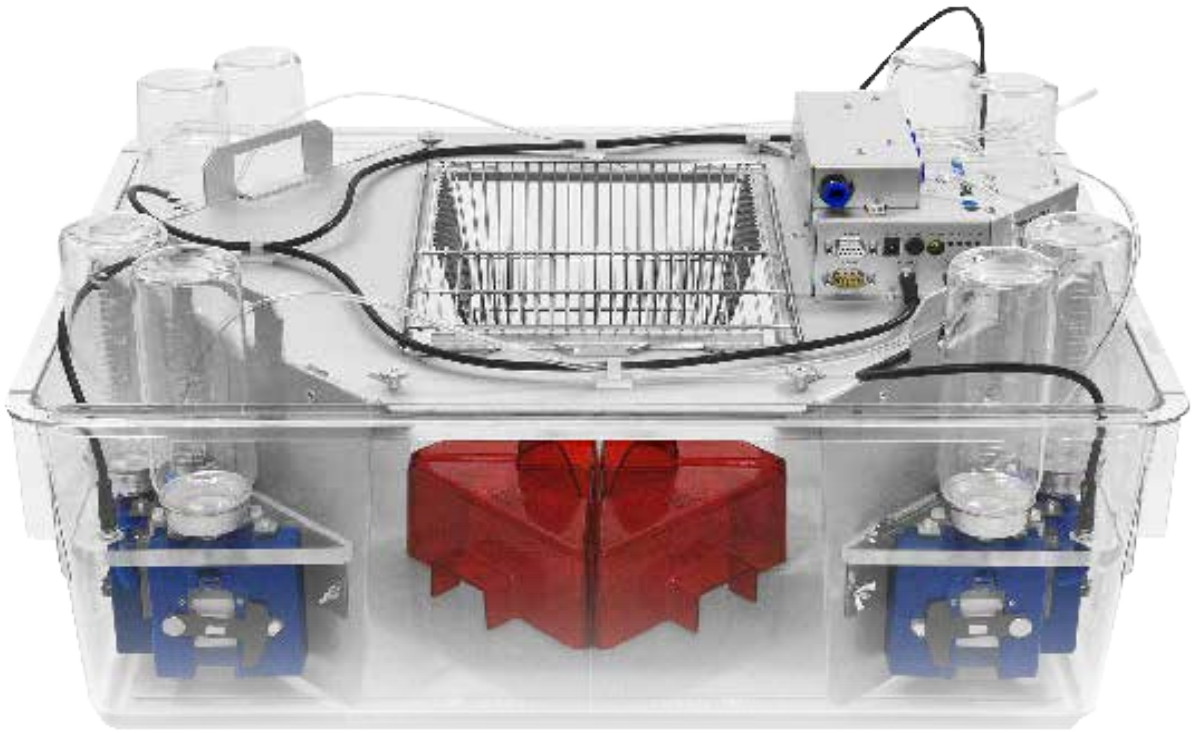
Sophisticated Life Science Research Instrumentation

NewBehavior

IntelliCage

Cognitive & Behavioral Screening of Individual Mice Living in a Social Group



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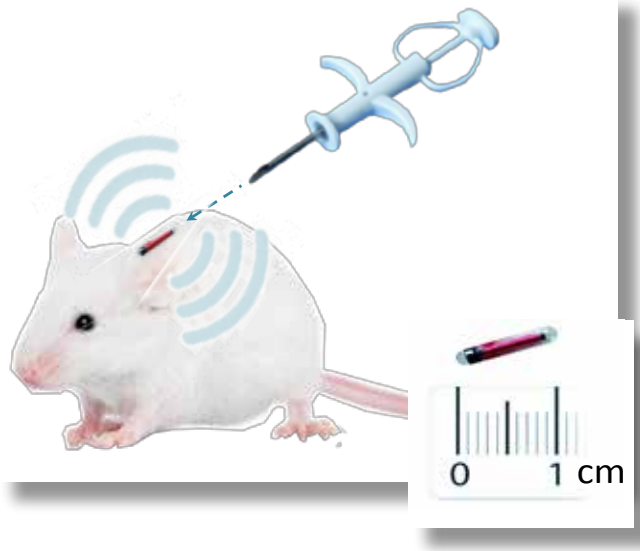
IntelliCage: System Description

The IntelliCage is designed for **long-term high-throughput investigation of cognitive abilities in laboratory mice**. Socially housed mice can perform a variety of freely programmable behavioral tasks in their home cage. Simple to complex conditioning tasks or experiments can be graphically designed in a uniquely flexible manner and controlled for each individual animal in the IntelliCage. The individually tailored experimental protocols are automatically run and analyzed for large numbers of transponder tagged animals simultaneously in the same cage. This allows the investigation of experimentally induced phenotypic or genotypic effects on cognitive abilities as well as activity patterns. Such behavioral screening is frequently required in biomedical and basic behavioral, neurobiological, pharmacological and genetic research, and can be conducted in the IntelliCage with exceptionally high efficiency, standardization and minimal work load.

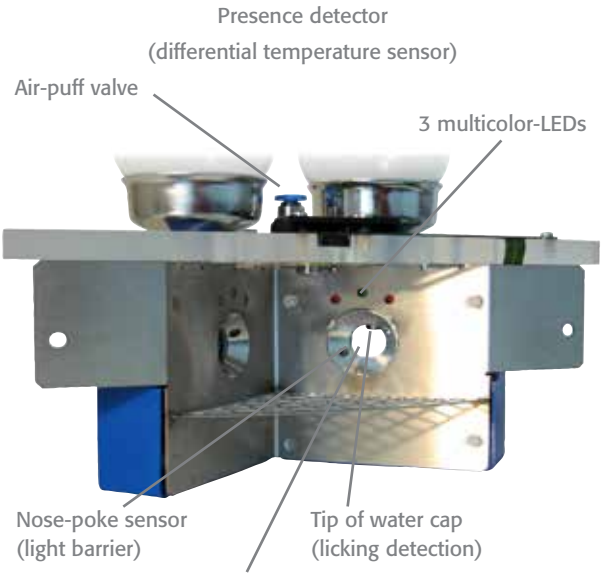


Transponder Technology

- Subcutaneously injected RFID-transponders allow animal recognition – a key feature of the system
- Up to 16 mice can be housed within one single IntelliCage
- For high-throughput testing up to 8 IntelliCages can be connected to a single computer

Operant Corner Technology

- A single IntelliCage contains 4 identical operant conditioning corners, each may accommodate one mouse at a time
- Each operant corner is equipped with a combination of actors for shaping the animal's behavior according to individualized reinforcement and conditioning protocols

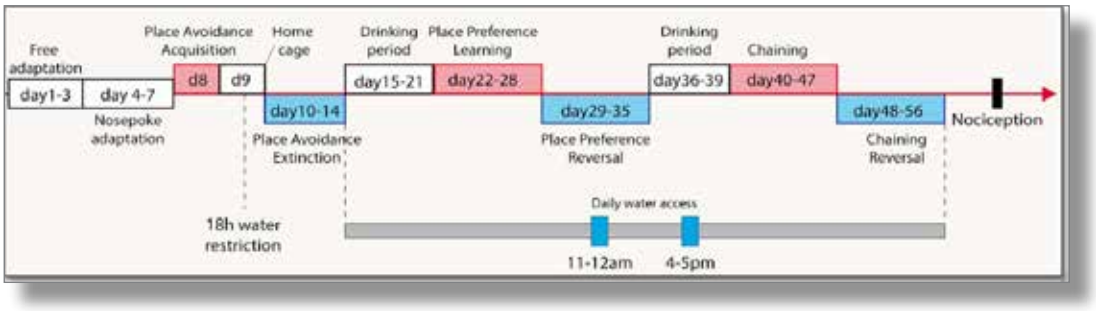


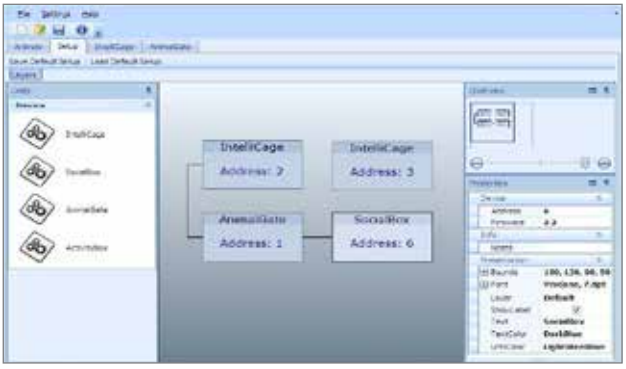
- **Actors include:**
  - An RFID antenna for recognition of individual transponderized mice
  - 2 motorized doors blocking or allowing access to water bottles on both sides of the corner (positive reinforcement)
  - Multicolor LEDs above the doors on both sides (conditional stimuli)
  - An air-puff valve for delivering negative reinforcement

- **Behavioral events detected in operant corners:**
  - Visits: the RFID antenna identifies individuals, a temperature-sensitive presence sensor detects the start, end, and duration of a corner visit
  - Nosepokes: interruption of a light-beam sensor at either door allows access to water bottles
  - Drinking: the number and duration of tongue-contacts with nipples of the bottles are registered by a lickometer

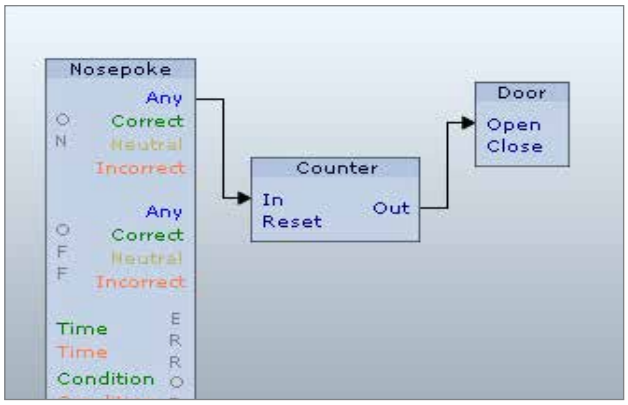
- Any of the behavioral activities or sequences can be connected to actions of the system in order to build up customized conditioning protocols (more detailed description in the Software section)

IntelliCage: Software

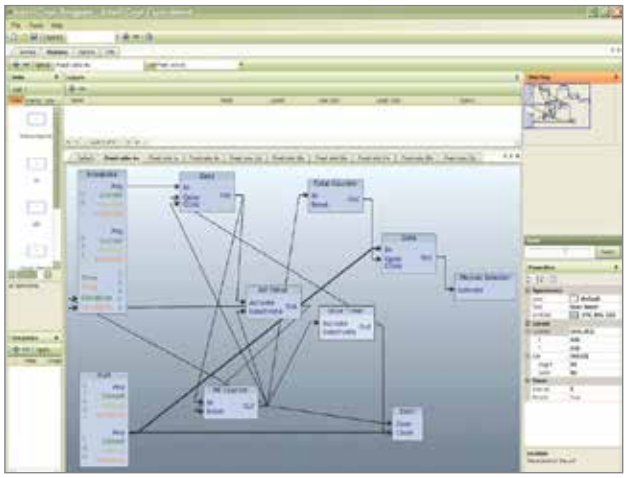




Designer - Hardware Setup



Designer - Simple Module



Designer - Advanced Module

IntelliCage Plus Software

- The functionality of the IntelliCage is achieved by the unique and user-friendly IntelliCage Plus Software consisting of three separate parts:
  - Designer
  - Controller
  - Analyzer

Designer

- The Designer software allows the definition of individual cognitive test schedules and protocols applied to the transponder-marked animals in the IntelliCage. Access to water (or other liquid) from bottles in specific corners can be used as positive reinforcement; air-puffs can be used for negative reinforcement and LEDs as conditional stimuli.The basic steps for configuring the experiment are the following:
  - Specify hardware settings
  - Create animal list (enter animal IDs & transponder numbers)
  - Create experimental designs by assigning specific clusters to individual animals and modules to groups of animals

- Clusters represent the status of cage components for each animal assigned to this cluster; each corner and each side within a corner can be independently defined as correct, neutral, or incorrect for any number of different clusters.

- Create modules (experimental designs) defining events in specific corners (according to cluster information): link animal behavior (visit, nosepoke, lick) to trigger hardware events (door opening, lights, air-puff) – resulting in full control over conditioning behavior.

- Experimenters can create several modules and imply switches between the clusters and/or modules that can be driven by the specific behavior of mice. Moreover, day patterns can be defined where links between the modules or clusters occur at specified times of the day.

IntelliCage: Software

Controller

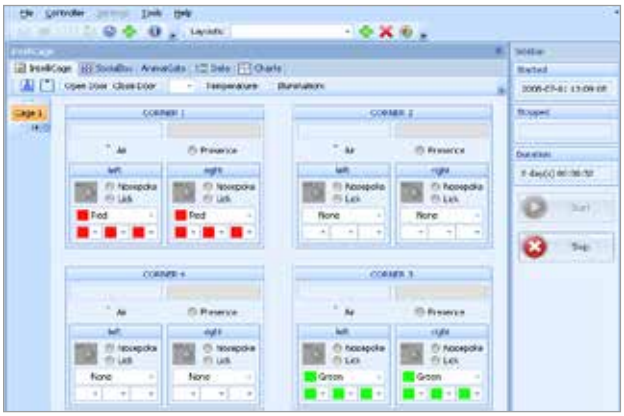
- The Controller extracts and stores all behavioral events (visits, nosepokes, licks) from the incoming stream of sensor data
- It provides an overview of data outputs - referred to the controlled design. Thereby, IntelliCage measures the correct or incorrect presence of individuals in the conditioning corners, location and correctness of nosepokes, the incidence and extent of drinking behavior, and the occurrence of negative reinforcement (air-puffs).
- All events can be monitored and visualized on the screen during the experiment in an overview console.
- The Controller further visualizes basic behavioral parameters during ongoing experiments, allowing online-monitoring of events and developments.
- The Controller saves experimental data into files of individual animals or of animals in groups.
- The Controller can be programmed to send alerts if an animal has no visits or licks during a specified period.

The Controller - in Short

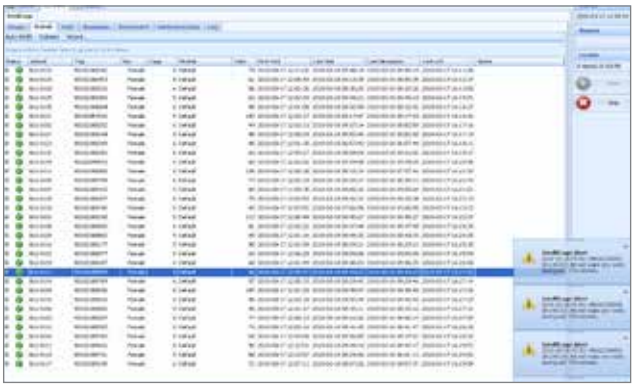
- Executes the designed protocols
- Monitors the progress of th experiment in real-time
- Saves experimental data into zip-archives

Analyzer - Data graph (e.g. visits)

Analyzer - Charts (number of licks, nosepokes, visits)



Controller



Controller - Animal list with alerts

Analyzer

- The Analyzer software takes advantage of the stored behavioral sequence data to derive the temporal development of the animals' behavior in response to the designed conditioning protocols.
- Figures, tables and filtered data can be saved for export to other computer programs such as standard graphical and statistical packages.

The Analyzer - in Short

- Open experimental data saved by the Controller
- Explore data, create and apply filters (e.g. by module, time, events etc.), create and export charts for customized views
- Save the filtered data into tab-delimited text files for further analysis by external statistical packages

IntelliCage: Tasks & Paradigms

Freely Programmable Tasks

- The IntelliCage offers enormous flexibility for designing behavioral and conditioning protocols – customer-specific demands can be adapted by a simple graphical design.

- Tasks can be programmed for the following behavioral domains:

1) Spontaneous behavior – anxiety, neophobia, exploration, behavioral stereotypies, habituation, circadian activity
2) Spatial and temporal behavior – place preferences and avoidance learning, reversal learning, spontaneous alternation, temporal conditioning, patrolling schedules
3) Discrimination learning – visual discrimination, gustatory discrimination, spontaneous drug preference or avoidance
4) Memory – habituation, working and reference memory, gustatory memory, procedural memory
5) Operant conditioning – fixed or progressive ratio conditioning, differential reinforcement of low responding (DRL)

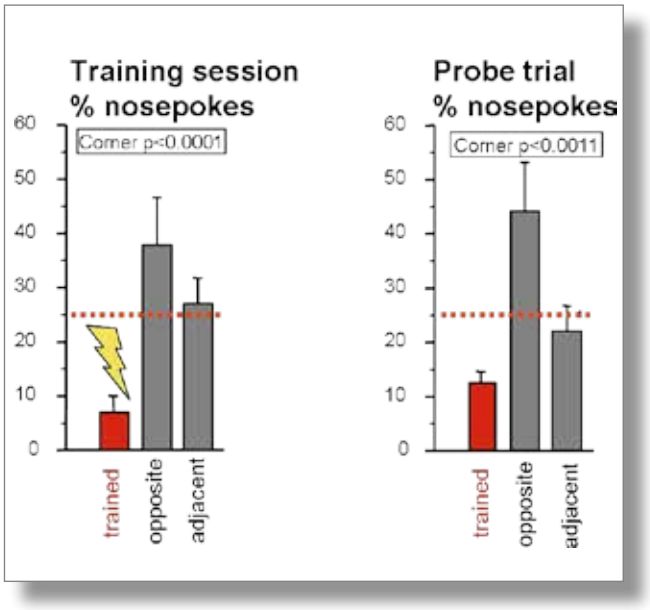
- Some of the most commonly used tasks are explained below. The scientific hypothesis and goal of the specific study define which tasks are to be performed or combined into a test battery.

- Preprogrammed behavioral tasks are available on request.

- **Important Note:** animals need not to be kept permanently in the IntelliCages. Test cohorts can be formed and kept in ordinary cages. Animals adapt very rapidly after being reintroduced to

Spontaneous Behavior
Basic activity levels, circadian activity
Spatial and Temporal
Stereotypical place preferences
Spatial preference and avoidance learning
Spatial reversal learning
Spontaneous alternation
Temporal conditioning
Temporo-spatial conditioning
Systematic patrolling schedules
Radial maze like patrolling
Discrimination Learning & Preferences
Visual discrimination
Gustatory discrimination learning
Spontaneous drug preference or avoidance

Memory
Procedural memory
Habituation
Spatial short-term (working memory)
Visceral / gustatory memory
Operant Conditioning
Procedural learning
Fixed ratio conditioning (motivation)
DRL (different reinforcement of low responding, response inhibition, timing)
Social & Others
Competition rank order
Approach-avoidance conflicts



Spatial avoidance during training and probe test (DP. Wolfer)

Spontaneous Behavior

Free adaptation

- Animals are released into the IntelliCage with all doors open for an adaptation period. Neophobia and habituation can be detected by latency and number of the corner visits and initiation of drinking. The number of corner visits also provides a reliable measure of activity, allowing the detection of individual circadian rhythms. The adaptation period is particularly useful for establishing individual baselines and detecting behavioral anomalies in mutant mice.

Nosepoke adaptation

- The doors are closed at the beginning of the module and can be opened only by nosepoking into the door area. This can be viewed as most simple fixed ratio 1 operant conditioning procedure in the IntelliCage.

Temporal conditioning

- Several conventional learning tests require food or water deprivation for increasing the motivation of the animals and the training is carried out in discrete trials. This can be programmed in the IntelliCage. Access to water can be restricted to certain time periods during the dark or light phase. Using temporally restricted drinking access provides information about temporal learning abilities and might be a prerequisite for subsequent learning tasks.

IntelliCage: Tasks & Paradigms

Spatial Conditioning Tasks

Nosepoke avoidance with probe trial

- One of the four corners is assigned as incorrect for each individual mouse, where nosepokes are punished with airpuffs and doors are not opened. Following a retention interval (e.g. 1 or 7 days) animals are returned to the IntelliCage for a 'probe trial period where nosepokes open the door in each corner and no airpuffs are applied. This task shares similarities with conventional spatial learning and memory procedures (e.g. water maze, but also fear conditioning or passive avoidance) where a certain delay is implied between training and testing.

Corner preference / avoidance

- Preference learning: one corner is assigned to be correct for each individual animal in which water is accessible. Acquisition of this task can be followed by reversal learning, where the opposite corner is assigned to be correct.
- Avoidance learning: the visits to one or more incorrect corners are punished with air-puffs.

Serial reversal

- The correct corner is changed for each drinking session.

Patrolling designs

- The position of the correct corner is rotated clockwise or anticlockwise after correct visit, or actual drinking, or after each visit.

Taste preference or aversion

Conditioned taste aversion (CTA)

- During training the conditioned group has access to bottles with sucrose and lithium chloride (LiCl) or water. During the test, the animals can choose between water and sucrose.

Other gustatory preference/avoidance tasks

- As one cage contains 4 corners with two bottles each, it can be seen as an excellent environment for testing spontaneous preference or avoidance to different concentrations of various compounds.

Operant Conditioning Tasks

Light discrimination

- The correct side for making the nosepoke and opening the door is indicated by LEDs at the beginning of a visit. The correct side is randomly changed for each corner visit.

Impulsivity and DRL procedures

- The animal initiates a trial with the first nosepoke and is required to wait for a certain delay before making the second nosepoke to open the door. The delay can be signaled by a light stimulus.

Attention

- Animals are required to make a nosepoke when LED is on. Stimulus duration can be shortened progressively along with random delay for switching the LED on after the beginning of the visit.

Delay discounting

- Each corner contains two bottles – one with plain water and one with sweet solution (e.g. sucrose). During training both doors are opened at the same time allowing animals to learn the position of sweet solution and to show preference for it. Following adaptation the door to water is opened immediately when the visit begins, while the other door will be opened only if the animals tolerate the pre-defined delay without drinking water. Thus, the mice can choose between immediate access to water and delayed access to sweet solution.

Practical examples for validation

Animals with a hippocampal lesion can be reliably distinguished from control mice already during early adaptation and in various learning tasks (Voikar et al., 2010):

- Initial inhibition (longer latencies to visit the corners) followed by hyperactivity
- Spatial stereotypies (increased percentage of corner re-entries)
- Impaired temporal conditioning (lack of increased activity before the drinking sessions)
- Impaired corner avoidance learning and memory

Animal entering operant corner

Lick during corner visit

End of corner visit

IntelliCage: Applications

Change in behavior is the most sensitive biological end-point informing about any alterations within the organism. Therefore, careful behavioral analysis of animal models is an important part in modern biomedical studies. The following fields of research profit enormously from using IntelliCages:

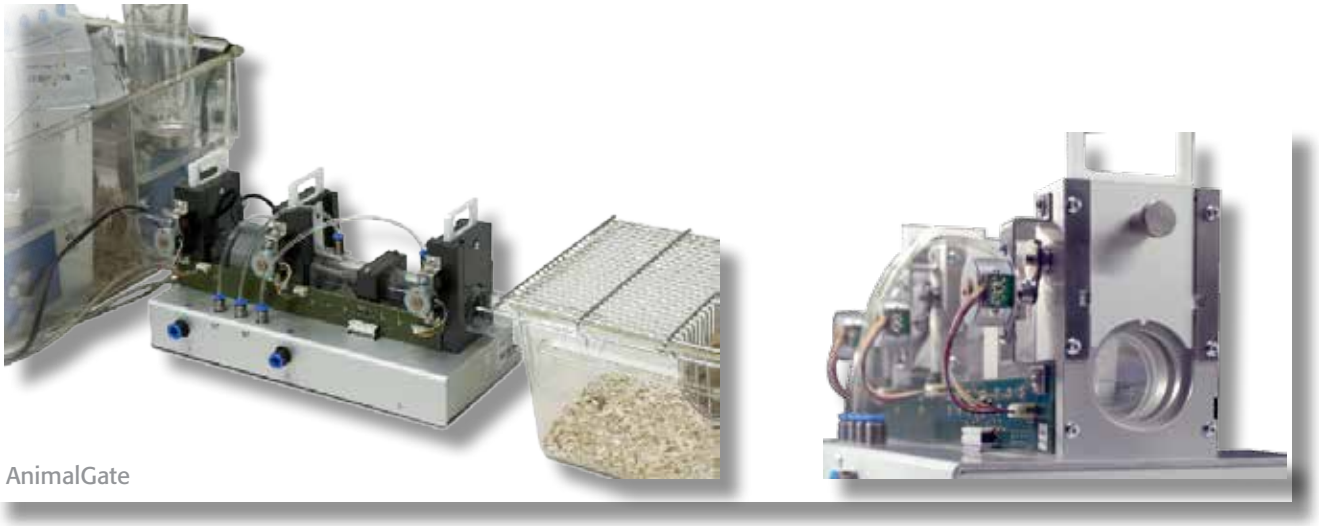
1) High-throughput behavioral phenotyping – several international initiatives aim at targeting most of the genes in the mouse genome with the long-term goal of identifying specific functions for every single gene. The success of these projects depends on highly standardized phenotyping procedures, such as offered by IntelliCage, that cover multiple behavioral and cognitive domains.

2) Assessment of disease models – IntelliCage allows true longitudinal studies, where animals either stay in the IntelliCage for a prolonged period, or re-visit the system several times during their life-span. Both approaches allow the detection of early or age-dependent signs and symptoms of a disorder (e.g. Huntington's disease, Alzheimer 's disease).

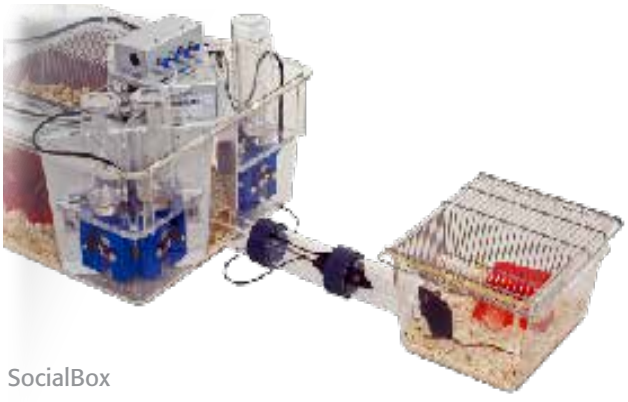
3) Mechanistic studies – e.g. brain lesion studies for testing involvement of specific structures in different behavioral domains, leading to better general understanding of mouse behavioral biology.

4) Behavioral genetics – a lot of information on inbred strain differences has been obtained during the past decades using conventional test setups. Experiments in the IntelliCage can advance these comparisons and bring them to a new level by also considering the social factor and by eliminating human interference and analysis bias.

5) Pharmacological studies – drugs can be administered orally in drinking water or delivered via osmotic mini-pumps.



AnimalGate



SocialBox

Add-Ons

AnimalGate

- Expands IntelliCages to a multi-area system
- Senses the direction of movement & allows/denies passage
- Measures body weight of passing mice
- Restricts access to food or liquid sources or any other area
- Expands IntelliCage to measuring food consumption
- Allows application of different treatments to specific animals without handling

SocialBox

- Expands IntelliCages to a multi-area system
- Allows studies on social and/or preference patterns
- Up to 4 additional rooms for each IntelliCage offer additional space for enriched environment if requested

Recent Publications

- **Vannoni E et al.** (2014) Spontaneous behavior in the social homecage discriminates strains,lesions and mutations in mice. J Neurosci Methods; 234:26-37
- **Smutek M et al.** (2014) A model of alcohol drinking under an intermittent access schedule using group-housed mice. PLoS One; 9(5):e96787
- **Too LK et al.** (2014) The pro-inflammatory cytokine interferon-gamma is an important driver of neuropathology and behavioural sequelae in experimental pneumococcal meningitis. Brain Behav Immun; 40:252-68
- **Puscian A et al.** (2014) A novel automated behavioral test battery assessing cognitive rigidity in two genetic mouse models of autism. Front Behav Neurosci; 8(140):1-11
- **Knapska E et al.** (2013) Reward learning requires activity of matrix metalloproteinase-9 in the central amygdala. J Neuroscience; 33(36):14591-14600
- **Branchi I et al.** (2013) Antidepressant treatment outcome depends on the quality of the living environment: a pre-clinical investigation in mice. PlosOne; 8(4): e62226
- **Knapska E et al**. (2013) Measuring basal and complex behaviors of rats in auto mated social home cage systems using IntelliCage for rat technology. J Neuroscience; 33(36): 14591-600
- **Kobayashi Y et al.** (2013) Genetic dissection of medial habenula-interpeduncular nucleus pathway function in mice. Front Behav Neurosci; 07: 17
- **Parkitna JR et al.** (2013) Novelty-seeking behaviors and the escalation of alcohol drinking after abstinence in mice are controlled by metabotropic glutamate receptor 5 on controlled by metabotropic glutamate receptor 5 on neurons expressing dopa mine D1 receptors. Biological Psychiatry; 73(3): 263-70

Service & Warranty

TSE Systems offers a Two (2) Years ALL-IN Premium Warranty with all new products, including:

- 24/7 technical hotline
- Remote maintenance and update function
- On-site visits upon necessity
- Free replacement parts during warranty

After the expiry of the warranty period, TSE Systems offers comprehensive extensions of the warranty or economical maintenance and repair contracts to ensure the continued smooth running of your instruments. Please contact us for further details.