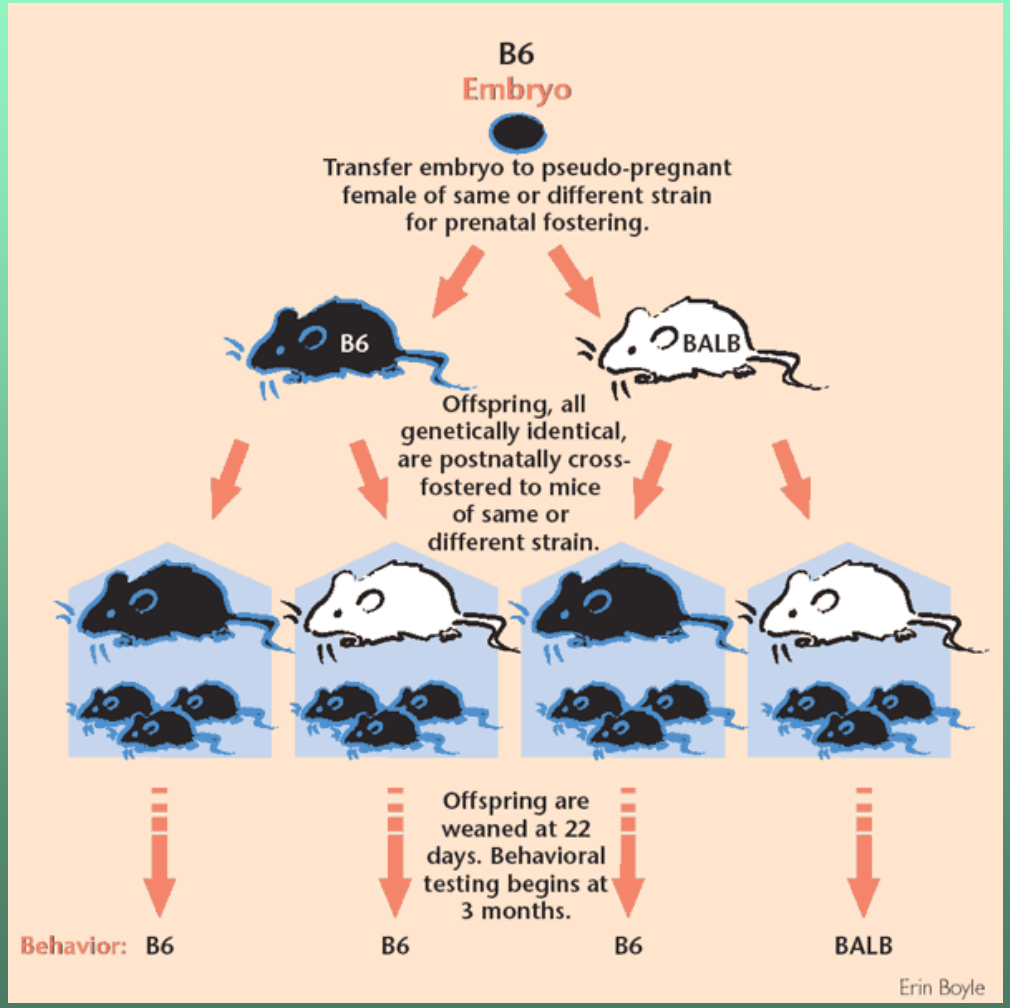
Wurbel, 2002

Housing and caging considerations

Climatic conditions	**Animals**	**Room specifications**	**Health & hygiene status**
Ventilated cages (vcs)	Max. animals/cage	Acoustic background	Health monitoring
Total air/h within vcs	Max. animals in total	Room space (m^2)	Health checks/year
Fresh air/h within vcs	Gender	Acoustic deprivation	Parasitology
Total air/h	Group composition	White noise	Bacteriology
Fresh air/h	Other species in room		Serology
Air condition			SPF-conditions
Temp. light phase			
Temp. dark phase			
Humidity			

Wurbel, 2002

Behavioral epigenetics



- Francis *et al*. experiments
- B6 offspring showed behavior typical of BALB/c only when they developed from embryos to weaning in the care of surrogate BALB dams

Crabbe and Philips, 2003

Translational validity?

E.g. How is “depression” detected in a rodent?

- Researching endophenotypes that are reactive to antidepressants
- Testing pharmacological compounds
- Test other antidepressant methods (e.g. effects of exercise or social status)

Is it valid?

- **Face Validity**
  *S*imilarity between the behavior exhibited by the animal model and the specific symptoms of the human condition

- **Predictive Validity**
  The ability of a test to predict a criterion of the human phenomenon

- **Construct Validity**
  The accuracy with which the test measures that which it is theoretically intended to measure

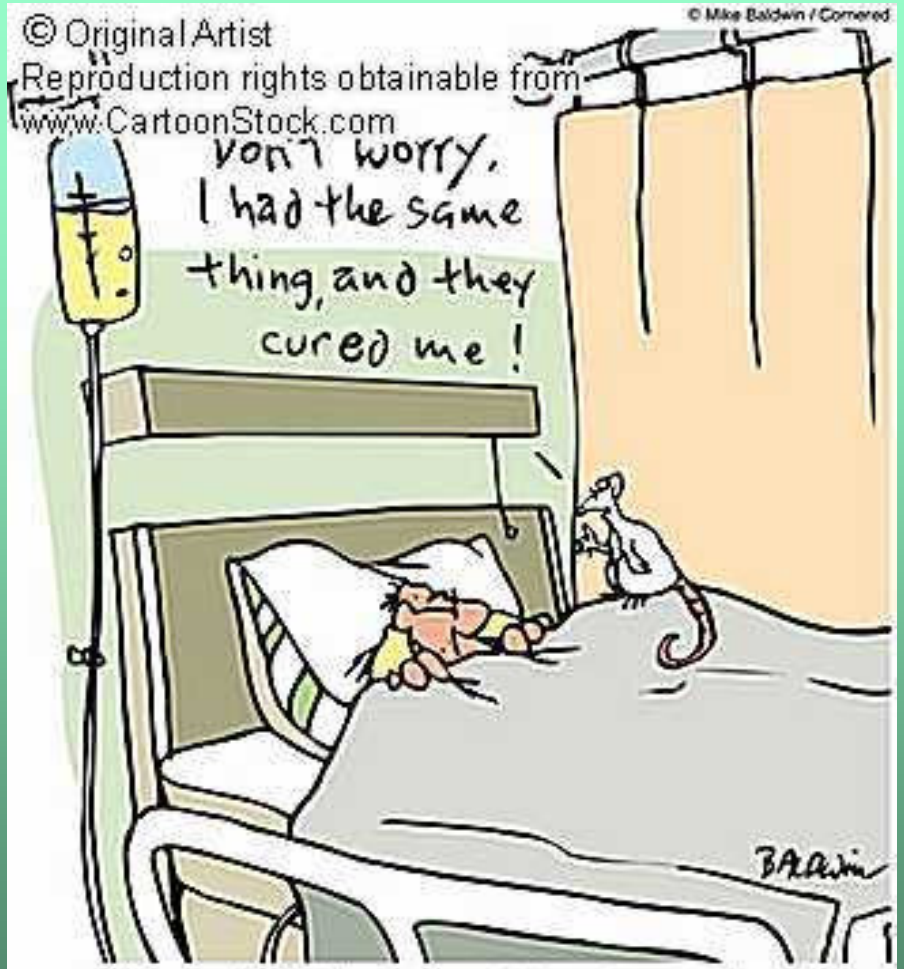
Purpose of animal models

To test hypotheses about disease pathogenesis, and to predict the human reaction to the treatment

An ideal model should be:

- Reproducible
- Robust
- Simple
- Quantitative
- Respond to effective (and not respond to ineffective) human treatments
- Have similar pathogenesis to human disorder
- Be analogous to the human disorder behaviorally, anatomically, neurochemically, etc.

Crawley, 2000



First clue that the latest medical breakthrough isn't quite there yet.