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Mouse models
of human (nervous system) disease

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Outline

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  - *ethical considerations, 3Rs principle*
- *Mouse models of nervous system disease*
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  - *diseases modeled in mice*
- *Behavioral tests for mice*
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  - *disease-typical behavioral changes*
- *Problems and developments*
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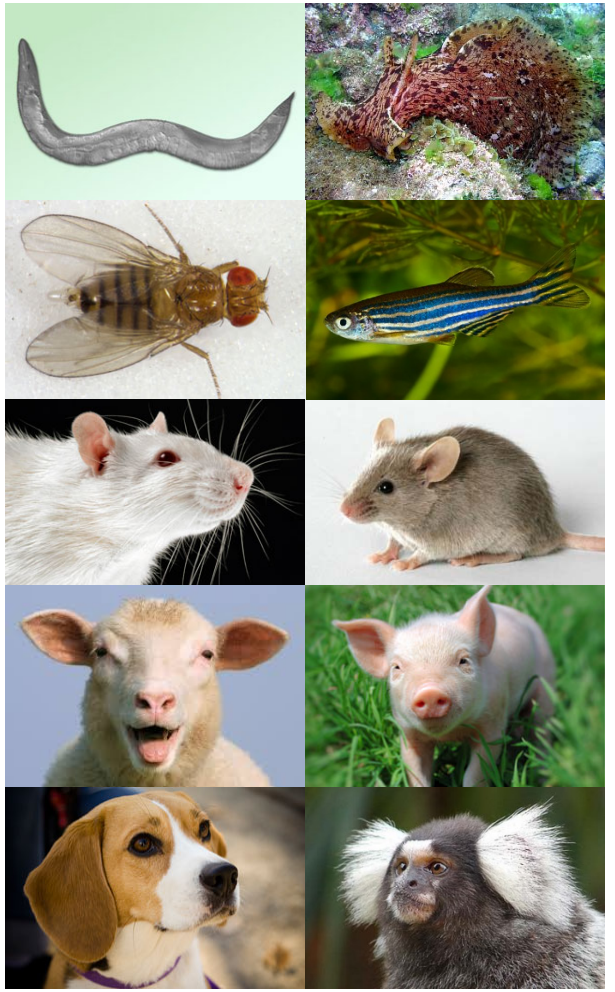
  - *endophenotypes, optogenetics, homecage systems*
- *Examples of behavioral tests*
  - *rotarod, light-dark transition, elevated O-maze, spontaneous T-maze alternation*
  - *8-arm radial maze, IntelliCage*

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*
  - *Alzheimers 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

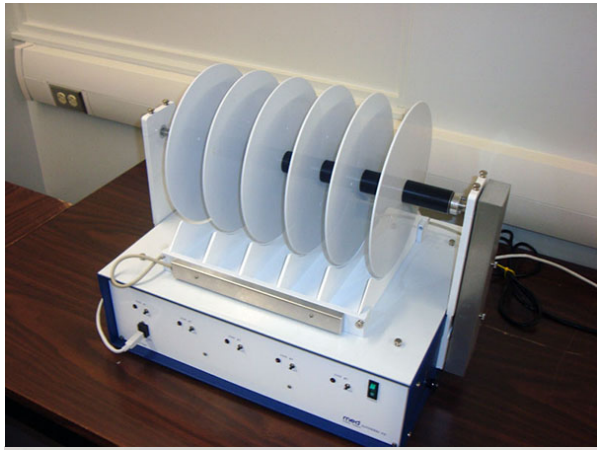
- *Selection of tests*
  - *individual test results depend on multiple factors, converging evidence from multiple tests needed*
  - *design of test batteries: presence and absence of changes equally important to demonstrate specificity*
- *Dementia & intellectual disability*
  - *similar behavioral profile, 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*

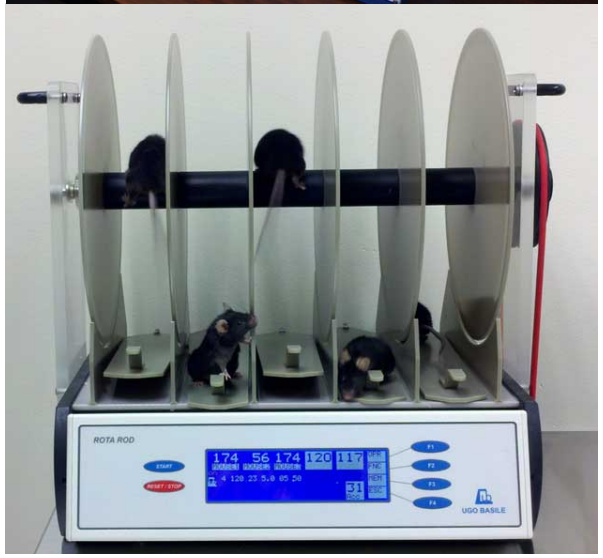
Problems and recent developments

- *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

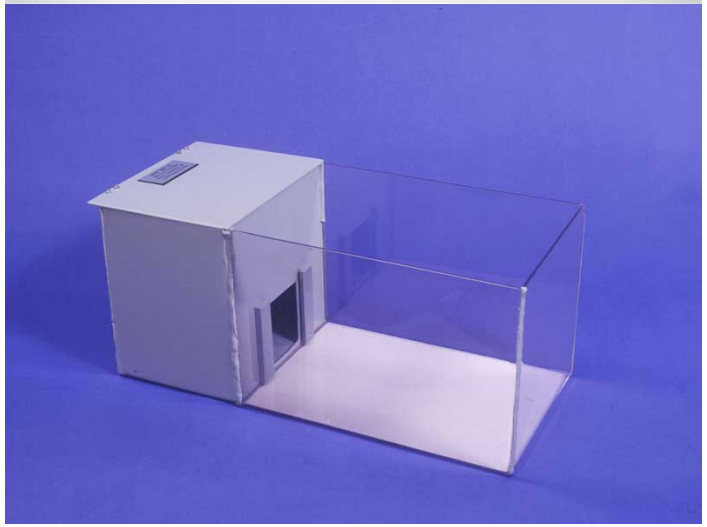
- *Apparatus and Procedure*
  - *elevated rotating rod / drum with accelerating rotation speed (0-40 RPM over 5 min)*
  - *mice avoid falling off by walking synchronously with rotating rod, sometimes grab the rod and rotate with it*
- *Measures*
  - *time to fall off / speed when falling off*
  - *time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

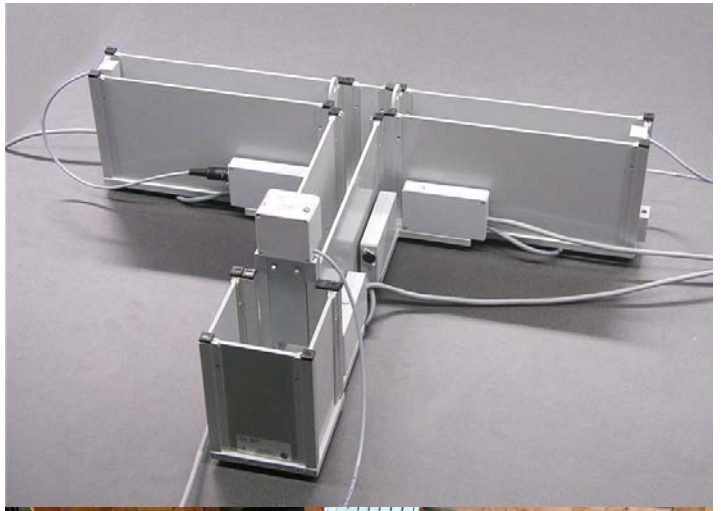


Elevated O-maze

- *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*

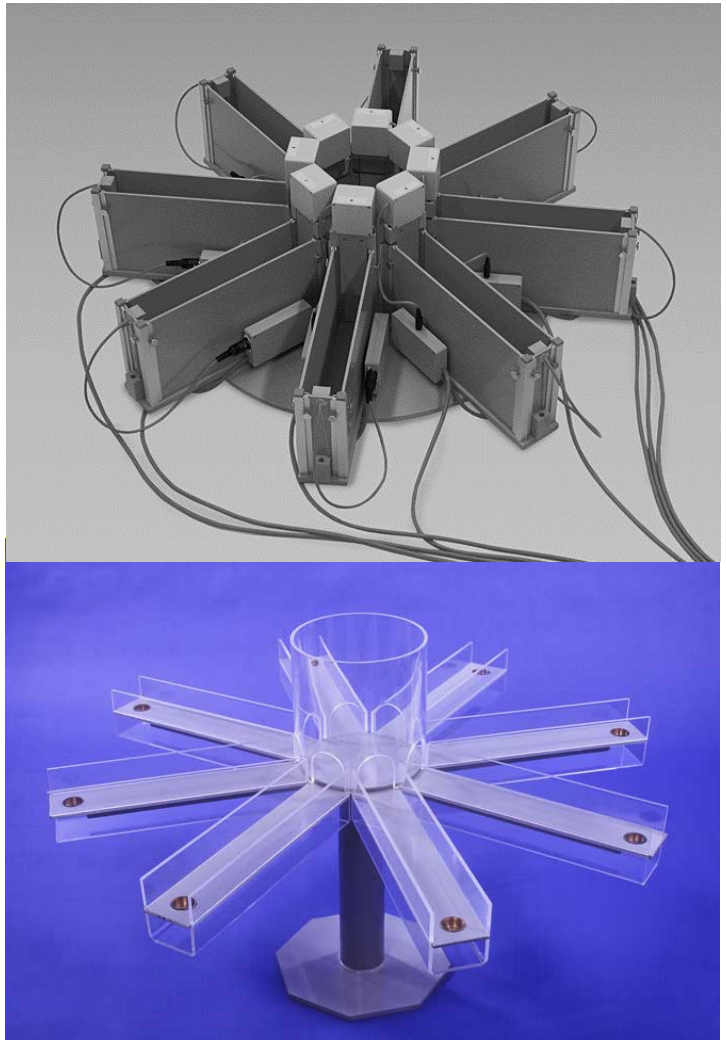
Spontaneous T-maze alternation

- *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*