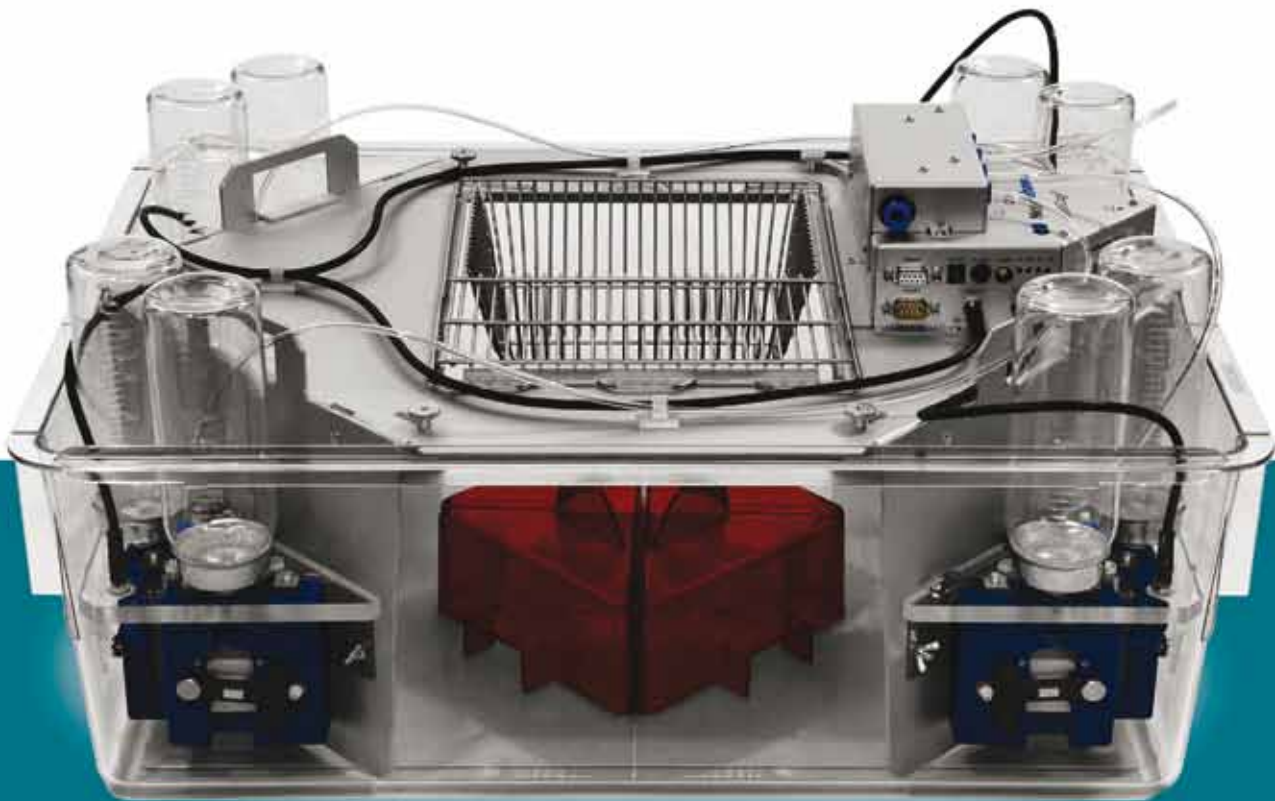


Sophisticated Life Science Research Instrumentation



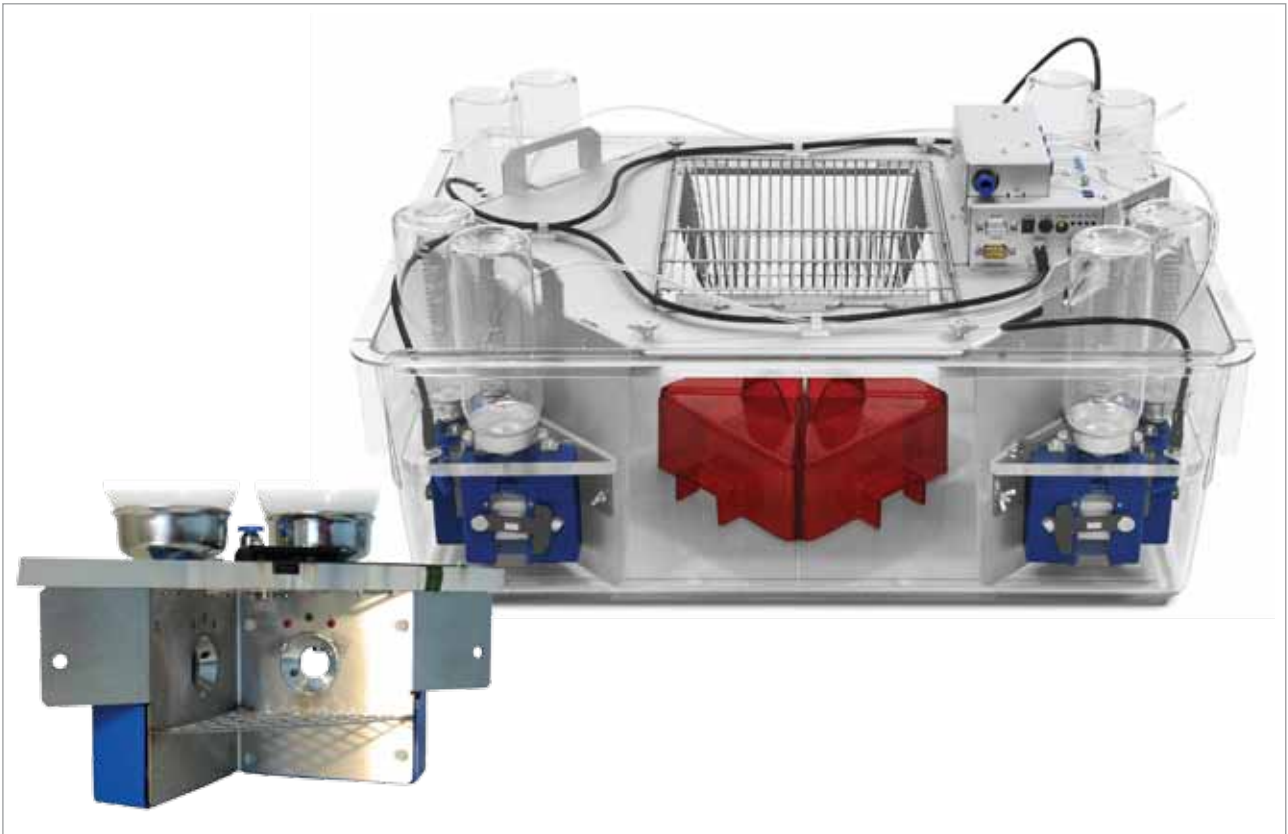
IntelliCage

Cognitive screening for mice

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– Specifications subject to change without notice –

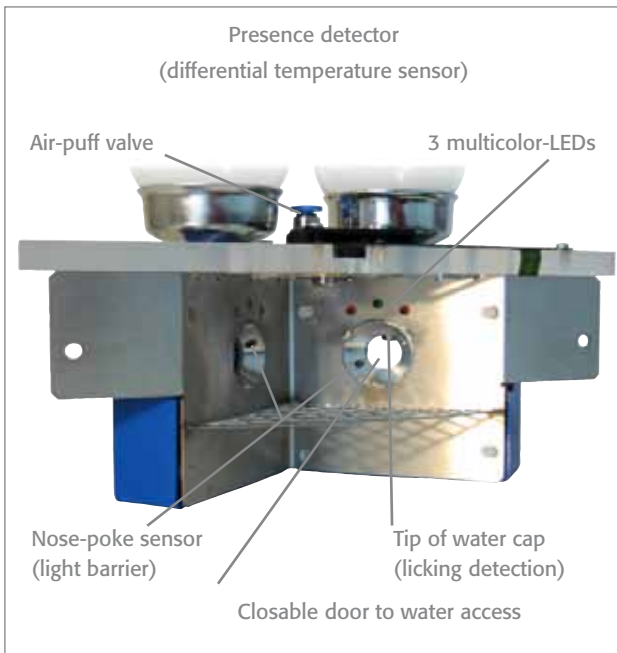


IntelliCage

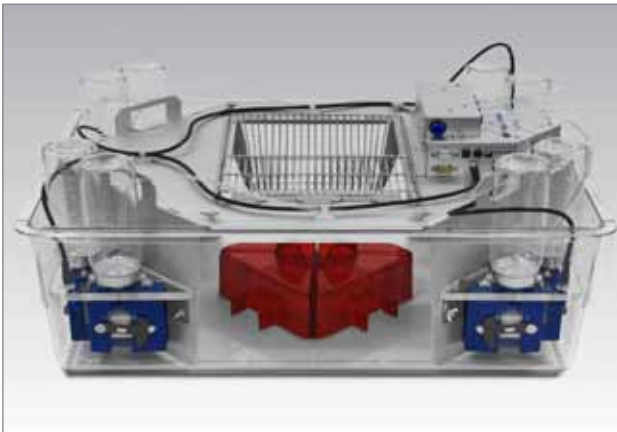
System Description and Benefits

The IntelliCage is designed for long-term high-throughput investigation of cognitive abilities in laboratory mice. Social housed mice can perform a variety of freely programmable behavioral tasks in their home cage. Simple 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.

- Investigation of individual mouse behavior in the social context excluding stress
- Fully automated screening of complex learning procedures with online monitoring
- Ability to monitor individual learning over long time periods
- High level of animal welfare



IntelliCage Corner



IntelliCage

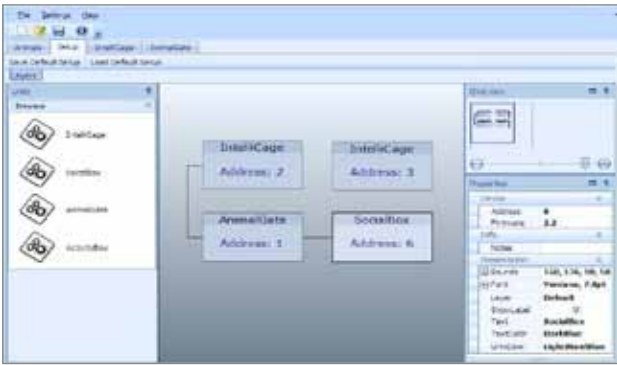


Transponder

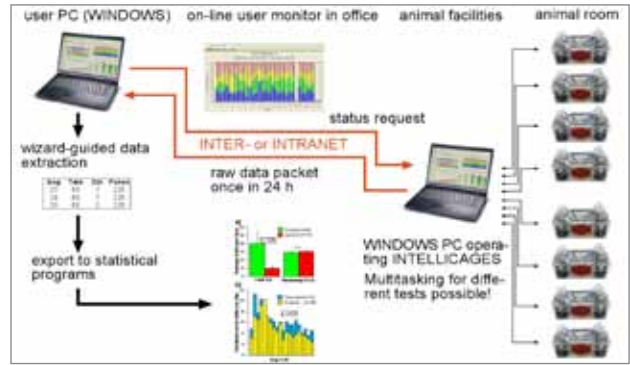
Hardware & Transponder Technology

- A single IntelliCage consists of four identical operant conditioning corners accommodating only one mouse at a time
- Each operant corner contains actors for shaping the animal's behavior according to individual reinforcement and conditioning protocols
 - one antenna for individual recognition of transponderized mice
 - two motorized doors blocking or allowing access to water bottles on both sides of the corner (positive reinforcement)
 - multicolor LED's above the doors on both sides (conditional stimuli)
 - one air-puff valve for delivering negative reinforcement
- Subcutaneously injected RFID-transponders for individual recognition of group-housed animals – a key feature of the system
- Up to 16 mice can be housed simultaneously within one single IntelliCage based on a standard cage (20 x 55 x 38 cm). For high-throughput testing up to 8 IntelliCages, each potentially containing 16 mice, can be connected to a single computer
- The following behavioral events of the animals are detected and recorded by specific sensors in the operant corners
 - Visits – RFID antenna identifies individual animals based on their transponder, a temperature-sensitive presence sensor detects the start, the end, and the duration of a corner visit
 - Nosepokes – interruptions of a light-beam sensor at both doors allowing access to water bottles
 - Drinking – the number and duration of tongue-contacts with nipples of the bottles are registered by a lickometer

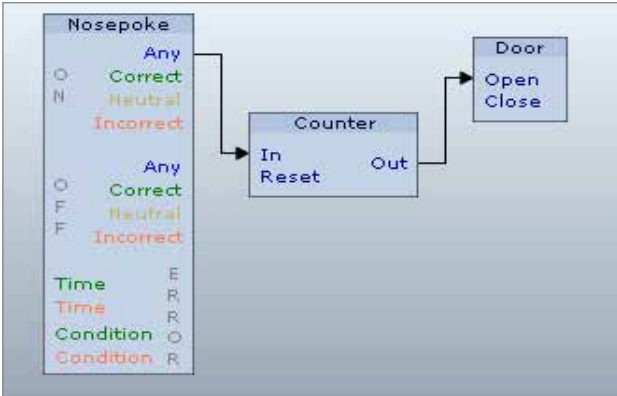
Any of the behavioral activities or sequences can be connected to actions of the system in order to build up customized conditioning protocols (more detailed description in the Software section).



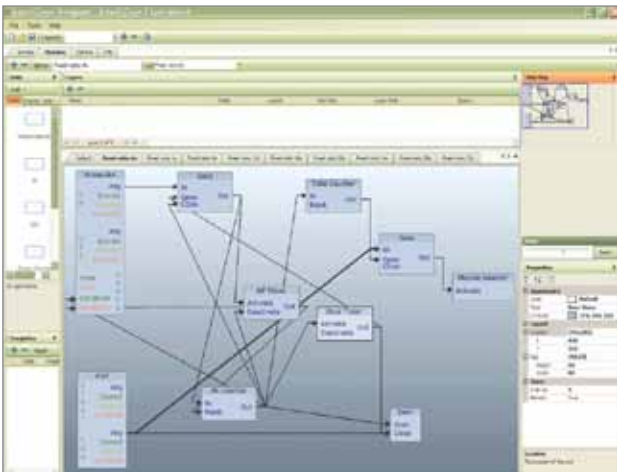
Designer - Hardware Setup



IntelliCage 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 each transponder-marked animal 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:

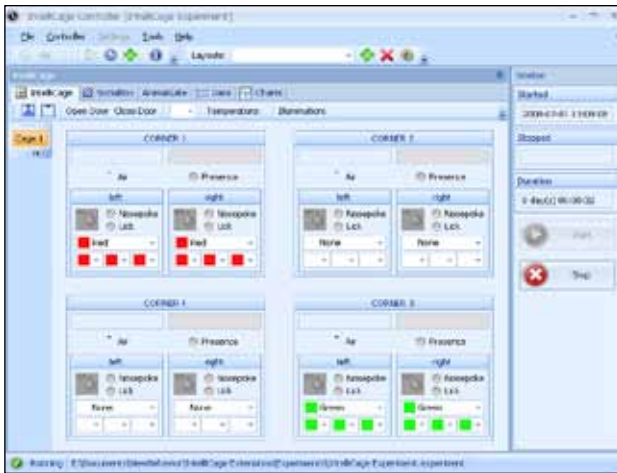
- Specify hardware settings
- Create animal list (enter incorporate animal names and corresponding transponder numbers)
- Create experimental designs by assigning specific clusters to individual animals and modules to groups of animals

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.

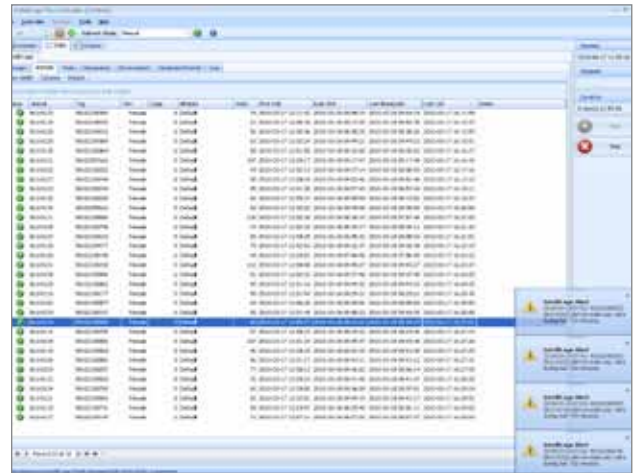
Create modules (experimental designs) defining events in specific corners (according to cluster information): link animal behavior (visit, nosepoke, lick) to trigger hardware events (door opening, lights, air-puff) – resulting in full control over conditioning behavior.

You can create several modules and imply switches between the clusters and/or modules that can be driven by the specific behavior of mice. Moreover, you can define day patterns where links between the modules or clusters occur at specified time of the day.

Custom schedules are available on request.



Controller



Controller - Animal list with alerts

Controller

The Controller extracts and stores all behavioral events (visits, nosepokes, licks) from the incoming stream of sensor data and 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 these 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 individual animals or of animals in groups. At specified intervals the Controller can be programmed to send alerts if animal has no visits or licks during a specified period.

The Controller

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

Analyzer

The Analyzer software takes advantage of the stored behavioral sequence data in order 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 into other computer programs such as standard graphical and statistical packages.

The Analyzer allows to

- 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



Analyzer - Data graph (e.g. visits)



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

Spontaneous Behavior
Basic activity levels, circadian activity
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

Social & Others
Competition rank order
Approach-avoidance conflicts

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 ration conditioning (motivation)
DRL (different reinforcement of low responding, response inhibition, timing)

Free programmable tasks

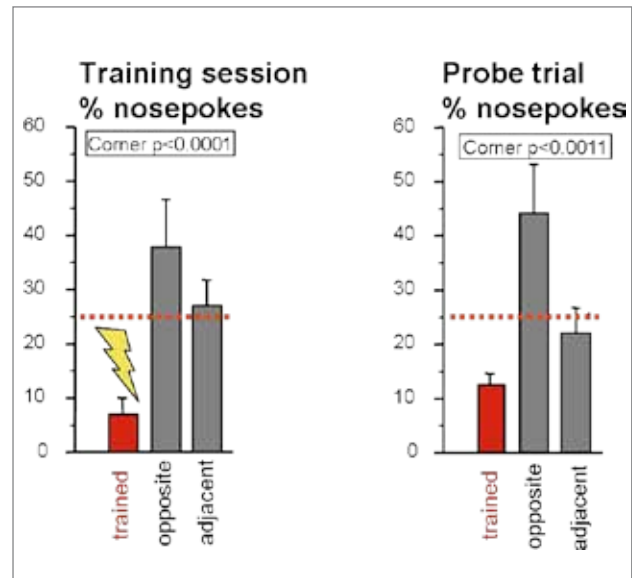
Freely programmable tasks

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

Behavioral domains covered by programmable tasks in the IntelliCage:

- 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)

A few most common and straightforward tasks are explained in the text sessions. The scientific hypothesis and goal of the specific study regulates which tasks need to be performed or combined into a test battery. It is important to note that the 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 the IntelliCage allowing efficient long-term monitoring.



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

Spontaneous behavior

Free adaptation

Animals are released in 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.

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 air-puffs 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 anti-clockwise after correct visit, or actual drinking, or after each visit.

Operant conditioning tasks

Light discrimination

The correct side for making the nosepoke and opening the door is indicated by LED in the beginning of visit. The correct side is randomly changed for each corner visit.

Impulsivity and DRL procedures

The animal initiates a trial with the first nose-poke and is required to wait for certain delay for making the second nose-poke 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 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, animals can learn the positions of sweet solution and are expected to show preference. Following adaptation the door to water is opened immediately when 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.

Taste preference or aversion

Conditioned taste aversion (CTA)

During training the conditioned group has access to bottles with sucrose and LiCl or water. For testing, 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.

Practical examples for validation

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

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

Applications of the IntelliCage

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 can take advantage of IntelliCage:

- 1) High-throughput behavioral phenotyping – several international initiatives aim at targeting most of the genes in mouse genome with long-term goal at identification of specific functions of every single gene. The success of these projects depends on phenotyping procedures applied for characterization of mutant mice.
- 2) Assessment of disease models – IntelliCage allows longitudinal studies, where animals can visit the system several times during their life-span, thereby allowing to detect 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 15 years in conventional tests, testing in the IntelliCage can advance these comparisons further.
- 5) Pharmacological studies – drugs can be administered orally in drinking water or delivered via osmotic mini-pumps.



AnimalGate



SocialBox

Add-on AnimalGate

- expands IntelliCages to a multi-area system with selective access for individual mice
- senses the direction of passage and distributes animals according to your directives
- measures precisely body weight of mice passing
- permits individual access to specific food or liquid sources, and any other connected area
- expands the capacity of IntelliCage for measuring food consumption behavior
- creates the opportunity to apply treatments differentially to specific animals without handling

Add-on 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

IntelliCage applications in selected articles

- **Barlind et al. (2010)** – IntelliCage was used to evaluate place learning after irradiation
- **Branchi et al. (2010)** – measuring anhedonia following social stress as an animal model of depression
- **Galsworthy et al. (2005)** – the first paper published with IntelliCage, where the comparison of behavioral profiles of two rodent species was shown. Spontaneous behavior, activity pattern, corner preference and reversal were analyzed
- **Jaholkowski et al. (2009)** – authors used IntelliCage for behavioural characterization of GM-mice; place preference and avoidance tests were applied
- **Kiryk et al. (2008)** – IntelliCage was used for behavioural characterization of GM-mice. Adaptation period was followed by place preference and place avoidance learning
- **Knapska et al. (2006)** – IntelliCage was used for appetitive and aversive conditioning, and the authors examined the role of amygdala in these processes. In the place preference test the mice were supposed to associate the sweetened water with a specific corner (appetitive learning), whereas in the aversive training they were learning to avoid the corner where air-puff was delivered
- **Krackow et al. (2010)** – cross-laboratory validation of the IntelliCage, documentation of consistent strain differences without interaction between strain and testing laboratory. Three mouse strains were tested in three replications by 4 laboratories. The following protocols were applied – free adaptation, nosepoke adaptation, sessions adaptation, place preference followed by reversal learning
- **Mechan et al. (2009)** – comparison of behavioural profiles of young and middle-aged mice (C57BL/6J) in the IntelliCage. The protocols included place learning and reversal with positive (drinking allowed in one correct corner) and negative reinforcement (air-puffs in three incorrect corners), also novelty induced exploration was examined (an object placed under the grid in the corner)
- **Onishchenko et al. (2007)** – the long-term effects of developmental exposure to methylmercury (MeHg) in mice were investigated. The IntelliCage appeared to be more sensitive than conventional tests in detecting alterations in activity and learning paradigms (adaptation, place preference learning and reversal, patrolling)
- **Rudenko et al. (2009)** – characterization of mouse model of Huntington's disease in the IntelliCage. The protocols included place learning and reversal learning, place avoidance learning, patrolling behavior, and side alternation task. The results indicated that the IntelliCage was a reliable system for recording exploratory activity, drinking behavior, circadian rhythm, spatial preference, and cognition in mice during prolonged periods of assessment; moreover, system detected early dysfunctional behaviors in transgenic mice
- **Safi et al. (2006)** – the authors showed that the IntelliCage can be used for assessment of anxiety and effect of anxiolytic drugs in a fast and efficient way by applying a Vogel conflict test adapted for use in the IntelliCage
- **Viosca et al. (2009)** – characterization of mouse model of Costello syndrome (a rare congenital disorder associated with cognitive and emotional impairments). The IntelliCage was used for assessment of general locomotor activity (number of corner visits)
- **Voikar et al. (2010)** - assessment of learning and memory in different strains; affects of hippocampal and striatal lesions
- **Zhu et al. (2010)** – memory impairments caused by isoflurane anesthesia in mice were shown in the IntelliCage (corner preference and reversal learning were applied)

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TSE Systems is a leading supplier of sophisticated research instrumentation in the global life science market. Our focus is on providing the total customer solution, with modular designs of integrated hardware and software platforms for neuroscience, metabolic and behavioral phenotyping, drug screening and toxicology. *NewBehavior* as part of the TSE Systems International Group is specialized in state-of-the-art technology for testing animal behavior within the social context.

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