Introduction to neurophenotyping research

(Part 3)

1st ISBS Summer School

St. Petersburg, Russia

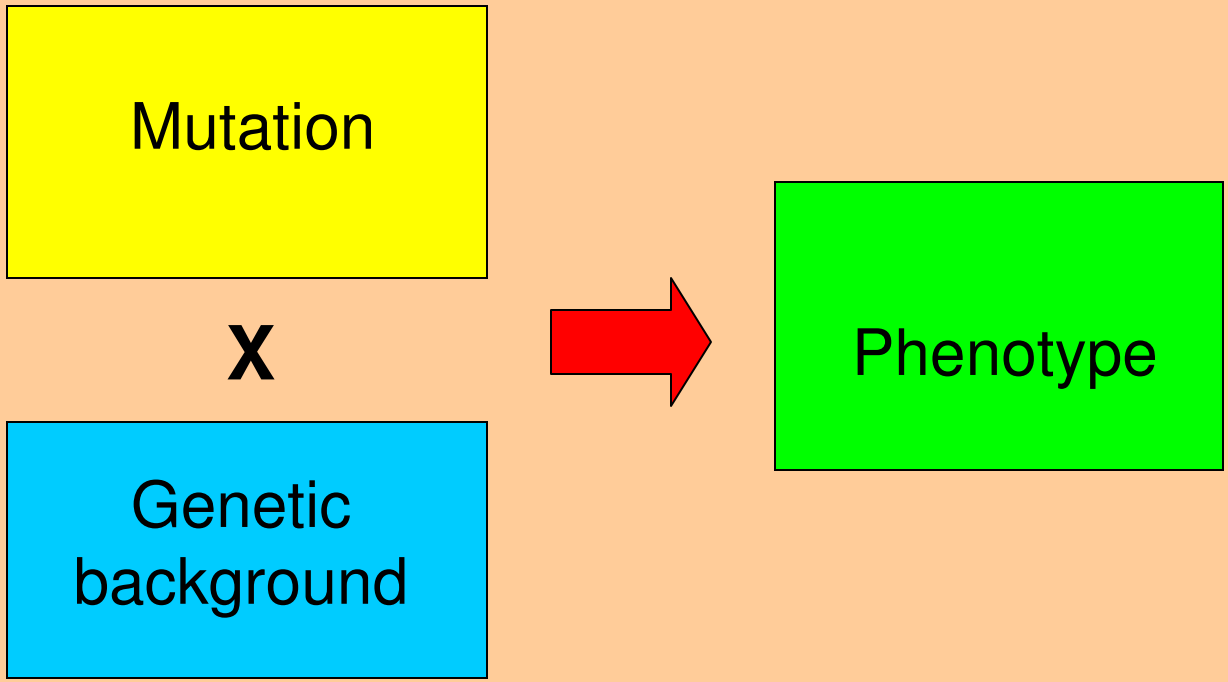
May 9th -15th,2008

Genetic caveats

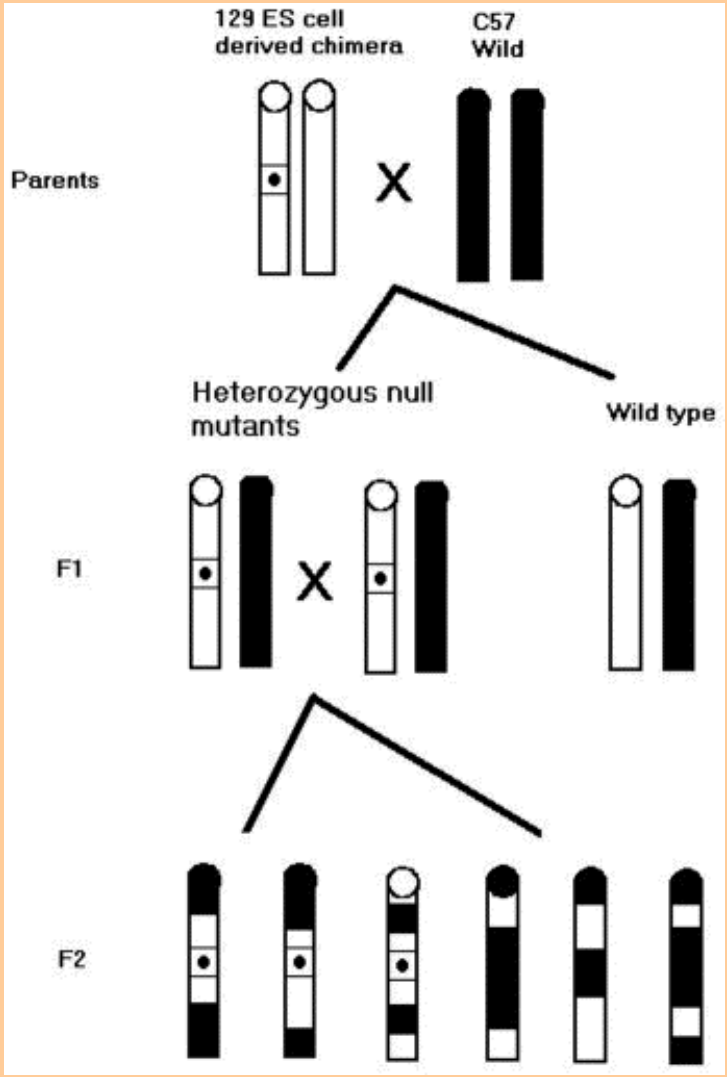
- Genetic background

- Flanking genes

Genetic background



Flanking genes



Chromosomal constitution of F2 mice from a cross between a C57BL/6 mouse and a 129-derived germline chimera generated by gene targeting. Because of crossing-over and recombination, F2 chromosomes will be a mixture of the parental chromosomes. F2 chromosomes carrying the null mutation will also differ from chromosomes carrying the wild-type allele by many 129-derived alleles for genes localized in the chromosomal region flanking the targeted gene.

Crusio, 2004

Setting priorities for phenotyping the mouse

The National Institute of Mental Health (NIMH) organized a meeting of 50 scientists in 2000 to discuss:

- New strategies for characterizing inbred strains with multiple phenotypical domains
- Developing batteries of tests to maximize cost/benefit
- Detection of subtle alterations by random mutagenesis
- Formation of a public database detailing phenotypical information on inbred and mutant strains
- Coordination between NIH and Jackson laboratory

Interpretation issues: be right to be right

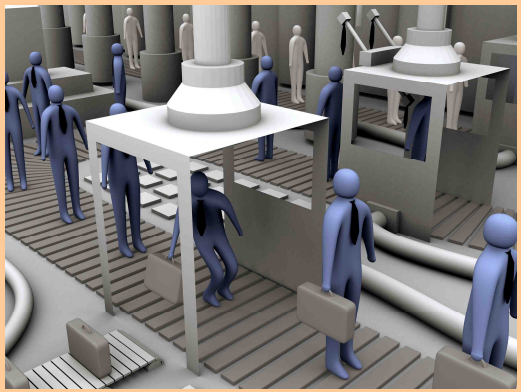
Tortuous ways to phenotype (Voikar, 2006)

- Kalueff et al. 2004: Anxiety in VDR KO mice
- Burne et al. 2005: Motor problems in VDR KO mice

But:

- Kalueff et al. 2004: special diet (Ca^{2+}, lactose) – very mild hypocalcemia in mice
- Burne et al. 2005: Ca^{2+} in drinking water – pronounced hypocalcemia in mice [likely to affect almost every behavior]

Standardize or not?



- Standardization of all experimental conditions increased validity of research
- Should be strictly implemented in all behavioral laboratories
- Should be based on universal standard protocols

Crabbe and Wahlsten

Standardize or not?

- Standardization does not consider individual variability of animals
- How can we know the right standards? Consider C. Hall example (1934)
- May preclude us from finding new brain phenomena
- Limits creativity of research
- Development of new models is a key part of behavioral neuroscience

Phenotyping neural and sensory function

- 9 inbred strains identified as a high priority for Jackson Laboratory's Phenome Project, which establishes baseline data for basic phenotypes
- Requires detailed characterization of sensory, vestibular, and autonomic domains
- Battery of 12 high-throughput screen was recommended
- Neurophenotyping high priority strains (129/SvImJ, A/J, BALB/cJ, BTBR, C3H/HeJ, C57BL/6J, CAST/Ei, DBA/@J, and FVB/NJ) was recommended

NIMH, 2000

Phenotyping complex behavior

High-throughput assays and multiple domain characterization are needed to investigate:

- Fear
- Anxiety
- Emotionality
- Social behaviors
- Learning and memory
- Sensory funcion
- Motor, exploratory behavior
- Feeding

High priority should be given to innovative behavioral assays in these areas

NIMH, 2000

Additional characterization

- Assessment of the effects of psychoactive substances on the nervous system and behavior are needed
- Establishment of baseline pharmacological data on drug administration, delivery, metabolism, and excretion is necessary
- Behavioral data, along with *in vitro* methods (radioligand binding procedures, second messenger Western blot, autoradiographic assays) should be employed
- Should focus on substances with robust behavioral effects, such as alcohol, amphetamine, MDMA, morphine, cocaine, etc.
- Psychotherapeutic compounds should be investigated so that genetic influences of these drug responses may be better understood

NIMH, 2000

Complex behavior

1. What new and existing paradigms could better model human behavioral disorders?
2. What are the cost/benefit ratios and priority levels for assays in testing batteries?
3. Is high-throughput screening practical, and for all phenotypes?
4. How can batteries be constructed so that data on subsequent assays is not effected?
5. How can the reliability, efficiency , and validity of these batteries be objectively quantified and monitored across labs?

NIMH, 2000

Complex behavior

Recommendations:

- Establish an inbred strain database for complex phenotypes and standardization of assays
- Encourage the development of new mouse behavioral paradigms
- Facilitate the training of scientists for examining complex behavioral traits via courses and workshops

NIMH, 2000

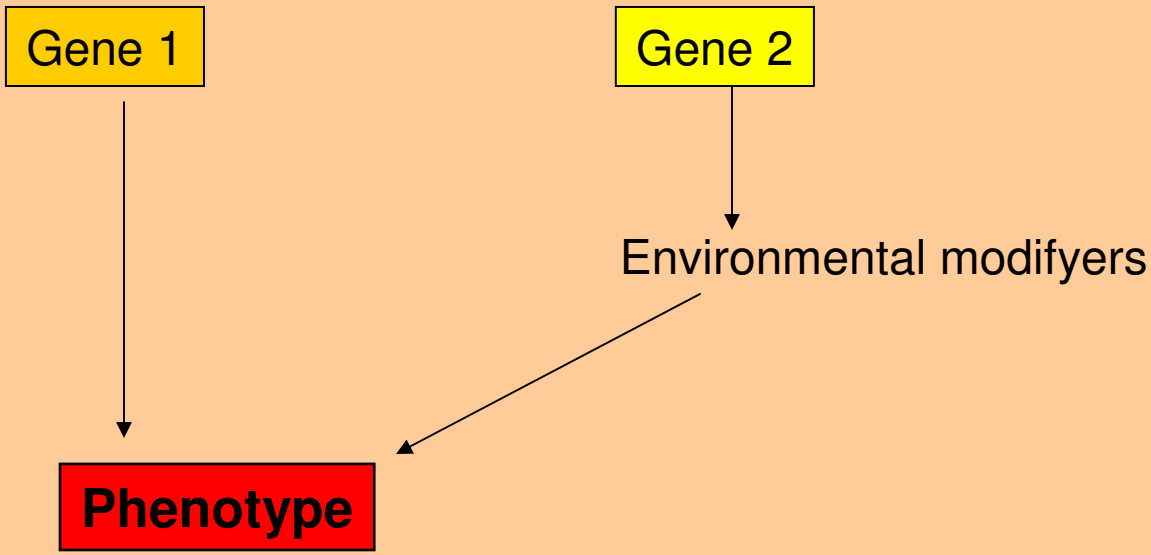
Compensatory mechanisms and secondary phenotypic alterations

- A null mutant (constitutive knockout) could possess alterations as a result of compensation by other genes (false negative findings) or secondary phenotypical alterations (false positive) that are not linked to the function of the gene of interest
- The abnormalities may be due to genetic redundancy
- Some related genes may take over the function of the targeted one to compensate
- This compensation could actually cause the observed abnormalities
- Pleitropy is a form of epistatic interaction where the gene has a role in several metabolic, developmental, or other processes. So different functions can be affected if upregulated or deleted genes have a pleitroopic effect

Voikar, 2006

Phenocopies

A phenotype that is not genetically controlled but looks like a genetically controlled phenotype. An environmentally induced phenotype that resembles the phenotype produced by a mutation



Economics of phenotyping

Strategy, management, and health policy
Early behavioral screening

Stages				
Enabling Technology Genomics Proteomics	Preclinical research	Preclinical Development Toxicology, Formulation Drug delivery Pharmacokinetics	Clinical development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

Castagne et al., 2006

Economics of phenotyping

Many factors of time and cost are involved in the use of genetically engineered animals, such as:

1) Breeding efficiency of the strain
2) Breeding scheme requires to perpetuate the colony
3) Colony management

Support stock mice - Could have many hidden costs

- Source – may be economical to buy some mice to supplement support stock
- Personnel and Space – consider the cost of maintaining the colony

Centralized animal facilities

- transgenic strain using F1 hybrid has lower cost of production than inbred strain based on higher egg yields and survival

Lake et al., 1999

Economics of phenotyping

Colony development

- Necessary to have quick and reliable genotyping protocol
- Find personnel with expertise in handling mice
- Consider the needs for both transgeneic and targeted mutations in mice

Nomenclature

- Newly-induced mutations need proper nomenclature
- This will avoid confusion and identify the strain in the literature

Importing genetically-engineered mice

- May offer savings but other restrictions should be considered (e.g. quarantine)

Lake et al., 1999

Economics of phenotyping

Breeding colony establishment

- All research personnel should be notified of the nature particularities of the strain
- All equipment for specialized mice should be obtained before the arrival of the animals

Colony management

- The research laboratory staff should remain involved with the maintenance of the colony
- Excellent record-keeping is required

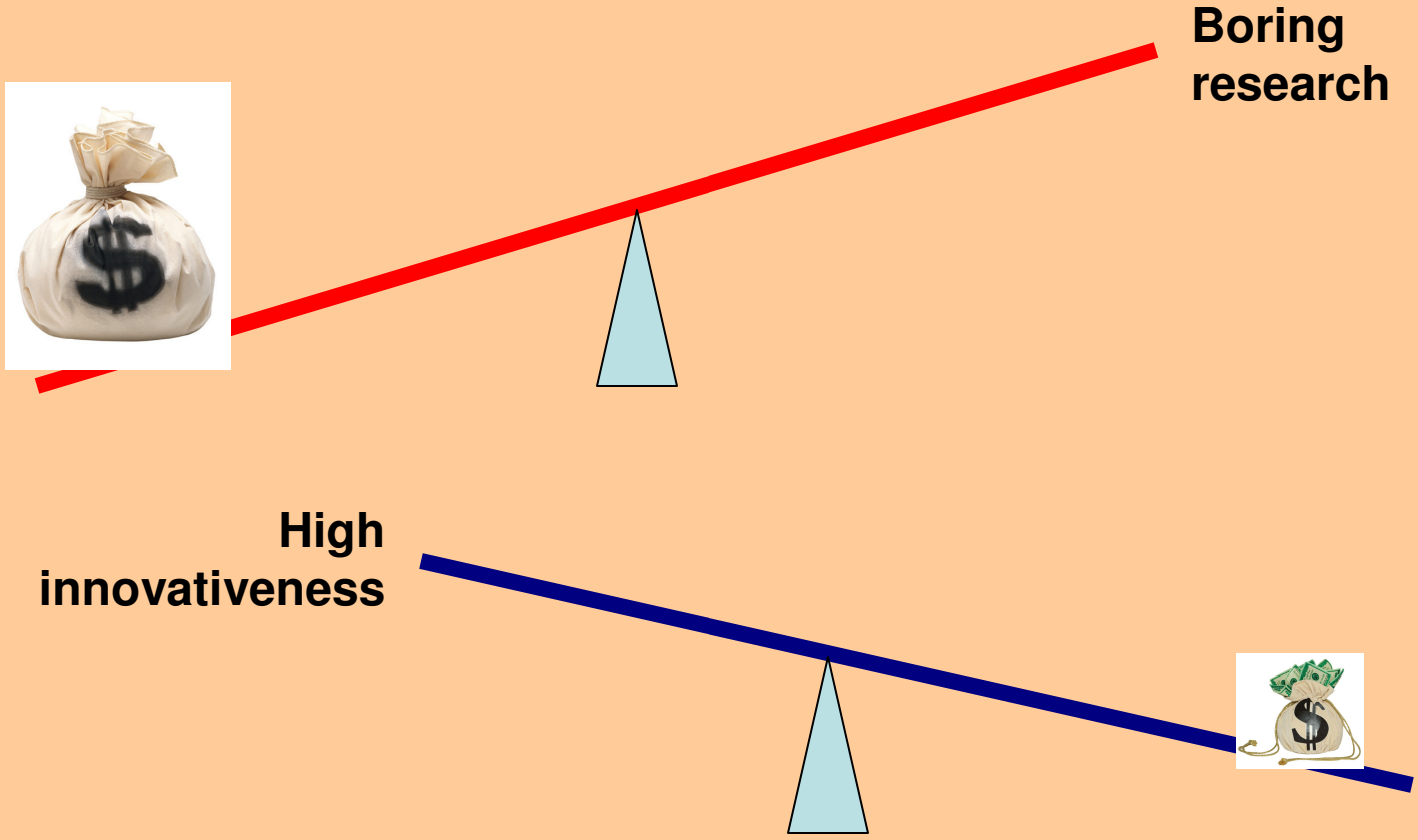
Colony maintenance

- Inbreeding, backcrossing, testing for homozygosity will be required for the perpetuation of the strain

Lake et al., 1999

Rich and poor labs

Good news for poor labs



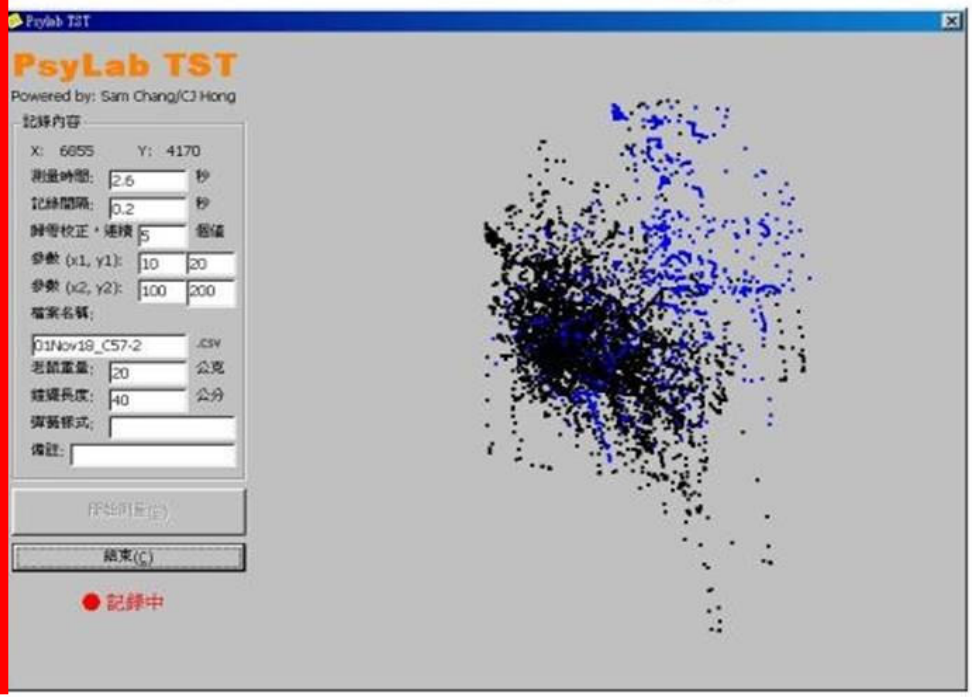
Innovation

- Necessary for progress in the field
- Can help create new models
- Can target newly-identified disorders
- Will integrate different areas of the field
- Can be employed with little or no expense
- Requires creativity, not necessarily money

"What's the opposite of 'Eureka!'?"

Example of innovative phenotyping

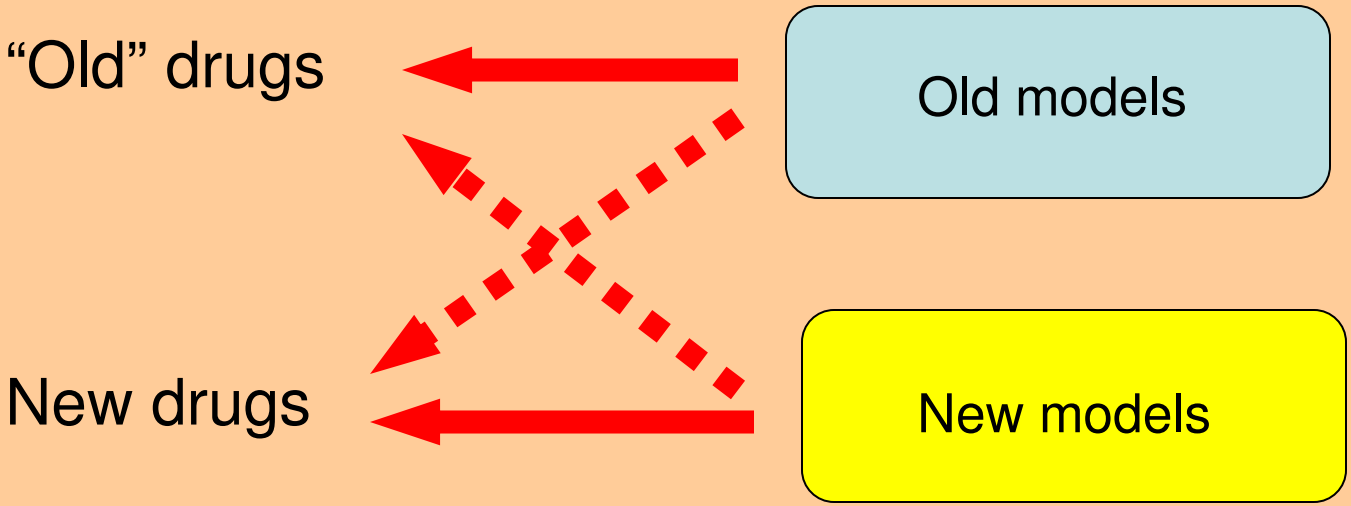
PsyLab Quantitative
Tail Suspension Test
(USA & Taiwan Invention Patents, 2005)



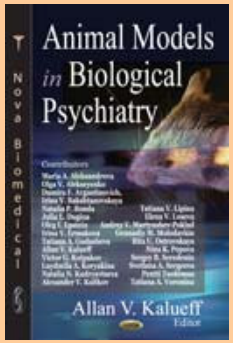
- Objective, quantitative measure of immobility in the TST
- Useful as an improved paradigm for measuring immobility

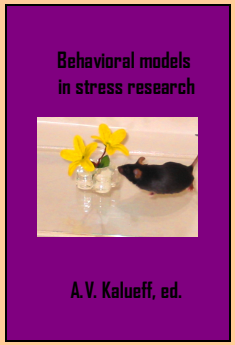
Innovative research:

Developing new models for screening psychotropic drugs



Neurophenotyping education





Forthcoming books:

Experimental models in behavioral research. Ed: A. Kalueff, Nova Science, NY, 2008.

Experimental models in serotonin transporter research. Ed: A. Kalueff, Cambridge University Press, 2009.

Educational activities:

1997-2008: 11 International "Stress and Behavior" Conferences

2008-2009: 2 International Summer Schools on Behavioral Genetics and Neuroscience

Papers on methodology and strategies of neurophenotyping research:

Kalueff, Tuohimaa (2004) Experimental models of anxiety and depression. Acta Neurobiol. Exper. 64, 123-127.

Kalueff et al. (2007). What's wrong with my mouse model? Advances and strategies in animal modeling of anxiety and depression. Behav. Brain Res., 179, 1-18.

Kalueff, Murphy (2007) The importance of cognitive phenotypes in animal models of anxiety and depression. Neural Plasticity, 2007, 1-7.

Eumorphia Project (2002-2006)

EC-funded creation of neurophenotyping databases

- **EMPReSS** contains Standard Operating Procedures (SOPs) for phenotypical characterization of mice

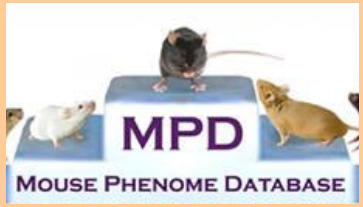
- **Europhenome** holds the phenome data acquired from EMPReSS

- **Eumodic** is a project that generates phenome data on mice using the EMPReSS SOPs

Online resources for mouse models

- **Nomenclature resources**, Jax Mice and Services articles and announcements:
  http//jaxmice.jax.org/info/nomenclature

- **Mouse Genome Informatics Database (MGI),** search by gene symbol, gene name or common name
  www.informatics.jax.org



- **Mouse Phenome Database (MPD),** A standardized collection of phenotype and genotype data on inbred, diabetic, and obese mice:
  www.jax.org/phenome

- **International Mouse Strain Resource (IMSR),** Search for mouse strains publicly available worldwide:
  www.informatics.jax.org/imsr

Expanding neurophenotyping batteries

OCD screens
Perseverations and stereotypies

Autism/sociability screens
Aggression screens

Chronic stress
Social defeat paradigm
Anhedonic depression

Maternal phenotypes
Cross-fostering
Nest-building phenotypes

Oto-vestibular phenotypes

Bipolar depression
Models of mania

Cognitive screens
Within- and between-trial habituation tasks
Barnes maze, 3D-maze
Spontaneous alternation tasks
Mismatch negativity

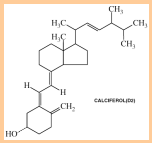
Schizophrenia-related tests

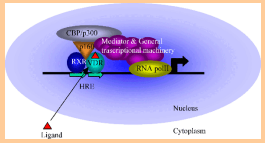
Early life stress
Behavioral effects of enrichment

Neurotoxicity syndromes
Spontaneous (serotonin syndrome)
Drug-potentiated

Drug abuse phenotypes
Ethanol-withdrawal anxiety
Ethanol-related behaviors
Drug preference
Screens for hallucinogenic drugs

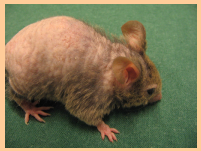
Step-by-by step phenotyping approach



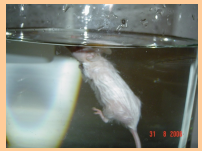


- Vitamin D is a steroid hormone
- Has multiple functions in the CNS
- Acts via nuclear receptors (VDR)

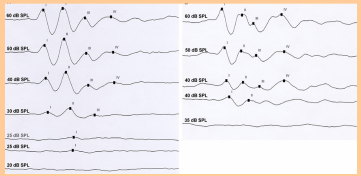
VDR-/- mice



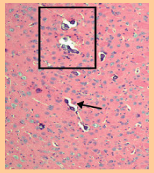
Aberrant swim



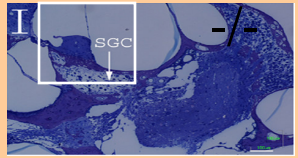
ABR waves +/+ -/-

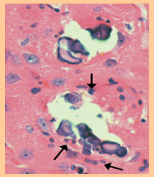


Thalamic calcification

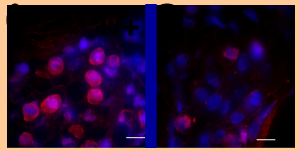


Spiral ganglion cell loss





Caspase 3 activation



1997 2003 2004 2005-2006 2007

Kato et al.
Generation of the mouse

Kalueff et al.
Increased anxiety
Cited>30 times

Kalueff et al.
Impaired swimming

Kalueff and Bart:
Hearing loss

Kalueff et al.
Thalamic calcification

Zou, Kalueff et al. (in press)
Aberrant inner ear morphology,
reduced caspase 3 activation

This confirms the important role of the vitamin D/VDR neuroendocrine system in the regulation of the brain functions, including anxiety, motor- vestibular functions, hearing and brain Ca++ metabolism.

Relevant to clinical data on the same affected domains in humans with mutated VDR

Research quality and progress

Investigators must be able to:

- discuss the hypotheses to be tested
- design the optimal battery of tests
- discuss potential effects of genetic background and other factors
- participate in behavioral tests of their animals
- get competent advise on how to interpret behavioral data
- get competent help on statistical analyses of their data

Research quality and progress

The laboratory must be able to:

- provide training for research staff
- perform multidisciplinary collaborative research
- constantly update the neurophenotyping platform
- develop new models of CNS disorders
- extend research activities into new fields (genomics, proteomics, bioinformatics)
- maintain high-quality research adhering to the mission of the research Institutes


© Original Artist
Reproduction rights obtainable from
www.CartoonStock.com
© Mike Baldwin / Cornered
"At first it's, we'll try this and we'll try that. But when there's a medical breakthrough, guess who takes all the credit."