Mouse models
of human (nervous system) disease

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Outline

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  • ethical considerations, 3Rs principle

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• Behavioral tests for mice
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• Problems and developments
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• Examples of behavioral tests
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  • 8-arm radial maze, IntelliCage
Purpose and validity of disease models

• Models, what for and why?
  • understand (normal function and) disease mechanisms and pathogenesis
  • develop disease prevention, diagnostics, therapy: translation bench to bedside
  • reductionistic approach: simple question, optimal experimental design, maximal control of conditions
  • analyses and experimental manipulations that are technically or ethically impossible in humans

• Levels of analysis and methods
  • cellular-molecular, tissue-organ, system-organism
  • genetics, biochemistry, omics
  • morphology, physiology, imaging
  • clinical symptoms, behavior, survival

• Types of models
  • cell, tissue, organ culture
  • induced pluripotent stem cells and organoids
  • animal models: vertebrate and invertebrate
  • simulation: mathematical and computer models, robotics

• Model validity
  • construct validity: model reproduces disease mechanism and important aspects of pathogenesis
  • face validity: model reproduces clinical disease symptoms that are observed in human patients
  • predictive validity: model responds to therapeutic intervention in the same way as human patients

• Ethical considerations, 3Rs principle
  • Use of vertebrae models is regulated, requires a license and needs ethical justification: expected benefit for patients (human or animal) > harm to experimental animals (Güterabwägung)
  • Replace: avoid using animals whenever possible, use simplest species possible
  • Refine: minimize pain, suffering, distress or lasting harm – improve animal welfare: optimal methods and well trained experimenters
  • Reduce: smallest possible (but sufficient!) number of test animals (definition of relevant effect size, power calculation, optimized experimental design)
Animal model species

**Invertebrates**
- *Caenorhabditis elegans*: small, very simple nervous system (synaptic and circuit plasticity, neurodevelopment, aging research)
- *Aplysia californica*: simple and large nervous system (synaptic and circuit plasticity, mechanisms of learning and memory)
- *Drosophila melanogaster*: small, elaborate behavioral repertoire, powerful genetic tools (circuit analysis, disease genetics, e.g. alcohol abuse)

**Vertebrates**
- *zebrafish*: small strongly visual and social vertebrate (increasingly important as model of nervous system disease and neurodevelopment)
- *rat*: similar to humans, rapidly reproducing, easy to handle, brain larger than in mouse (classical model animal in experimental psychology, lesion studies)
- *mouse*: similar to human, strain diversity, efficient tools for genetic manipulation (mouse models for virtually all types of human disease)
- *sheep & pig*: comparable to humans in size and weight (osteoarthritis, experimental heart surgery)
- *dog*: developed social cognition, breed diversity (cognitive research, behavior genetics, disease genetics)
- *primates*: brain and immune system most similar to humans (infectious disease, higher cognitive function)
Techniques to create mouse models

- **Forward genetics: phenotype to gene**
  - selective breeding: comparison of classical lab strains (fancy mice), association studies based on crosses of lab strains, ad hoc selective breeding
  - random chemical mutagenesis: selection of relevant phenotypes to identify responsible genes

- **Reverse genetics: gene to phenotype**
  - random insertion transgene: gain of function, dominant negative, expression of Cre-recombinase to activate conditional alleles
  - targeted mutagenesis by homologous recombination in ES cells: constitutive knockout (null mutation) or knockin (e.g. point mutation), conditional (“floxed”) alleles to be activated by Cre-recombinase
  - Gene editing using CRISPR/Cas9: rapidly developing technique suitable for all species including humans
  - viral vectors for gene delivery: adenovirus (not integrating), retrovirus (integrating), rabies (circuit tracing)

- **Non-genetic models**
  - trauma, mechanical or chemical lesion, irradiation
  - substance application: pharmacology, toxicology
  - environmental manipulation: enrichment, acute or chronic (unpredictable) stress, social stress
  - infection and immune challenge: models of infectious and autoimmune disease (e.g. EAE = experimental autoimmune encephalitis as model of multiple sclerosis), immune challenge during pregnancy as model of neurodevelopmental disorder

- **Combined models**
  - dual hit models for gene x environment interactions: disease associated mutations + environmental enrichment, stress or developmental challenge
  - testing of drugs or treatments in disease models
  - pharmacologically controllable mutations (doxycycline, tamoxifen)
  - chemogenetic models using DREADDs (designer receptors exclusively activated by designer drugs)
Mouse models of nervous system disease I

• **Neurological disease**
  - Stroke: medial cerebral artery occlusion (MCAO), multiple sclerosis: EAE
  - Huntington’s (monogenic disease, loss of striatum neurons): R6 mice express human mutant huntingtin as transgene driven by human huntingtin promoter
  - Parkinson’s (mostly sporadic, loss of dopamine neurons, aggregation of αSyn may be mechanism): αSyn, A53T αSyn transgenic mice, DA-neuron destruction by MPTP or 6-OHDA
  - ALS (amyotrophic lateral sclerosis, loss of motor neurons, mostly sporadic, 40 associated genes): mice with ALS-linked mutations (SOD1G93A transgenic, C9orf72 BAC, UBQLN2P497H transgenic mice)

• **Dementia**
  - Alzheimer’s disease (mostly sporadic): expression of human mutant genes as transgene, alone (PDAPP, TG2576, APP23, TgCRND8, J20), with mutant PS1 and/or TAU (APP/PS1, 5xFAD, 3xTg-AD).
  - Frontotemporal dementia (FTD) linked to ALS

• **Intellectual disability**
  - Down syndrome: mouse orthologs of human chromosome 21 genes distributed on mouse chromosomes 10-16-17 → subsets expressed as transgenes (Ts65Dn mouse, BAC transgenics)
  - single gene mutations (syndromic or non-syndromic): KO of ortholog mouse gene (Rsk2/Coffin-Lowry syndrome, NONO, Gdi1, αPix/Arhgef6)

• **Learning disability**
  - ADHD (attention deficit hyperactivity disorder, high heritability, risk genes are many, have small effects and act in combination, diagnosis based on clinics/behavior): genetic (DAT-KO, mouse expressing DAT Val559 variant) manipulations leading to hyperactivity, pharmacological models: stimulants, drugs modifying serotonin transmission
  - Dyslexia (high heritability, many risk genes): mice with mutated orthologs of risk genes (Dcdc2-KO, Dyx1c1-KO, Cntnap2-KO)
Mouse models of nervous system disease II

- **Schizophrenia**
  - high heritability, many risk genes with small effect
  - diagnosis based on behavior/symptoms (DSM-5)
  - genetic models: mutation or KO of risk genes (Shank3, DISC1 = disrupted-in-schizophrenia-1, neuregulin-1, calcineurin), genetically induced transmitter imbalance (NR1neo mouse, D2R transgenic mouse)
  - neonatal ventral hippocampal lesion, dual hit models (e.g. complexin2-KO x brain trauma), pharmacological models

- **Autism spectrum disorder**
  - high heritability, many risk genes with small effect that are often shared with schizophrenia
  - diagnosis based on behavior/symptoms (DSM-5): deficient social communication, repetitive behavior
  - Fragile X syndrome (lack of FMRP, autism, intellectual disability, other deficits): Fmr1-KO mouse
  - other genetic models: mutation or KO of risk genes (neuroligin-1,3,4; shank-1,2,3, neurexin-1)

- **Mood disorders**
  - heritability less than schizophrenia, little knowledge about risk genes, poorly understood relation to stress and adverse life events
  - diagnosis based on behavior/symptoms (DSM-5)
  - major depression: traditional “models” based on predictive validity (forced swim / tail suspension test, learned helplessness), stress-based models (chronic unpredictable stress, social defeat stress), olfactory bulbectomy, forward genetic models
  - bipolar disorder (mania ↔ depressive episodes): no true model, manipulations leading to hyperactivity
  - anxiety disorders (phobia, posttraumatic stress disorder, panic disorder): see lecture Sophie Masneuf

- **Substance abuse**
  - alcohol, nicotine, opiates, stimulants
  - models based on substance exposure: mice get addicted in ways similar to humans
  - forward genetic models to identify genes & mechanisms of resilience and susceptibility
Behavioral tests for mice

• Spontaneous behavior
  • appearance, posture and general health
  • species-typical behaviors: nest-building, burrowing, spontaneous T-maze alternation

• Sensory-motor function
  • hotplate / tail-flick test, shock reactivity, van Frey filaments, acoustic startle, pre-pulse inhibition, optokinetic reflex, vestibulo-ocular reflex, visual cliff test, optomotor drum, visual discrimination tests, chocolate search task, odor and taste discrimination
  • rotarod, beam walking, grip test, reaching tasks
  • water-maze cue navigation: sensory and motor control test for water-maze place navigation task

• Ingestive behaviors
  • metabolic cages, home cage systems

• Exploration, anxiety and fear
  • open field, light-dark transition, plus and O-maze, Vogel conflict test, novelty suppressed feeding
  • fear learning, fear extinction

• Learning and cognitive function
  • spatial learning: water-maze place navigation; dry mazes: radial-maze, T-maze, Hebb-William-maze
  • associative learning: cued / contextual / trace fear (Pavlovian) conditioning, operant conditioning
  • executive function: 5-choice serial reaction time task (motor impulsivity, attention), delay discounting tasks (choice control, cognitive impulsivity)
  • visual discrimination: touchscreen learning

• Motivation, reward
  • operant conditioning, drug self administration
  • choice / preference tests, progressive ratio schedule, cognitive bias (ambiguous cues), gambling task

• Social and reproductive behavior
  • 3-chamber test: sociability, social memory
  • resident intruder test: male (and female) aggression, tube dominance test
  • sexual and maternal behavior, pup retrieval test
  • ultrasonic vocalizations in pups and adult males
Overlapping! behavioral profiles of nervous system disease models

• Selection of tests
  • individual test results depend on multiple factors, converging evidence from multiple tests needed
  • design of test batteries: presence and absence of changes equally important to demonstrate specificity

• Dementia & intellectual disability
  • similar behavioral profile, different time course
  • deficits in tests of learning & executive function, focus on hippocampus-dependent tests (long- and short-term spatial memory, contextual memory)
  • impaired species-typical and exploratory behaviors

• Schizophrenia
  • positive symptoms: hyperactivity
  • negative symptoms: anhedonia, social withdrawal
  • cognitive symptoms: specific working memory deficit, motor impulsivity, attentional deficits
  • impaired prepulse inhibition of acoustic startle
  • behavioral response to neuroleptic drug: additional criterion, alone not sufficient

• Autism
  • social symptoms: social neglect in 3-chamber test, reduced ultrasonic vocalization
  • repetitive behavior & restricted interests: impaired reversal learning, stereotyped self grooming

• Depression
  • psychological symptoms: reduced activity and exploration, anhedonia, cognitive bias (negative interpretation of ambiguous cues), anxiety
  • social symptoms: social withdrawal, low sex drive
  • physical symptoms: disturbed sleep or food intake
  • improvement by (chronic!) antidepressant drug: additional criterion, alone not sufficient

• Bipolar disorder
  • hyperactivity alone does not make a manic mouse
  • long-term fluctuations of motivation, activity and sleep: behavioral monitoring in the homecage?
  • improvement of phenotype by chronic lithium
Problems and recent developments

• **Problems – disappointed public**
  • “translation crisis”: translation less successful in neuroscience than in other domains (e.g. cancer)
  • “reproducibility crisis”: 10% success in reproducing experimental results (not only in neuroscience)
  • scientists promise too much and make too many mistakes (like politicians)

• **Common mistakes**
  • mice are not small humans: overly simplistic and anthropocentric interpretation of behavior, false face validity of models – mice are not small rats either
  • overemphasis of predictive validity of models
  • flawed study design: insufficient power, environmental bias, bias by genetic background
  • pressure to succeed → biased / selective observation
  • inappropriate use and interpretation of statistics: multiple testing, inflated N, misinterpretation of p-values (misjudgment of negative / positive predictive validity)

• **Endophenotypes**
  • emphasis moved from system to circuit/organ level: bridge between construct and face validity, more similarity between mice and human patients
  • electrophysiology, EEG, prepulse inhibition
  • imaging, neurochemistry

• **Optogenetics**
  • expression of optical calcium sensors
  • expression of artificial genes that render particular types neurons responsive to light, used for silencing or stimulation
  • integration of cellular, circuit and behavioral analysis

• **Behavioral testing in the home cage**
  • higher throughput, continuous long-term observation
  • improves animal welfare: familiar (and social) environment, no handling by humans
  • more standardization, less impact of lab environment
  • test development and validation still in progress
Rotarod

• **Apparatus and Procedure**
  • elevated rotating rod / drum with accelerating rotation speed (0-40 RPM over 5 min)
  • mice avoid falling off by walking synchronously with rotating rod, sometimes grab the rod and rotate with it

• **Measures**
  • time to fall off / speed when falling off
  • time to rotate / speed at first rotation

• **Interpretation**
  • measures motor coordination, to lesser degree muscle force. Improvement over time can be used as measure of motor skill learning.
  • confounds: motivational changes, body weight changes (lighter mice are better), differences in diameter, surface and material of rod
Light-dark transition test

• **Apparatus and Procedure**
  - dark and brightly illuminated chamber connected by small opening. Conflict between light avoidance and exploratory drive, forced exploration test
  - spontaneous behavior observed

• **Measures**
  - time in dark
  - number of transitions, distance traveled
  - risk assessment postures, rearing / leaning, grooming

• **Interpretation**
  - dark time: anxiety – decreased by anxiolytic drugs (classical test for drug screening)
  - rearing, leaning: exploratory drive
  - confounds: blindness, altered activity, freezing in brightly illuminated chamber
Elevated O-maze

• **Apparatus and Procedure**
  • elevated circular runway, 2 90° sectors protected by sidewalls, 2 90° sectors open
  • elevated plus maze: original cross-shaped configuration with central platform and corners to retract to
  • spontaneous behavior observed

• **Measures**
  • % time in open/closed sectors, % entries to open sectors, number of entries to closed sectors, total distance moved
  • free and protected (with body between sidewalls) head dips, risk assessment postures (stretch attend & retract)

• **Interpretation**
  • open sector time: anxiety – increased by anxiolytic drugs (classical test for drug screening)
  • closed sector entries, distance moved: activity
  • head dips: measure of exploration
  • confounds: inactivity, freezing on open sectors, stereotyped head dipping
Spontaneous T-maze alternation

• **Apparatus and Procedure**
  • T-shaped corridor with doors
  • sample trial with confinement to chosen arm after choice, free choice trial after short delay
  • variant: continuous free running in an Y-maze
  • not to be confounded with rewarded T-maze tasks assessing discrimination, spatial or habit learning

• **Measures**
  • rate of alternation over repeated trial pairs
  • choice latency

• **Interpretation**
  • alternation depends on exploratory drive and spatial working memory
  • highly sensitive to hippocampal lesions
  • confounds: altered activity, loss of motivation
Radial-maze

• Apparatus and Procedure
  • 8-arm maze, small invisible baits at end of all or part of the arms, salient distal room cues
  • food deprived mice (kept at 85% of normal body weight) collect baits, walking freely or confined to central platform between choices

• Measures
  • working memory errors: reentry to emptied arm
  • reference memory errors: entry to unbaited arm
  • neglected and omitted baits, locomotor activity, choice patterns

• Interpretation
  • hippocampus-dependent spatial task, may differentiate between working and reference memory deficits
  • confounds: lack of motivation, motivation, reduced tolerance of food deprivation, use of local olfactory cues, stereotyped choice patterns
IntelliCage

- **Apparatus and Procedure**
  - large home cage with 4 operant learning corners giving access to 2 drinking bottles each. Food available at libitum.
  - up to 16 RFID tagged mice can be tested per cage in a large variety of fully automated computer controlled protocols

- **Measures**
  - individually recorded events: corner visits, nosepokes, licking
  - ambient variables: light, temperature

- **Interpretation**
  - depends on protocol / task: spontaneous behavior, hippocampus-dependent learning, anxiety, motivation and anhedonia, impulsivity
  - confounds: cheating by imitation in learning tasks, competition and fighting of male mice, corner hugging, poking with both ends of the body, two mice entering corner at the same time